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Urinary Tract infection, Drug Resistance Profile and Fetal Outcomes among Pregnant women in Two Health centers and Tikur Anbessa Specialized Hospital , Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Wegayehu Zebene, entitled:

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ABBREVIATIONS

ANC	Antenatal Care
ANC	Antenatal care clinic
APGAR	Pulse Grimace Appearance Activity Respiration
ART	Antiretroviral therapy
AST	Antimicrobial SusceptibilityTests
AYH	Adolescence and youth health
BAP	Blood Agar Plate
CFU	Colony forming unit
CoNS	Coagulase- Negative Staphylococcus
EPHI	Ethiopian Public Health Institute
EPI	Expanded Program on Immunization
IMNCI	Integrated Management of Newborn and Childhood Illness
IUGR	Intrauterine Growth Retardation
MAC	MacConkey Agar Plate
NRLCBM	National referral laboratory clinical bacteriology and mycology
PMTCT	Prevention of mother-to-child transmission
PROM	Prerupture of Membrane
SOP	Standard Operating
VCT	Voluntary counselling and testing
UTI	Urinary Tract Infection

ABSTRACT

Background: Urinary tract infection is commonly encountered health problem among pregnant women. Untreated urinary tract infection may result in fetal complications like preterm birth, low birth weight, intra uterine growth retardation, and intrauterine fetal death.

The problem of urinary tract infection is further compounded by the development of drug resistance. Determining drug susceptibility pattern of bacteria from urinary tract helps to identify effective drugs and minimize further adverse perinatal outcomes.

Objective: The objective of this study was to describe the magnitude and drug resistance profile of pregnant women and related fetal outcomes during their third trimester period at Gerji, Felege Meles health centers and Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia.

Methods: A cross-sectional study was conducted from March to June 2019. Socio-demographic data of the study participants were collected by administering structured questionnaire after obtaining full consent of the participants. Clean catch mid-stream urine was collected from the study participants and the samples were transported to the Ethiopian Public Health Institute laboratory with screw-capped container. Blood and MacConkey agar was used to culture the urine sample. Bacterial colonies were isolated and identified by their biochemical properties. Antibacterial susceptibility test was done on Muller-Hinton agar using different antibiotic discs and confirmed by VITEK2 Compact machine. At the time of delivery, infant's birth weight and gestational age was recorded on the log book as part of the daily work. Fetal outcome data required for the study were recorded from the log book and entered to EPI data management software then transferred to SPSS for analysis.

Result: Out of 424 urine samples processed, 63 (14.9%) yielded significant bacteriuria. Out of sixty three samples, fifty nine (93.7%) cases of UTIs were recorded among age group of 15-34years. *Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus* were the three dominant bacterial isolates.

Most of the gram negative bacterial isolates were resistant to ampicillin and ceftazidime (43.2% each) while gram positive were resistant for tetracycline (36.8%). The result of this study showed that UTI had statistically significant association with the occurrence of Intrauterine fetal death (IUFD) and prerupture of membrane (PROM) ($P<.05$).

Conclusion: Adverse fetal outcomes are significantly linked with the presence of UTI during pregnancy. The condition can be managed if early diagnosis and treatments are made for the mother. The study highlights that, UTI related adverse fetal outcomes reaches to the level that requires early interventions.
Key words: UTI, Fetal outcomes, AST, IUFD, APGAR score

1. INTRODUCTION

1.1.Background

The urinary tract includes the organs that collect and store urine and release it from the body and these organs include the kidneys, ureters and bladder, urethra and accessory structures [1]. A urinary tract infection (UTI) is an infection caused by growth of microbes to different structures involved in formation and elimination of urine namely the kidney, ureters, urinary bladder and urethra [1]. Based on the structures involved, UTIs are medically classified as cystitis (if the infection is in the bladder), pyelonephritis (if kidney is affected by the infection), ureteritis (ureter infection) and urethritis (infection in the urethra) [1].

UTI affects both genders but it is more prevalent (75%) in females [2], due to short urethra and contamination of the urinary tract with fecal flora from the anal opening [3-5]. In non-pregnant female, the uterus lies behind and partly over the bladder [6]. During the course of pregnancy, the enlarging uterus creates a pressure on nearby ureters and bladder which results a delay in urine flow and prevent complete emptying of urine; this condition places an opportunity for growth of microbes within the urinary tract [7]. In addition to the static urine in dilated urethra, development of glycosuria further enhances bacterial growth in the urethra and ascending tract. Furthermore; an increased bladder volume along with decreased bladder and urethral tone enhances bacterial growth in pregnant women [6, 8, 9].

UTI is common health problem for almost 50% of women during their child bearing age (16-35) and second most common illness after anemia for more than 10% of pregnant women [1, 10, 11]. UTI-related adverse fetal outcomes in untreated case include; low birth weight, intrauterine growth retardation (IUGR), intrauterine fetal death and preterm delivery [4, 10, 12].

Escherichia coli is responsible for the majorityof UTI cases and *Staphylococcus saprophyticus*, *Klebsiella* species, *Proteus* species and *Enterobacter* species account for the remaining cases [1, 13, 14].

Nitrofurantoin, amikacin, ofloxacin, ciprofloxacin and cefotaxim were highly effective drugs over most bacterial isolates in Libya, while Alemu, A., et al., reported similar finding for ceftriaxon, ciprofloxacin, norfloxacin, gentamicin, amoxicillin-clavulanic acid, co-trimoxazole. In this study, chloramphenicol produced the highest sensitivity over all isolates [6, 15]. According to the report from similar study conducted in Dire Dawa, Eastern Ethiopia, Gram negative bacteria were highly sensitive for ceftriaxone, gentamicin, and ciprofloxacin [12].

All the isolates were resistant to nalidixic acid, cotrimoxasole and cefaloxine in Nigeria, [16]

E.coli was highly sensitive to nitrofurantoin, cefotaxim, amikacin, ciprofloxacin and ofloxacin in Libya [15] to ceftriaxone, gentamicin, ciprofloxacin, trimethoprim–sulfamethoxazole, and chloramphenicol in Dire Dawa [12], to ceftriaxon, chloramphenicol, ciprofloxacin, norfloxacin and gentamicin in Gondar [6], and to nitrofurantoin in Bahir Dar [17].

The finding of the study conducted by Derese et.al indicates that Gram-positive isolates were susceptible to gentamicin, erythromycin, ceftriaxone, ciprofloxacin and nitrofurantoin. According to this study, the isolates were resistant to ampicillin, tetracycline, and trimethoprim–sulfamethoxazole [12]. Similar study conducted in Bahirdar reported the high sensitivity of gram positive bacterial isoltes to gentamicin, nitrofurantoin and amoxicillin-clavulnic acid [17].

In crossectional retrospective study conducted in Iran, UTI during pregnancy and fetal weight were highly associated [18]. Similarly, a study in Ghana revealed that babies birth weight, birth outcome (live birth or still birth), Pulse Grimace Appearance Activity Respiration (APGAR) score at 1 minute and gestational age are significantly dependant on the UTI status of the mother during pregnancy [19]. Iqbal et. al. also reported that, 11.3 % of the deliveries from UTI positive pregnant mothers produced low birth weighted babies [4].

1.2.Statement of the problem

Symptomatic and asymptomatic UTIs are commonly encountered infections during pregnancy. If they are left undetected and untreated, they may cause adverse maternal and fetal outcomes [20].

Since pregnant women are at a high risk of development of UTIs, it is recommended that special attention is paid to them, especially for the management of bacterial UTIs. To this end determining

uropathogen isolates and their drug susceptibility pattern among pregnant women is of a highest priority before deciding to manage UTI cases [21].

Most of the times, physicians tend to prescribe broad-spectrum antibiotics when they suspect UTI. Two factors- Poor patient compliance and incomplete course of antibiotic therapy are responsible for the evolution of resistant bacterial strains to most of antibiotics [2, 22].

The distribution of antimicrobial susceptibility data of UTI causing microorganisms changes from time to time and from place to place [23]. This urges for the isolation of bacterial strains and their drug resistance profile should be determined for that specific location before treatment is given based on sign and symptoms of patients. There should be periodic monitoring of etiologic agents ofUTI and their resistance pattern to avoid further complication of the case [24]. Therefore, screening for UTI in pregnant women is very important if the prevalence rates are ≥2 % [25].

The studies regarding uropathogens and their antimicrobial susceptibility patterns in pregnant women and the related fetal complications in Ethiopia are limited [26].

Studies have shown the strong association that exist between maternal UTI during pregnancy and fetal complications including low birth weight, APGAR score, PROM, and IUFD [4, 18, 19].

Thus; the result of this study provide vital information on type of bacterial isolates and the drug resistance pattern in relation to fetal complications in pregnant women attending at study sites .

1.3.Significance of the study

This study determined the etiologic agent and antimicrobial susceptibility pattern of bacterial uropathogens among pregnant women in Gerji, Felege Meles Health Centers, and Tikur Anbessa Speclised Hospital.

Pregnant mothers infected with UTI face a two fold problem; their own infection and consequence of the UTI on the fetus.

Increasing multidrug resistance as a result of indiscriminate use of antibiotics is becoming an important and emerging public health problem. This needs regular monitoring of the antibiotic susceptibility of uropathogens in a particular area.

According to different literatures, maternal urinary tract infections are responsible for most of preterm births, low birth weight, IUGR, low fetal APGAR score and if worsen IUFD. These infections remain undetected and untreated in developing countries. The finding of this research produced important information for future use for physicians regarding UTI related birth complications.

The outcome of this research could be used as a reference for further studies in related field of study and it can provide updated information.

2. LITERATURE REVIEW

Worldwide the disease burden of UTI is estimated to be 150 million cases annually, with different types of UTI posing serious health problems. The financial burden is also understandably enormous with an estimated annual cost of community-acquired UTI of approximately $1.6billion in United States of America [27].

Out of 120 pregnant women studied at India, 42 (35%) cases were UTI positive with incidence rate of 48%, 45%, and 7% during third, second and first trimester respectively [28]. Related study in Iraq reported 21.8%bacteriuria among pregnant women. The result further indicates that the highest proportion of the cases (33.8%, 45.1%) were recorded for ages of 25-29 years and during the time third trimester respectively [29]. Hamdan et.al reported a prevalence of UTI 14.0% among pregnant women attending at Khartoum north teaching hospital [30]. A higher prevalence (26.7%) was reported in a study conducted by Onwuezobein in South Nigerian city and similar study in Nigerian teaching hospital indicates a prevalence of 5.8% [31, 32].

A study conducted in Gondar reveal prevalence of UTI 10.4%among recruited pregnant women. The finding of the research further indicates that history of catheterization and previous history of UTI had significantly associated with UTI [6]. A hospital based cross sectional study aimed to identify asymptomatic bacteriuria was conducted in 2012 in Dessie referral hospital, Northeast Ethiopia. The result indicates a prevalence of significant bacteriuria 15.6% (56/358) among asymptomatic pregnant women [33]. Similar study on asymptomatic bacteriuria conducted in Adigrat, Northern Ethiopia, shows a prevalence of asymptomatic bacteriuria 21.2% (55/259) [34].

2.1.Fetal outcomes

Bacterial endotoxins together with inflammatory chemokines and cytokines such as interleukin and tumor necrosis factor released in response to UTI stimulate production of prostaglandins. Prostaglandins and degrading enzymes in turn stimulate uterine contractions, premature rupture of membranes and eventually lead to preterm birth [35].

The study conducted in Iran on factors associated with preterm labor, UTI was the leading factor responsible for 263 (35.8%) of the total preterm cases [35].

Out of 72 deliveries from UTI positive mothers, 9 (12.5%) were underweight newborns and 24 (33.3%) gave normal birth weight [28].

Studies by Mate Siakwa et.al have reported UTI related maternal and perinatal outcomes including live birth/still birth; baby's Apgar score at 1 minute; gestational age and baby's birth weight [19].
According to the study, pregnant mothers with UTI had higher probability giving preterm birth with underweight babies than mothers without UTI. Most babies born from UTI positive mothers had APGR score ofless than 7 at one minute than babies born from mothers without UTI.
Their finding also shows that UTI in pregnant mothers had strong association with foul smelling vaginal discharge; prematurerupture of membranes and intra-partum bleeding (both APH and PPH) [19].
However; UTI in pregnancy was not significantly associated withoccurrence of Pregnancy-induced hypertension (PIH), history of previous abortion and mode ofdelivery (spontaneous vaginal delivery/instrumental delivery/ Caesarian section) [19].

2.2.Bacterial isolates

According to the study conducted in Khartoum, most of the cases 18/33 (54.5%) are caused by gram negative bacteria and the ratio of individual isolates are *E.coli* 14/33 (42.4%), *S. aureus*13/33 (39.3%), *K. pneumoniae* 3/33 (9%), group B streptococcus 2/33 (6%) and *P. aeruginosa* 1/33 (3%).[30]Findings from the Al-Kadassy AM study revealed that, *E.coli* is the commonest causative agent of UTI accounting for 19 (63.4%) of the total case [8].

Similar results (40%-50.0%) was reported in different partsofNigeria for *E. coli* while *K. pneumonia, S. aureus, P. aeruginosa,* and *P. mirabilis* took a prevalence of (11.1%-31%),(17.9%-21.00%) , *(3*.0%) and (3%) respectively [31, 32, 36].

There were 55.17% of *E.coli* isolates in a study done by Lavrinenko and it accounts for 50% of the total isolates in a study conducted by Al-Kadassy AM [8].

From the total number of bacteria isolated by Workineh, Gram negative bacteria accounts for 90.3% and *E. coli* accounts for 45.2%. *Proteus Spp, K. Pneumoniae, S. aureus* and *P aeroginosa* had incidence rate of 22.6%, 16.1%, 9.7% and 6.4% respectively [33].

Similarly, Ali et.al reported a prevalence of 62% for *S. aureus* and *E. coli, 31*% each. CoNS, *K.pneumonia,* GBS, and *Enterobacter spp* constitute 29.3%, 3.4%, 3.4% and 1.7% respectively [33].

2.3.Antibiotic susceptibility test

Alsamarai et al. in Indiareported that *E. coli* shows more than 70% resistance to ampicillin, amoxicillin, cefixime, cefprozil, ceftazidime, ceftriaxone and tobramycin. According to the researcher, low resistance of *E.coli* was observed for imipenem (1.5%), amikacin (5.9%), nitrofurantoin (17%) and, moderate resistance (50-70%) was recorded to gentamycin, cefaclor, trimethoprim, pipercillin,cefotaxime, Nalidicxic acid, azitreonam and tetracycline [20].

Majority of *E. coli* strains were sensitive to amikacin, streptomycin, ciprofloxacin, azithromycin, ceftriaxone and tetracycline while resistant to penicillin G, amoxicillin, amoxicillin-clavulanic acid, cephalexin, vancomycin [29].

The study conducted by Dougnon TV et.al indicates different degree of multidrug resistance for different strains of bacteria in urine. 36% of E. coli strains show multidrug resistance and all strains of *Citrobacterfreundii*, *S. aureus*were drug resistant [37].

Demilieet.al reported resistance pattern of gram-negative bacterial isolates. According to the result, 82.6%, 78.3% and 69.6% resistance were observed to ampicillin, amoxicillin and tetracycline respectively [17].

2.4.Risk factors of UTI

The obstetric status of patient had no significant relation with presence of UTI in a study conducted in Saudi Arabia [38].

Pregnant women with age groups of 25-29 and gestation period of third trimester had higher incidence of UTI in the study conducted in Iraq [29].

Conceptual framework

This conceptual frame work is adopted from different literatures for explaining the factors associated with UTI (the blue colored arrows) and variables affected by UTI (the green colored arows). This study mainly focuses on those variables indicated by colored arrows only.

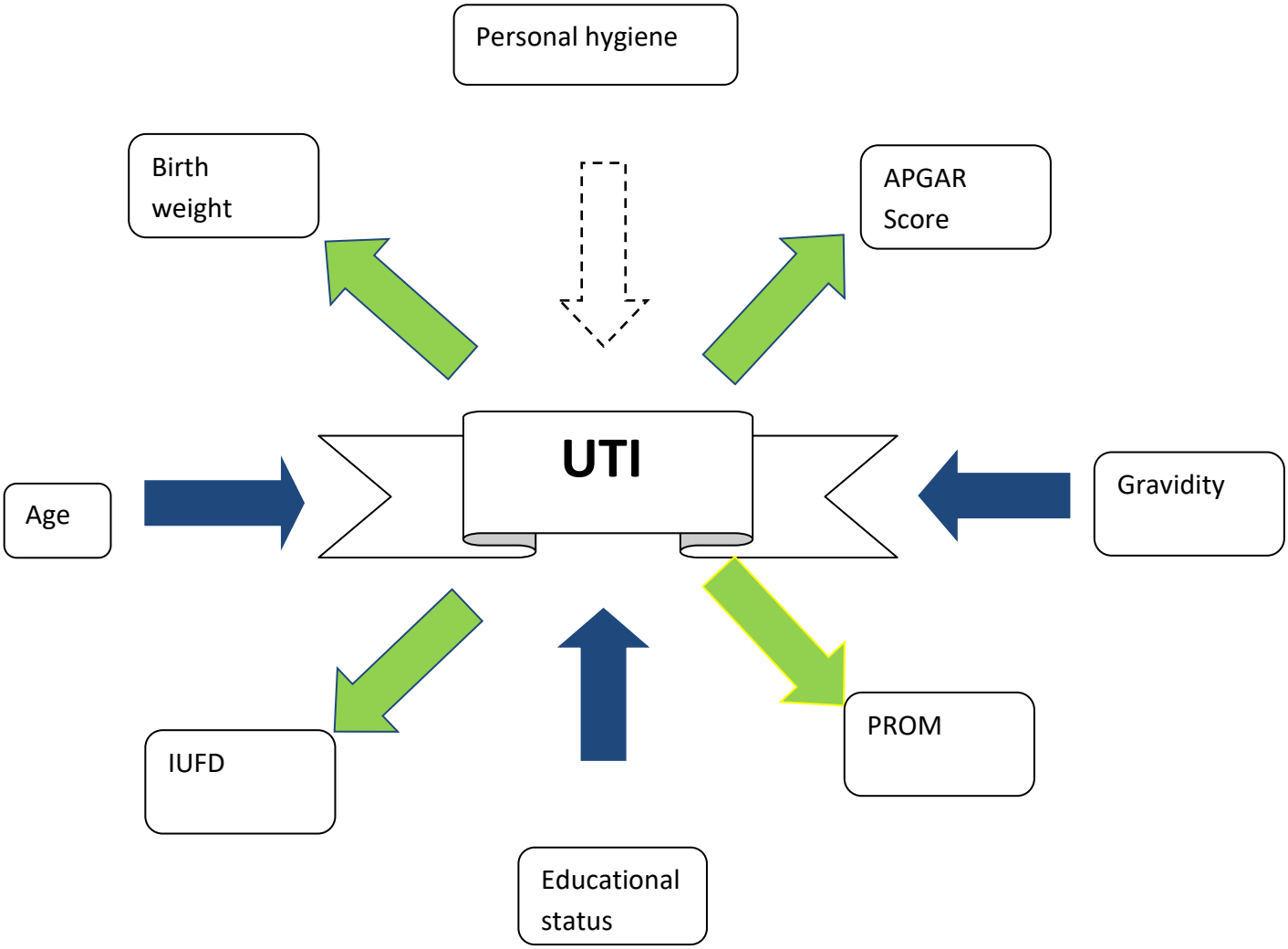

Figure 1: Risk factors of UTI taken from different literatures [5, 12]

3. OBJECTIVE

3.1.General objective

To assess the magnitude and drug resistance profile of pregnant women and related perinatal outcome at Gerji, Felege Meles Health Centers, and Tikur Anbessa Speclizsed Hospital, Addis Ababa, Ethiopia, from March to June 2019.

3.2.Specific Objective

- To determine the magnitude of UTI among pregnant women attending during their third trimester period at Gerji, Felege Meles Health Centers, and Tikur Anbessa Speclised Hospital Addis Ababa, Ethiopia, from March to June 2019.
- To determine drug resistance pattern of bacteria isolates from urine of pregnant women during the third trimester at the study sites
- To assess the association of perinatal outcomes on pregnant women with UTI attending at Gerji, Felege Meles Health Centers, and Black Lion Specialized Hospital, Addis Ababa, Ethiopia, from March 2019 to the end of June 2019.

4. HYPOTHESES

HO: There is no difference between pregnant women with UTI and without UTI on the perinatal outcome.

5. MATERIALS AND METHODS

5.1.Study Area

The study was conducted at Gerji Health Center, Felege Meles Health Centers, and Tikur Anbessa Speclised Hospital. Gerji Health Center established in 2013, is located at Gerji, Bole Sub City woreda 13. The health center is organized in to health care service case team and disease prevention case team, laboratory case team and finance case teams. Delivery ward, ANC room, EPI, family planning, IMNCI, VCT, ART and AYH are under disease prevention case teams. ANC room provides services related to PMTCT. Delivery ward is staffed with seven BSc midwives and equipped with seven beds and other materials necessary for providing 24-hour delivery services.

Felege Meles Health center was established in 2012, located in Addis Ketema subcity, woreda 06. The healthcenter has similar case teams as Gerji health center and has 9 midwiferies. The ANC room has 9 beds for delivery service.

Tikur Anbesa Tertiary Specialized Hospital is one of the largest university hospitals in Ethiopia with 700 bed capacity accepting referrals from across the nation for specialized cares.

5.2. Study Design and Period

A cross-sectional study was conducted from March to June 2019.

5.2.1. Source population

All pregnant women attending antenatal clinic at the two health centers, and Tikur Anbessa Speclised Hospital during the study period.

5.2.2. Study population

All pregnant women on their third trimester during the study period and attending antenatal clinic at the two health centers, and Tikur Anbessa Speclised Hospital that fulfil the inclusion criteria were the study population.

5.3.Inclusion and Exclusion criteria

5.3.1. Inclusion criteria

Pregnant women with the gestational age of third trimester and those who were attending antenatal clinic during the study period.

5.3.2. Exclusion criteria

Pregnant women who were on antibiotic therapy for UTI during 14 days of data collection time.

Pregnant women in labour.

5.4.Study Variables

5.4.1. Dependent variables

- Perinatal outcomes
  - ✓ Fetal low birth weight
  - ✓ IUFD
  - ✓ APGAR score

5.4.2. Independent variables

- UTI Magnituide

Covatiates (age, marital status,educational status,gravidity)

5.5.Sample Size Calculation and Sampling Method

5.5.1. Sample size determination

A single proportion formula was used to calculate the sample size,

$n=Z^2 p (1-p)/d^2$

Where Z= Z score for 95% confidence interval= 1.96

P= Prevalence, =0.5

d= Tolerable error=5%

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

(3.842)(0.5)(0.5)/(0.0025)=385with 10% anticipated non- response rate, the total sample size were (385+38.5) =424 participants.

5.5.2. Sampling method

Every pregnant mothers attending the antenatal clinic who were willing and available at the time of study, fulfilling the inclusion and exclusion criteria were enrolled by non-probable consecutive technique until the number reaches the required sample size.

5.6.Measurement and Data Collection

5.6.1. Socio-demographic and fetal outcome information

A structured questionnaire was used to collect socio-demographic data and clinical history of the study participants. Age, marital status, educational status, UTI sign and symptoms and questions related to fetal outcomes were included on the questionnaire. Participant's clinical data and fetal outcome data were taken from the individual card and ANC log books. Three trained BSc degree holder midwifes were involved for data collection.

5.6.2. Specimen collection

Midstream urine sample were collected from the study participants attending antenatal room at Gerji Health Center, Felege Meles Health Centers, and Tikur Anbessa Speclized Hospital from March to June 2019. Participants were informed on how to bring "mid-stream" urine of 15–20 ml volume through the following process.

- Thorough hand washes with water and dry
- Wash the genitalia
- Void the first 20-30 ml of urine in the toilet then collect the remaining urine in to a sterile clean container.

Then, the samples were transported to EPHI microbiology laboratory within an hour after it was collected. All urine specimens were collected in a well labeled, sterile, dry,wide-necked, leak proof and screw capped container.

5.6.3. Laboratory Methods

5.6.3.1.Culture

A calibrated sterile wire and plastic disposable loops were used to inoculate 1µl of urine specimen on MacConkey and blood agar plates.

After 24 hours of incubation at 37°C, a specimen considered positive for UTI if a single organism was cultured at a concentration of $>10^5$cfu/ml.

Identification of the isolated bacterial pathogens were done on the basis of culture morphology and biochemical characters. Indole test, citrate, urea, coagulase, catalase, motility agar, lysine iron agar and novobosin disk were used for identification.

5.6.3.2.Antimicrobial Susceptibility testing

Antimicrobial Suceptibility testing were performed on isolates according to the criteria of ClinicalandLaboratory StandardsInstitute (CLSI, 2019) using the Kirby–Bauer disc diffusion method on Muller-Hinton Agar . A homogeneous bacterial suspension was prepared by mixing a loop full of pure bacterial colony in 5ml of 0.85% saline solution. After adjusting the 0.5 McFarlandstandard of the suspension, it was evenly innoculatedon MullerHinton agar plate using sterile cotton swab and antibiotic disks were placed at 15mm and 24mm distance from the edge and from each other respectively. The plates were then incubated for 24 hours at 37^oc. The isolates were categorized as sensitive (S), resistant (R) or intermediate (I) based on the inhibition zone they produced around the antibiotics used.

Confirmation of AST result was done by Vitek 2 Compact system. The Vitek2 Compact (30card capacity) system uses a fluorogenic methodology for organism identification and a turbidimetric method for susceptibility testing.

AST- GN73 and AST-GP74 cards were used for the susceptibility testing of gram negative and gram positive bacteria respectively.

The drugs that were used in the susceptibility test of gram negative bacteria are Ampicillin, Ampicillin/Sublactam, Piperacillin/ Tazobactam, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Trimethoprim/ Sulfamethoxazole. While for gram positive bacteria, Amoxicillin, Penicillin, Cefotaxime, Ceftriaxone, Chloramphenicol, Ertapenem, Erythromycin, Levofloxacin,

Linezolid, Meropenem, Moxifloxacin, Ofloxacin, Telithromycin, Tetracycline, Trimethoprim/Sulfamethoxazole, Vancomycin are used.

5.6.4. Data Quality Assurance

During data collection and sample collection, data quality were ensured through the use of standardized questionnaire, proper orientation and intensive supervision by the principal investigator. In addition, the questionnaires are translated in to Amharic language for easy understanding. SOPs of the microbiology laboratory were followed.

Sterility of the media was checked by over night incubation of the media at 37^0c prior to inoculation. Quality control bacteria were used to check the growth support of the media. For blood agar plate (S. pyogen, E. faecalis, S pneumonia), for Mackonkey agar plate (E.coli, P. mirabilis, E. faecalis) and for Muller Hinton agar plate (S. aureus, E.coli, E. faecalis) were used.

Well-trained and experienced laboratory professionals were involved during culturing, isolation, and drug susceptibility test and data analysis stage. All the data were checked for their completeness and representativeness prior to entry.

5.7.Data Analysis and Interpretation

Data entry was done using EpiData Manager (version 4.4.2.1) and statistical analysis was performed using IBM SPSS (version 23) software.

Findings of the study was further explained in words and tables. Binary and multiple logistic regression analysis was used to see if there is an association with different variables. In all cases P-value less than 0.05 considered as statistically significant.

5.8.Operational Definition

Previous antibiotic use: - patients who had received antibiotic therapy before 15 day of the sample collection.

Preterm labor: - The parturition before completion of 37 weeks of pregnancy (less than 259 days)

Low birth weight: - Birth weight (measured within the first 72 h of life) of <2500 g.

Urinary tract infection/ significant bacteriuria:- A culture that grew $\geq 10^5$ CFU/ml in single voided midstream urine.

Fetal outcome:- Stillbirth, pre-term birth, low birth weight below 2500gm, low APGAR score in 1 minute or normal birth .

5.9.Ethical considerations

The ethical approval for this study was obtained from Department Research and Ethical Review Committee (DRERC) of Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, and Addis Ababa Health Bureau prior to initiation of the study. Support letters were written from the department to Addis Ababa Health Bureau, EPHI, Gerji Health Center, Felege Meles Health Center and Tikur Anbessa Speclised Hospital.

Proper explanation about the study was given to the study participants and the study was conducted after obtainingfull Verbal and written consent from pregnant women attending at antenatal clinics at the study sites.

The purpose of the study was explained to the study participants and also they were informed that the procedures used in the study do not cause any harm to them or their child. They were told that they have a full right to participate or not, to withdraw the consent and stop participation at any time without any form of prejudice.

Study participants were also informed that confidentiality of the information and privacy of the respondents will be assured byusing codes instead of their names at each step of the study process. In addition, they were informed about the benefit of being part of the study. Positive UTI results were given to clinician and the study participants got appropriate treatment.

5.10. Dissemination of the results

The result of the study will be submitted to Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences. Oral presentation of the thesis will be made. A printed and soft copy of this material will be submitted to EPHI Laboratory, Ob-Gyn Department, Health Science College, Addis Ababa University, annual conferences of professional societies and other concerned bodies.The finding of the study will also be presented to the medical scientific community and manuscript will be submitted to peer reviewed journals for publication.

6. RESULTS

6.1. Socio demographic characteristics

There were a total of 424 participants and the age distribution of study subjects ranges from 18-40 years with the mean (standard deviation) of 27 ± 4.6 years and 393 (92.7%) of them belongs to the age group 15-34 years.

It was observed that around 1.4% of the study participants were divorced. Only 41 (9.7%) of the study participants had higher education and 293 (69.1%) were housewives. Based on their gravidity, more than half of the study participants (62.3%) were multigravida and 80 (18.9%) of the study participants had history of previous urinary tract infection. None of the study subjects were cigarette or alcohol addicted (Table 1).

Table 1: Sociodemographic, obstetric, and clinical variables of pregnant women

Variables	**Total No of participants = 424**				
	Nº (%)	**No Bacteriuria Nº (%)**	**Bacteriuria Nº (%)**	**Chi^2**	**P value**
Age					
15-24	138 (32.4)	118 (27.8)	20 (4.7)	0.147	0.929
25-34	255 (60.3)	216 (50.9)	39 (9.2)		
35-44	31 (7.3)	27 (6.4)	4 (0.9)		
Marital Status					
Married	417 (98.6)	356 (84.2)	61 (14.4)	1.633	0.201
Divorced	6 (1.4)	4 (0.9)	2 (0.5)		
Educational level					
Illiterate	68 (16.0)	55 (13)	13 (3.1)	9.451	0.051
Elementary	173 (40.8)	147 (34.7)	26 (6.1)		
High school	142 (33.5)	127 (30)	15 (3.5)		
Higher education	41 (9.7)	32 (7.6)	9 (2.2)		
Occupation					
Housewife	293 (69.1)	248 (58.5)	45 (10.6)	0.622	0.891
Government	78 (18.4)	67 (15.8)	11 (2.6)		
Non-Government	29 (6.8)	26 (6.1)	3 (0.7)		
Student	24 (5.7)	20 (4.7)	4 (0.9)		
Gravidity					
Primigravida	160 (37.7)	133 (31.4)	27 (6.4)	1.417	0.234
Multigravida	264 (62.3)	228 (53.8)	36 (8.5)		
History of UTI					
Yes	80 (18.9)	68 (16)	12 (2.8)	0.002	0.968
No	344 (81.1)	293 (69.1)	51 (12)		
Cigarette/ alcohol addiction					
Yes	0	0 (0)	0 (0)	--	--
No	424 (100)	361 (85.1)	63 (14.9)		
Symptoms of UTI					
Yes	82 (19.3)	68 (16.0)	14 (3.3)	4.915	0.555
No	342 (80.7)	293 (69.1)	49 (11.6)		

6.2. Magnituide of UTI and distribution of bacterial isolates

Four hundred twenty-four (424) urine samples were collected from pregnant women attending antenatal clinic at the study sites, out of which 63 (14.9%) were positive for the presence of significant bacteriuria. From the total study subjects, 80.7 % of them had no symptoms of UTI out of which 11.6 % had significant bacteriuria in their urine.

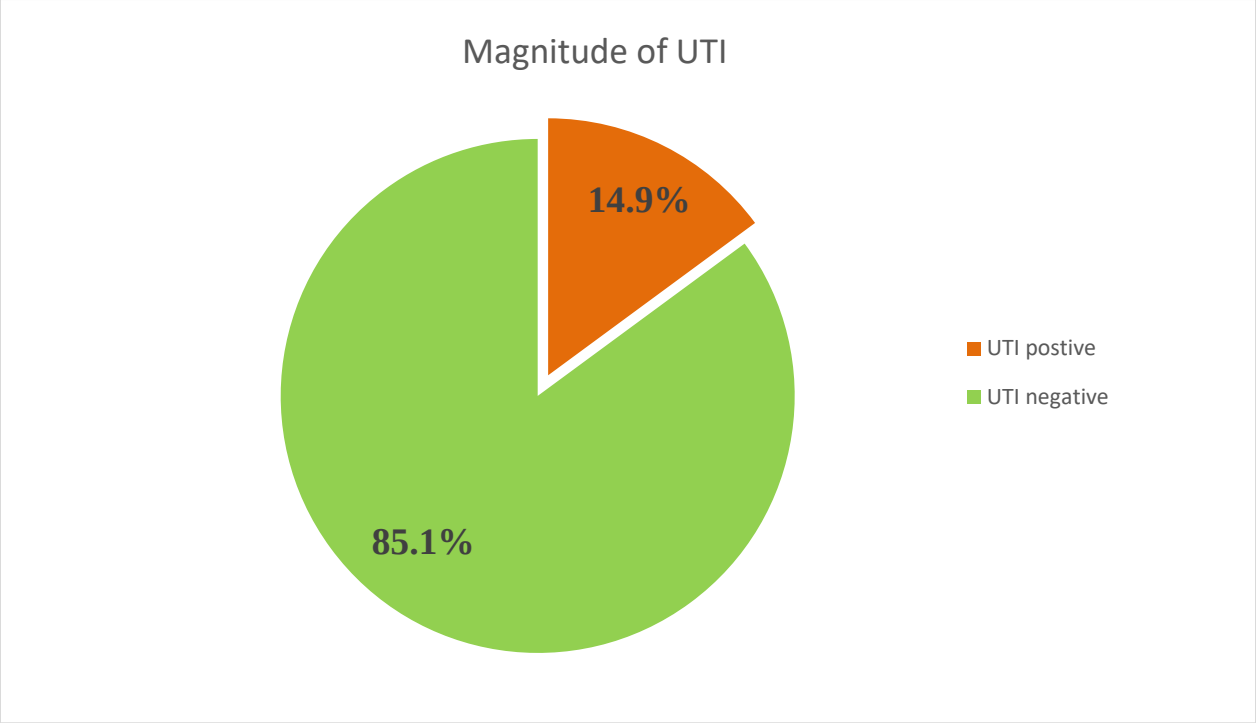

Figure 2: Magnitude of UTI

Of the total isolates, gram negative bacteria were the most common accounting for 44 (68.75%) of the total isolates. *E. coli*, *K. pneumoniae* and *S. aureus* were the three predominant bacteria consisting of 28 (44.4%), 6 (9.5%) and 6 (9.5 %) of the total isolates respectively. The least abundant bacterial isolates were *C. freundii*, *K. oxytoca*, and *P. stuartii*, each represented by one (1.6%) isolates.

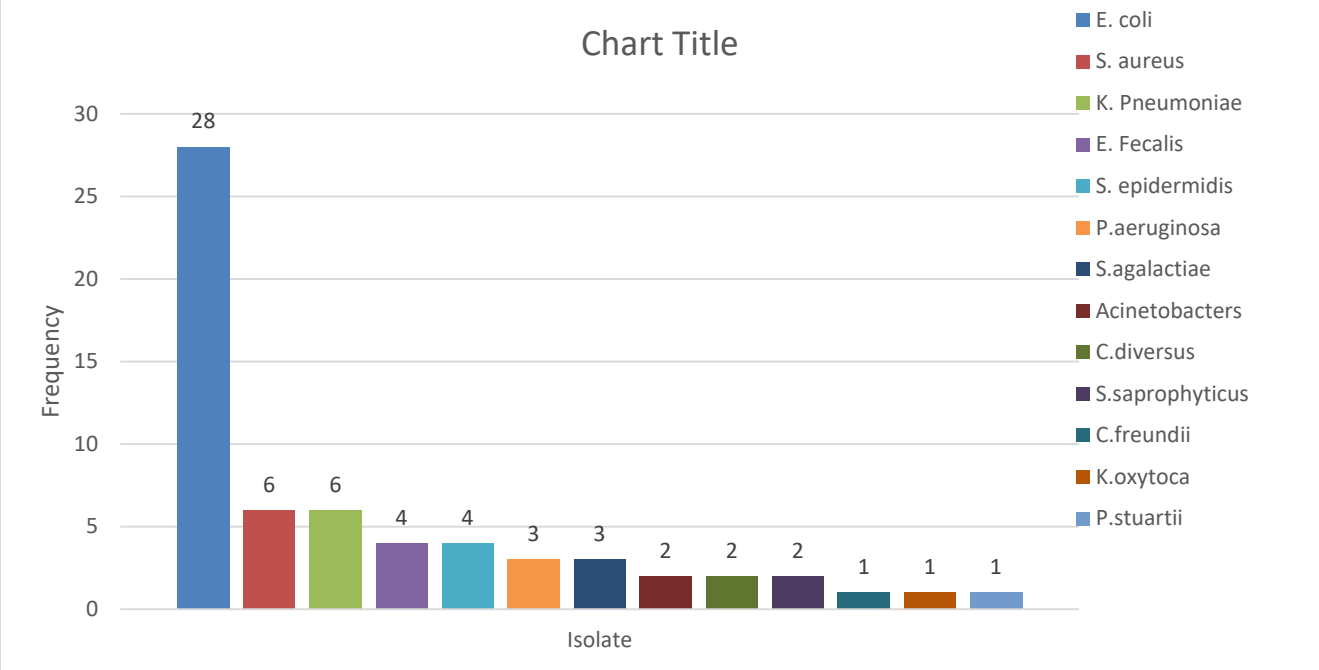

Figure 3: Distribution of bacterial species isolated from pregnant women

6.3.Antimicrobial susceptibility pattern of isolated bacteria

Tables 2 and 3 summarized antibiotic susceptibility patterns of gram negative and positive isolates respectively.

E. coli were sensitive for cefoxitin (92.9%), levofloxacin (92.9%), cefepime (92.9%), amikacin (92.9%), piperacillin/ tazobactam (85.7%), cefazolin (89.3%), tobramycin (96.4%), nitrofurantoin (89.3%), trimethoprim/ sulfamethoxazole (82.1%), ciprofloxacin (89.3%), gentamicin (89.3%), ceftriaxone (82.1%), and resistant for ampicillin (57.1%), ceftazidime (39.3%).

K. Pneumonia isolates showed resistance to ceftazidime (66.7%), cefepime (50%), meropenem (50%), while it was susceptible for cefoxitin (100%), levofloxacin (100%), piperacillin/ tazobactam (100%), cefazolin (83.3%), amikacin (83.3%), gentamicin (100%), tobramycin (100%), nitrofurantoin (83.3%), trimethoprim/ sulfamethoxazole (100%), ciprofloxacin (100%).

Table 2: Antibiotic susceptibility pattern of Gram negative isolates

Bacterial Isolates	**№**	**Pattern**	**Antimicrobial agent tested**															
			AM	**SAM**	**TM**	**CZ**	**FOX**	**CAZ**	**CRO**	**FEP**	**MEM**	**AMK**	**GM**	**TOB**	**CIP**	**LEV**	**FT**	**SXT**
A. baumannii	2	S	1 (50)	1 (50)	2 (100)	2 (100)	2 (100)	1 (50)	2 (100)	2 (100)	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50)
		R	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)
		I	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
C. diversus	2	S	0 (0)	1 (50)	2 (100)	1 (50)	2 (100)	0 (0)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50)	1 (50)
		R	1 (50)	0 (0)	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)
		I	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)
C. freundii	1	S	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
		R	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
E. coli	28	S	11 (39.3)	18 (64.3)	24 (85.7)	25 (89.3)	26 (92.9)	14 (50)	23 (82.1)	26 (92.9)	20 (71.4)	26 (92.9)	25 (89.3)	27 (96.4)	25 (89.3)	26 (92.9)	25 (89.3)	23 (82.1)
		R	16 (57.1)	7 (25)	4 (14.3)	2 (7.1)	2 (7.1)	11 (39.3)	5 (17.9)	2 (7.1)	2 (7.1)	2 (7.1)	3 (10.7)	1 (3.6)	3 (10.7)	2 (7.1)	1 (3.6)	5 (17.9)
		R	2 (33.3)	2 (33.3)	0 (0)	1 (16.7)	0 (0)	4 (66.7)	2 (33.3)	3 (50)	3 (50)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)

AM= Ampicillin, SAM= Ampicillin/Sublactam, TM= Piperacillin/ Tazobactam, CZ= Cefazolin, FOX= Cefoxitin, CAZ= Ceftazidime, CRO= Ceftriaxone, FEP= Cepepime, MEM= Meropenem, AMK= Amikacin, GM= Gentamicin, TOB= Tobramycin, CIP= Ciprofloxacin, LEV= Levofloxacin, FT= Nitrofurantoin, SXT= Trimethoprim/ Sulfamethoxazole, S= Susceptible, R= Resistant, I= Intermediate

Table 2: Antibiotic susceptibility pattern of Gram negative isolate

Bacterial Isolates	**№**	**Pattern**	**Antimicrobial agent tested**															
			AM	**SAM**	**TM**	**CZ**	**FOX**	**CAZ**	**CRO**	**FEP**	**MEM**	**AMK**	**GM**	**TOB**	**CIP**	**LEV**	**FT**	**SXT**
K.oxytoca	1	S	0	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1
			(0)	(100)	(100)	(100)	(100)	(0)	(0)	(100)	(0)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
		R	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0
			(100)	(0)	(0)	(0)	(0)	(100)	(100)	(0)	(100)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
		I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
P. stuartii	1	S	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
			(100)	(0)	(0)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
		R	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
			(0)	(100)	(100)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
		I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
P. aeruginosa	3	S	2	3	3	3	3	1	1	3	2	2	3	2	2	3	3	3
			(66.7)	(100)	(100)	(100)	(100)	(33.3)	(33.3)	(100)	(66.7)	(66.7)	(100)	(66.7)	(66.7)	(100)	(100)	(100)
		R	1	0	0	0	0	2	2	0	1	1	0	1	1	0	0	0
			(33.3)	(0)	(0)	(0)	(0)	(66.7)	(66.7)	(0)	(33.3)	(33.3)	(0)	(33.3)	(33.3)	(0)	(0)	(0)
		I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Total № of isolates	44	S	19	28	39	38	42	19	33	38	28	40	41	42	40	42	39	37
			(43.2)	(63.6)	(88.6)	(86.4)	(95.5)	(43.2)	(75)	(86.4)	(63.6)	(90.9)	(93.2)	(95.5)	(90.9)	(95.5)	(88.6)	(84.1)
		R	23	11	5	5	2	21	11	6	8	4	3	2	4	2	1	7
			(52.3)	(25)	(11.4)	(11.4)	(4.5)	(47.7)	(25)	(13.6)	(18.2)	(9.1)	(6.8)	(4.5)	(9.1)	(4.5)	(2.3)	(15.9)
		I	2	5	0	1	0	4	0	0	8	0	0	0	0	0	4	0
			(4.5)	(11.4)	(0)	(2.3)	(0)	(9.1)	(0)	(0)	(18.2)	(0)	(0)	(0)	(0)	(0)	(9.1)	(0)

AM= Ampicillin, SAM= Ampicillin/Sublactam, TM= Piperacillin/ Tazobactam, CZ= Cefazolin, FOX= Cefoxitin, CAZ= Ceftazidime, CRO= Ceftriaxone, FEP= Cepepime, MEM= Meropenem, AMK= Amikacin, GM= Gentamicin, TOB= Tobramycin, CIP= Ciprofloxacin, LEV= Levofloxacin, FT= Nitrofurantoin, SXT= Trimethoprim/ Sulfamethoxazole, S= Susceptible, R= Resistant, I= Intermediate

Table 3 summarizes antibiotic susceptibility pattern of gram positive bacteria. Gram positive bacteria showed variety of susceptibility pattern ranging from 0% to 100%. The most dominant gram positive bacteria, *S. aureus* were resistant to trimethoprim/ sulfamethoxazole (50.0 %) and tetracycline (66.7%) while it showed sensitivity to amoxicillin (100%), penicillin (83.3%), cefotaxime (83.3%), chloramphenicol (83.3%), erythromycin (83.3%), ertapenem (83.3%), linezolid (100%), moxifloxacin (100%), telithromycin (100%).

Similarly, *S. saprophyticus* were susceptible to amoxicillin (100%), penicillin (100%), cefotaxime (100%), ertapenem (100%), erythromycin (83.3%), levofloxacin (100%), linezolid (100%), meropenem (100%), moxifloxacin (100%), telithromycin (100%), ofloxacin (100%), tetracycline (100%), trimethoprim/ sulfamethoxazole (100 %), vancomycin (100%) and developed resistance to erythromycin (50 %), chloramphenicol (50%), ceftriaxone (100 %).

Table 3: Antibiotic susceptibility pattern of Gram positive isolates

Bacterial Isolates	**№**	**Pattern**	**Antimicrobial agent tested**															
			AMX	**P**	**CTX**	**CRO**	**C**	**ETP**	**E**	**LEV**	**LNZ**	**MEM**	**MXF**	**OFL**	**TEL**	**TE**	**SXT**	**VA**
E. faecalis	4	S	2 (50)	3 (75)	3 (75)	4 (100)	4 (100)	3 (75)	2 (50)	4 (100)	3 (75)	4 (100)	4 (100)	4 (100)	2 (50)	2 (50)	3 (75)	4 (100)
		R	2 (50)	1 (25)	1 (25)	0 (0)	0 (0)	1 (25)	2 (50)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	1 (25)	2 (50)	1 (25)	0 (0)
		I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)
S. agalactiae	3	S	1 (33.3)	3 (100)	2 (66.7)	2 (66.7)	1 (33.3)	2 (66.7)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	1 (33.3)	0 (0)	3 (100)	2 (66.7)
		R	1 (33.3)	0 (0)	1 (33.3)	0 (0)	2 (66.7)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (66.7)	3 (100)	0 (0)	1 (33.3)
		I	1 (33.3)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
S. epidermidis	4	S	3 (75)	4 (100)	4 (100)	4 (100)	3 (75)	4 (100)	1 (25)	2 (50)	3 (75)	4 (100)	2 (50)	3 (75)	4 (100)	1 (25)	3 (75)	3 (75)
		R	1 (25)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	3 (75)	2 (50)	1 (25)	0 (0)	1 (25)	1 (25)	0 (0)	3 (75)	1 (25)	1 (25)
		I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
S. aureus	6	S	6 (100)	5 (83.3)	5 (83.3)	4 (66.7)	4 (66.7)	4 (66.7)	5 (83.3)	4 (66.7)	6 (100)	4 (66.7)	6 (100)	4 (66.7)	5 (83.3)	2 (33.3)	2 (33.3)	3 (50)
		R	0 (0)	0 (0)	0 (0)	2 (100)	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
Total № of isolates	19	S	14 (73.7)	17 (89.5)	16 (84.2)	14 (73.7)	13 (68.4)	15 (78.9)	11 (57.9)	15 (78.9)	16 (84.2)	17 (89.5)	17 (89.5)	16 (84.2)	14 (73.7)	6 (31.6)	13 (68.4)	14 (73.7)
		R	4 (21.1)	2 (10.5)	3 (15.8)	4 (21.1)	5 (26.3)	3 (15.8)	7 (36.8)	4 (21.1)	2 (10.5)	2 (10.5)	1 (5.3)	3 (15.8)	4 (21.1)	12 (63.2)	5 (26.3)	4 (21.1)
		I	1 (5.3)	0 (0)	0 (0)	1 (5.3)	1 (5.3)	1 (5.3)	1 (5.3)	0 (0)	1 (5.3)	0 (0)	1 (5.3)	0 (0)	1 (5.3)	1 (5.3)	1 (5.3)	1 (5.3)

AMX=Amoxicillin, P=Penicillin, CTX=Cefotaxime, CRO= Ceftriaxone, C= Chloramphenicol, ETP= Ertapenem, E= Erythromycin,LEV=Levofloxacin, LNZ= Linezolid, MEM= Meropenem, MXF= Moxifloxacin,OFL= Ofloxacin, TEL= Telithromycin, TE= Tetracycline, SXT= Trimethoprim/Sulfamethoxazole, VA= Vancomycin, S= Susceptible, R= Resistant, I= Intermediate

6.4.Magnitude of fetal outcome and its association with UTI

From the total of 424 pregnant women participating in the study, there were 57 (13 %) and 367 (87%) poor and good fetal outcomes respectively. Poor fetal outcome includes the occurrence of either one or more of low APGAR score in 5-min (below 7), low birth weight (below 2500 gm), Premature rupture of membrane, Preterm labor, or IUFD. The presence of bacteriuria, had a significant association with fetal outcomes with (p value =0.000). Maternal age, gravidity, educational status, occupation, marital status and history of UTI had no association with the UTI status of the mother.

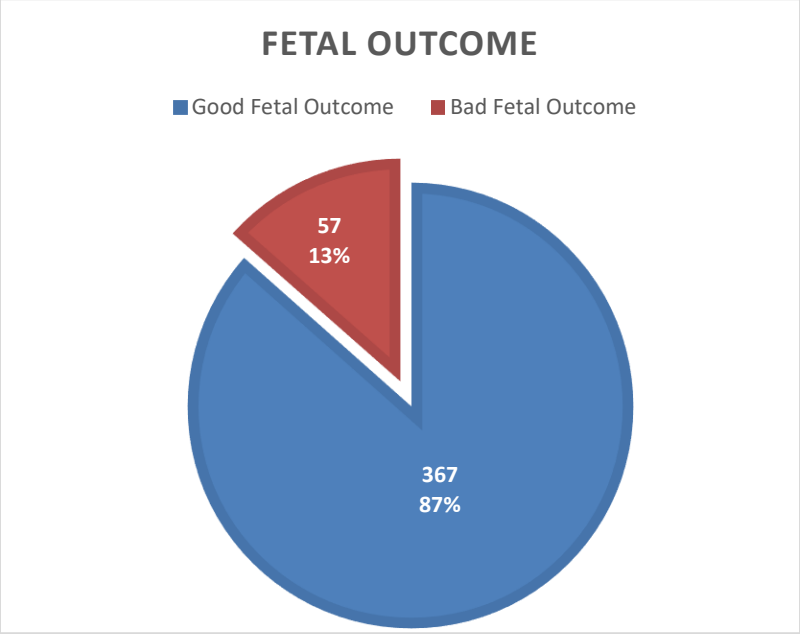

Figure 4: Fetal outcome of pregnant women

PROM had a significant association with the occurrence of maternal UTI (P=0.000). However, no significant differences were noted between pregnant mothers with or without UTI in terms of IUFD, birth weight, 5-min Apgar scores and preterm labor, (20.0% vs. 80.0%; P= 0.876, 20.0% *vs*. 80.0%; *p* = 0.521, 17.4% vs. 82.6%; p= 0.817 and 20.0% vs. 80.0%; p= 0.846) respectively. (Table 4)

Table 4: Association of significant bacteriuria with IUFD, PROM, birth weight, APGAR score and GA at birth

Variables		**Bacteriuria**		**COR**	**AOR**	***P***
		№ (%)		**(95%CI)**	**(95%CI)**	**value**
		No	**Yes**			
IUFD	No	357 (85.2)	62 (14.8)	1.4	1.2	
	Yes	4(80.0)	1 (20.0)	(0.16, 3.09)	(0.13, 11.29)	0.876
PROM	No	349 (87.7)	49 (12.3)	8.3	8.4	0.000*
	Yes	12 (46.2)	14 (53.8)	(3.63, 18.99)	(3.60, 19.41)	
Birth weight	Normal	345 (85.4)	59 (14.6)	1.5	1.5	
	Under	16 (80.0)	4 (20.0)	(0.47, 4.53)	(0.47, 4.50)	0.521
APGAR	Normal	342 (85.3)	59 (14.7)	1.2	1.1	0.817
score	Low	19 (82.6)	4 (17.4)	(0.40, 3.71)	(0.37, 3.50)	
Gestational	Term	357 (85.2)	62 (14.8)	1.4	1.2	
age at birth	Preterm	4 (80.0)	1 (20.0)	(0.16, 13.09)	(0.14, 11.55	0.846

*P value were from multiple logistic regression adjusted for covariats with significant level at p <0.01.

As indicated in table 5 below, out of the 63 pregnant mothers with bacteriuria, 18 (28.6%) of them gave birth with poor fetal outcome. The mothers with positive bacteriuria were found significantly associated with having a poor fetal outcome which is a summative effect of individual adverse fetal outcomes (95% CI= 1.742,6.261).

Table 5: Association of significant bacteriuria with fetal outcome, keeping other variables constant

Variables	Good fetal outcome	Poor fetal outcome	COR (95%CI)	AOR (95%CI)	P value(95%CI)
UTI					
Negative	322 (89.2%)	39 (10.8%)	1	1	0.000*
Positive	45 (71.4%)	18 (28.6%)	3.3	3.2	(1.742, 6.261)
Educational level					
No formal education	61 (16.6%)	7 (12.3%)	1	1	
Primary school	148 (40.3%)	25 (43.9%)	0.5	0.6	0.223
Secondary school	125 (34.1%)	17 (29.8%)	0.7	0.8	(0.900, 1.568)
Higher education	33 (9.0%)	8 (14.9%)	0.6	0.6	
Gravidity					
Multigravida	235 (89.0%)	29 (11.0%)	1	1	0.058
Primigravida	132 (82.5%)	28 (17.5%)	1.7	1.6	(0.981, 3.013)

*P value were from multiple logistic regression adjusted for covariates with significant level at p <0.01

7. DISCUSSION

The study was done to determine the magnitude and drug resistance profile of bacterial species isolated from pregnant women during the time of their third trimester and to check the association between UTI and adverse fetal outcomes. The overall prevalence of significant bacteriuria in this study was 14.9 % (63/424), the result correlates with the finding of similar study conducted in Dire Dawa (14%), Harar (15.5%), Gondar (10.4%) but lower than the studies conducted in Nigeria (85%), Benin (74 %) and Goba and Sinana Woredas, Bale Zone, South-East Ethiopia, (35.3 %) [6, 12, 37, 39-41]. Sample size, trimester of pregnant women and demographic difference might be mentioned as the possible reason for the variation.

The prevalence of UTI among symptomatic pregnant women in this study was lower than the prevalence of asymptomatic at a rate of 3.3% and 11.6 % respectively. The prevalence of symptomatic bacteriuria in the study site was in agreement with the result from similar studies done in Gondar (3.4%), lower than the result from Harar (21%), Dire Dawa (8%), Yemen (66.6%) but slightly higher than the result from Bahir Dar (1.9%) [6, 8, 12, 17, 39].

With respect to asymptomatic bacteriuria, the finding from Harar (13.0%), matches with this study but lower incidence rate was reported in Bahir Dar (7.6%), Dire Dawa (5.9%), and Gondar (7.0%) [6, 12, 17]. Higher prevalence of asymptomatic and symptomatic UTI reported in Yemen might be attributed to the less sample size, geographical variation or may also be due to difficulties in interpretating the symptoms by the study participants or the data collectors .

Sixty eight percent (68.75%) of the total bacterial isolates belongs to gram negative similar to the study conducted in Gondar (67.5), Dire Dawa (73.1), Yemen (63.4%), Tanzaniya (61.9%), but lower than the report from Harar (90.3%), and India (100%)[6, 8, 12, 28, 39, 42]. In addition to the sample size variation, the environmental conditions, population difference and socioeconomic status of the participants can be mentioned as possible reason for the result variation.

With regard to bacterial isolates, *E. coli* was the most dominant bacterial isolate (44.4% and 63.6%) from the total and from gram negative isolates respectively. Similarly, high proportions of *E. coli* from UTI positive pregnant women were reported in Nigeria (66.6%), India (43.9%), Dire Dawa (34.6%), Gondar (47.5%), and Tanzania (33.3%) [1, 6, 12, 42, 43]. In most of these cases, either *Kleibssiela spp* or coagulase negative *Staphylococcus* are the second predominant organisms next to *E. coli*. In contrast to the above findings, *Citrobacter freundii* were reported by

Tula and Lyoha in Nigeria as the most dominant bacterial isolate from UTI positive samples [44].

In this study, *K Pneumoniea* and *S. aureus* were equally ranked as the second predominant pathogens while *S. epidermidis* and *E. faecalis* ranked third. These findings indicate that even though there are a number of UTI causing bacterial species, majority of UTI cases are limited to few groups of bacteria.

Amikacin, ciprofloxacin, gentamicin, cefoxitin, tobramycin and levofloxacin were the most effective drugs over gram negative bacteria by producing more than 90% susceptibility.

In the study conducted by Alemu et al., ceftriaxone, ciprofloxacin, norfloxacin, gentamicin, amoxicillin-clavulanic acid, co-trimoxazole and tetracycline produced the highest level of sensitivity over gram negative bacteria [6]. Disk diffusion and MIC breakpoints for norfloxacin are deleted from CLSI M100, 29th ed. [45]. Similarly, ceftriaxone, gentamicin and ciprofloxacin were the most effective drugs against gram negative bacteria in the study done by Derese [12].

In Libya, nitrofurantoin, amikacin, ofloxacin, ciprofloxacin and cefotaxime were reported as drugs that produced high susceptibility to most of the isolates [15].

From gram negative bacteria, *E. coli* showed sensitivity to all the antibiotics tested. On contrary, a study done in Mekelle, Northern Ethiopia, reported a resistance of *E. coli* to amoxicillin-clavulnic acid and trimethoprim / sulfamethoxazole and ampicillin [46]. A study conducted in Dire Dawa, reported resistance of E.coli to Ampicillin and amoxicillin [12]. Similarly in a study conducted in Nigeria, *E.coli* was least susceptible to most of the gram negative antibiotics [1].

S. aureus also showed resistance to tetracycline but was sensitive to most of the antibiotics tested, this result differs from the result reported in Mekelle [46].

When we compare the rate of isolates of Gram negative and Gram positive bacteria, gram negative bacteria were the dominant causative agent of UTI which is in line with others report [12, 46].

In this study, the association of low birth weight and maternal UTI were not significant (P=0.521). About 16 (80%) and 4 (20%) underweight deliveries were recorded from 361 UTI negative and 63 UTI positive pregnant mothers respectively. This proportion of low birth weight were in line with the study conducted by Iqbal et al., However; in a study conducted by Amiri and his colleague in Ghana, birth weight were significantly associated with maternal UTI status

[18, 19, 47]. The difference in sample size, difference in settings and sampling method can explain the diference in the association of the two variables in different geography.

Like birth weight, APGAR score (in the 1st and 5th minute), IUFD and gestational age were not statistically associated with maternal UTI; however, in a study conducted by Siakwa in Ghana, occurrence of low APGAR score (less than 7) and preterm labor respectively were 3.8 and 3.4 times than their occurrence in UTI negative mothers. A recent study done by Farah Iqbal also reported similar association of preterm delivery and low birth weight with UTI. A retrospective and observational study in Brazil further points out the significant association that exist between preterm birth and UTI but with respect to APGAR score, the result had similar finding with this study. The difference in significance level and odds of occurrence may be attributed to the socio-demographic difference of study subjects. [18, 19, 47].

The finding of this study indicates that UTI during pregnancy had significant association with the occurrence of PROM (P= 0.000, AOR=8.4, CI=3.60, 19.41). This finding agrees with the finding of Siakwa M., et al. in Ghana, Antonio E. in Brazil. [18, 19, 47].

According to different studies, UTI induces macrophages to release metalloproteinase enzymes that can degrades amnioticmembranes and ultimately leads to rupture of amniotic membranes. [18, 19, 47].

8. CONCLUSION

The finding from this study showed that the prevalence of UTI in the study area was high in general and higher proportion was taken by pregnant women with out symptoms. Gram negative bacteria remained as the dominant causative agents of UTI. *E. coli, S. aureus and K. Pneumoniae* were the frequent isolates. And the antibiotic susceptibility test showed variety of drug resisitance pattern among different bacterial isoltes. *E. coli* were susceptible for most of the antibiotics tested. Majority of gram positive isolates were resistant for Tetracycline and the two antibiotics: Tetracycline and Trimethoprim/Sulfamethoxazole were not effective against the most prevalent gram positive bacteria, *S. aureus.* UTI was found significantly associated with fetal outcome after keeping all other variables constant.

9. STRENGTH AND LIMITATION

- The result of this study will provide a baseline information on the association of UTI with fetal outcome,inititating further studies in related areas.
- This study was done on small sample size to assesses UTI related fetal outcome.
- The other limitation of the study was that it considered pregnant women at the time of their third trimester so future studies should include all trimesters to measure and compare the prevalence of UTI at different terms of pregnancy.

10. RECOMENDATIONS

Further studies should be performed on a larger sample of pregnant women at different study sites for evaluation of the role UTI among pregnant women in relation to its fetal outcome.

There should be periodic check-up of pregnant women to know their UTI status and proper treatment should be given according to the culture result. Urine culture should be done regularly for pregnant women at least once in every trimester.

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Annexes

Annex I: Information sheet and consent form

Date.....

Introduction

My name is Wegayehu Zebene and I am MSc student of Addis Ababa University, School of Medical laboratory Sciences. I am doing my thesis with a tittle '' Urinary Tract infection, Drug Resistance Profile and Fetal Outcomes among Pregnant women in Two Health centers and Tikur Anbessa Speclised Hospital , Addis Ababa, Ethiopia.

The objective of this study is to determine magnitude and drug resistance profile of bacterial isolates from urine of pregnant women, to explore related fetal outcomes and to relate demographic factors with occurrence of bacteriuria in pregnant women during the time of third trimester at Gerji Health Center, Felege Meles Health Centers, and Black Lion Specialized Hospital.

Dear participants, the information and the specimen that you provide are valuable inputs for this research.

As a study participant, you are free to ask any question if you feel discomfort about the research or resign from the research at any time of the research period without informing your decision. Your participation in the research has no relation with the type and quality of service you need to get from the health center.

When you are found to be positive for the bacterial micro-organism, you will be informed by the health worker and receive proper treatment. But your name and address will not be disclosed rather an identification code will be used in such conditions.

If you agree to participate in the study and sign on the consent form, you will be asked to fill questionnaire designed for the study and urine sample will be collected from you for laboratory diagnosis. There is no anticipated risk in relation to your participation in the study and you have the right to get a copy of this consent form.

ስለጥናቱ

ቀን----------

መግቢያ

ስሜ ወጋየሁ ዘበነ ሲሆን በአዲስ አበባ ዩኒቨርሲቲ የህክምና ላቦራቶሪ ሳይንስ የማስተርስ ተማሪ ነኝ፡፡ ‹‹የሽንት ቱቦ ውስጥ የሚያድጉ ባክቴሪያ የተባሉት ረቂቅ ህዋሳት መድሃኒት የመቋቋም ደረጃ እና በጽንስ ላይ የሚያመጡት ችግሮች›› በሚል ርዕስ የመመረቂያ ጥናት በሁለት ጤና ጣቢያ እና በጥቁር አንበሳ ሆስፒታል እየሰራሁ ነው፡፡

የዚህ ጥናት ዐላማዎች በገርጂ ጤና ጣቢያ ፤ ፈለገ መለስ ጤና ጣቢያ እና በጥቁር አንበሳ ሆስፒታል የቅድመ ወሊድ ክትትላቸውን ከሚያደርጉ እናቶች ውስጥ የመጨረሻዎቹ ሶስት ወራት ከገቡ ነፍሰ ጡር እናቶች ሽንት ውስጥ ባክቴሪያ የተባሉትን ረቂቅ ህዋሳት መለየት እና መድሀኒት የመቋቋም አቅማቸውን ማጥናትና ለህዋሳቱ ተመራጭ የሆኑ መዴሃኒቶች መምረጥ ፤ በጽንስ ላይ የሚያመጡትን ተጽኖ መለየት፤የተለያዩ ማህበራዊ ሁኔታዎች እና የእነዚህ ረቂቅህ ዋሳት ያላቸውን ዝምድና መለየት ናቸው፡፡

ውድ የጥናቱ ተሳታፊዎች ለጥናቱ የሚሰጡት መረጃ እና የሽንት ናሙና ለጥናቱ ትልቅ ግብዐት ናቸው፡፡ በጥናቱ ሂደት ውስጥ ያልተመቻችሁን ነገር በነጻነት መጠየቅ ወይም ውሳኔያችሁን ሳታሳውቁ በማንኛውም ሰዓት ከጥናቱ ተሳታፊነት መውጣት ትችላላችሁ፡፡

በጥናቱ የሚኖርዎት ተሳትፎ በጤና ጣቢያው ሊያገኙ ከሚፈልጉት የህክምና አይነት እና የአገልግሎት ጥራት ጋር ምንም ግንኙነት የለውም፡፡

በሽንትዎ ውስጥ ለህክምና የሚያበቃ ባክቴሪያ ከተገኘ በህክምና ባለሙያዎች በኩል ተነግርዎት አስፈላጊው ህክምና ይደረግልዎታል፡፡ ስምዎትም ሆነ አድራሻዎት ለማንም አይገለጽም በምትኩ ሚስጥራዊ ኮድ እንጠቀማለን፡፡

በጥናቱ ለመሳተፍ ከተስማሙ እና የፈቃደኝነት መግለጫ ቅጽ ላይ ከፈረሙ በሁዋላ ለጥናቱ የተዘጋጁ መጠይቆችን ሞልተው ለላቦራቶሪ ምርመራ የሚውል ሽንት ይሰጣሉ፡፡በጥናቱ በመሳተፍዎ ምክንያት በእርስዎ ላይ ሊደርስብዎ የሚችል ጉዳት አይኖርም የፈረሙበትን የስምምነት ቅጽ ቅጂ የማግኘት መብት አለዎት፡፡

Annex II:Consent form

I. Consent form (English version)

I, the undersigned, confirm that, I give consent to participate in the study with a clearunderstanding 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 thelanguage I understand.

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

Date----------

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

Date----------

Annex III: Questionnaire

This questioner will be filled by the researcher or by the health care provider at antenatalcare unit. After the completion of the questionnaire, clean catch midstream urine will be collected using sterile urine cup.

The information can be taken either from the selected study subject and/ or from their follow-up card. All the information obtained will be used only for the research purpose and will be kept confidential.

Contact address of the researcher: 0911- 74- 19- 51

E-mail: sowegayehuzebene@gmail.com

I. Personal/ socio-demographic data

1. Age ----------
2. Address: Woreda _____
3. Marital status: Married ☐ Single ☐ Divorced ☐ Widowed ☐
4. Gestational period: 7-9 months
5. Gestational status: Primigravida ☐

   Multigravida ☐
6. Educational status: Illiterate ☐ literate ☐
7. Education level: Grade 1-8 ☐ Grade 9-12 ☐ Certificate ☐

   Diploma ☐ First degree ☐ Second Degree ☐ PhD ☐
8. Occupational status: House wife ☐ Merchant ☐

   Government employee ☐ Student ☐

II. Clinical Data

9. Questions to differentiate 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 ☐

11. History of previous UTI: Yes ☐ No ☐

12. History of catheterization: Yes ☐ No ☐

13. History of diabetes mellitus: Yes ☐ NO ☐

II. Laboratory Data

14. Urine Culture and identification:

SB: Yes ☐ No ☐

Name of Bacteria isolated……

I. የፈቃደኝነት መጠየቂያ ቅፅ

እኔ 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፡፡

የተሳታፊ ስም_____ የተሳታፊ ፊርማ _____

ቀን_____

የተመራማሪው ስም_____ የተመራማሪው ፊርማ_____

ማንኛውም ጥያቄ ወይም ጥርጣሬ ካለዎት ይህንን አድርሻ ይጠቀሙ፡

የዋናው ተመራማሪ አድራሻ

ወጋየሁ ዘበነ

በአዲስ አበባ ዩኒቨርስቲ ህክምና ፋኩሊቲ የህክምና ላቦራቶሪ ት/ት ክፍል በድህረ ምረቃ መርሀ ግብር እጩ ተመራቂ ተማሪ

ስልክ፡-0911-741951

ኢሜይል፡- sowegayehuzebene@gmail.com

II. መጠይቅ

ይህ መጠይቅ የሚሞላው ጥናቱን በሚያጠናው ወይም በጤና ባለሞያ ነው። የቅድመ ወሊድ ክትትል የሚያደርጉ ነፍሰ ጡር እናቶች ወይም የህክምና ካርዳቸው የመረጃ ምንጭ ይሆናሉ። መጠይቁ ከተሞላ በኃላ የጥናቱ ተሳታፊዎች የሽንት ናሙና ይሰጣሉ።ከመጠይቁ የሚገኙት መረጃዎች በሙሉ ሚስጢራዊነታቸው እንደተጠበቀ ሆኖ ለጥናቱ አላማ ብቻ ይውላሉ።

ለማንኛውም ጥያቄ የጥናቱ አቅራቢ ስልክ ቁጥር 0911- 74- 19- 51

ኢሜይል sowegayehuzebene@gmail.com

I. ክፋል አንድ፡- የግል እና ማሕበራዊ ሁኔታ መጠይቆች

1. እድሜ፡- _____

2. የትዳር ሁኔታ

ትዳር ያላት☐ የተፋታች☐

ትዳር የሌላት☐ በሞት የተለያት☐

3. የትምህርትደረጃ

መደበኛ ትምህርት ያልተማረች☐ መደበኛ ትምህርት የተማረች☐

አንደኛ ደረጃ (1 - 8 ክፍል)☐ ሁለተኛ ደረጃ(9– 12 ክፍል)☐

ሰርተፍኬት☐ ዲፕሎማ☐ የመጀመሪያ ዲግሪ ☐

ሁለተኛ ዲግሪ☐ የዶክትሬት ዲግሪ ☐

4. የሰራ ሁኔታ

የቤት እመቤት☐ የመንግስት ሰራተኛ ☐

ነጋዴ☐ ተማሪ ☐

ሌላ ካለ_____

II. ክፍልሁለት፡- የጤና ነክ መጠይቆች

5. የአሁኑ እርግዝናዎት ስንተኛዎት ነው?

የመጀመርያ ጊዜ ☐ ለሁለተኛ ጊዜ እና ከዛ በላይ ☐

ምልክት ያለዉ እና የሌለው መለያ

6. ሽንት ሲሸኑ የማቃጠል ስሜት አለዎት? እዎ አለኝ ☐ የለኝም ☐

7. ሽንትዎት ቶሎቶሎ ይመጣብዎታል?

እዎ ይመጣብኛል ☐ አይመጣብኝም ☐

8. ሽንትዎትን መቆጣጠር ይችላሉ?

አዎ እችላለሁ ☐ አልችልም ☐

9. ደም የቀላቀለ ሽንት ይሸናሉ?

አዎ ☐ አልሸናም ☐

10. የሆድህመምአለዎት?

አዎ ☐ የለኝም ☐

11. ትኩሳት እና የማንቀጥቀጥ ስሜት ይሰማዎታል?

አዎ ☐ አይሰማኝም ☐ የሙቀት መጠን (____^{O}C)

12. ጎንዎ አካባቢ ህመም ይሰማዎታል?

አዎ ☐ አይሰማኝም ☐

13.ከዚህ ቀደም የሽንት መሸኛ ቱቦ ሕመም አጋጥምዎት ያውቃል?

አዎ ☐ አያዉቅም ☐

14. ከዚህ ቀደም የሽንት መውጫ ቱቦ ተደርጎልዎት ያዉቃል?

አዎ ☐ አያዉቅም ☐

15. የስኳር ሕመም አጋጥሞት ያውቃል? አዎ ☐ አያውቅም ☐

Annex IV Laboratory procedures (Adopted from EPHI laboratory SOP)

Gram staining technique

Method

1. After making a smear, leave the slide in a safe place for the smear to air-drythen 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 notclean, use filtered water or clean boiled rainwater.
4. Tip off all the water, and cover the smear with Lugol's iodine for 30–60seconds.
5. Wash off the iodine with clean water.
6. Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediatelywith clean water. *Caution*: Acetone–alcohol is highly flammable; therefore use it well awayfrom 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 toair-dry.
10. Examine the smear microscopically, first with the 40x objective tocheck the staining and to see the distribution of material, and then with the oilimmersion objective to report the bacteria and cells.

Novobiocin disks test

Principle

Novobiocin disks are recommended for the differentiation of coagulase-negative *Staphylococcus saprophyticus* based on novobiocin resistance. This method is based on the antibiotic disk diffusion test of Kirby and Bauer. Laboratory identification of *S. saprophyticus* is made on the basis of the absence of hemolysin and coagulase and resistance to novobiocin. Novobiocin susceptibility test results are 100% sensitive and 96% speci*fic. S. saprophyticus is innately resistant to the antibiotic novobiocin.* Therefore, screening coagulase-negative staphylococci from urine cultures for novobiocin resistance is a reliable presumptive identification of *Staphylococcus saprophyticus.*

Method

1. Using sterile inoculating loop, select 4 – 5 well-isolated colonies.
2. Suspend growth in a tube of sterile saline or broth.
3. Adjust the turbidity to form a suspension comparable to a McFarland 0.5 standard.
4. Agitate this suspension thoroughly.
5. Dip a swab into the suspension and express excess fluid by rotating the swab against the inside wall of the test tube.
6. Inoculate the entire surface of a Mueller Hinton medium plate, streaking in three directions by rotating the plate 60^{o} after each streaking. If the inoculum is satisfactory, there will be a confluent lawn of growth.
7. Allow the inoculums to dry approximately 5 minutes with the lid in place.
8. Using sterile forceps, one Novobiocin disk on the inoculated surface. Gently press the disk down to ensure complete contact with the agar. Do not move a disk once it has touched the agar because the Novobiocin diffuses almost immediately.
9. Invert and incubate at $35^{o}C$ for 16 – 18 hours.
10. Measure the zone of inhibition, if present, with a ruler or caliper.

- Novobiocin (5μg) disks are intended for screening of novobiocin resistance only. They are not intended for determining the susceptibility of coagulase-negative staphylococci to novobiocin.

Other less significant coagulase-negative Staphylococcus strains are also novobiocin resistant, such as *S.cohnii, S.xylosus* and *S.sciuri.*

Result interpretation

S.saprophyticus is resistant to novobiocin and produces a zone of inhibition ≤ 16mm.

- For Gram-positive cocci in clusters which are catalase positive, coagulase negative and are novobiocin resistant: in a urine culture, report as *S.saprophyticus.*

Reporting *S.saprophyticus* should be limited to urinary tract isolations only unless further tests for identification to the species level are performed.

Catalase test

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. The catalase enzyme neutralizes the bactericidal effects of hydrogen peroxide and protects them. An organism is tested for catalase production by bringing it into contact with hydrogenperoxide. Bubbles of oxygen are released if the organism is a catalase producer. Anaerobes generally lack the catalase enzyme.

To find out if a particular bacterial isolate is able to produce catalase enzyme, small inoculums of bacterial isolate is mixed into hydrogen peroxide solution (3%) and the rapid elaboration of oxygen bubbles occurs.

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), removeseveral colonies of the test organism and immerse in the hydrogen peroxidesolution.

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

Principle

The coagulase test is used to differentiate *Staphylococcus aureus*, the most pathogenic of the staphylococci, from other commonly isolated staphylococci. Coagulase is a thermostable thrombin-like substance that activates fibrinogen to form fibrin, resulting in a fibrin clot. This is demonstrated in the test tube by the formation of a clot when plasma is inoculated with *S. aureus*. The substance is known as free coagulase, since it is liberated by the cell. In most but not all *S. aureus* organisms, fibrinogen binding cell surface receptor is also present in the cell wall, called "bound coagulase" or "clumping factor." Clumping factor is demonstrated by the ability of the organism to act directly on the fibrinogen on the plasma to clump it in a slide method. The test for clumping factor is rapid but requires several colonies, and, as stated above, the factor is not present in all *S. aureus* organisms.

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.

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 the organisms.

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

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 breakdown the amino acid tryptophan with the release of indole.

Principle

The test organism is cultured in a medium which contains tryptophan. Indole productionis detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethylaminobenzaldehyde. This reacts with the indole to produce a red coloredcompound. The indole test also can be performed by culturing the organism in tryptonewater or peptone water containing tryptophane, and detecting indole production byaddingKovac'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 color

Negative test: No red color

Urine culture

Bacteriuria is considered by most clinicians to be a definitive marker of UTI. Urine is normally a sterile body fluid. However, unless it is collected properly, it can become contaminated with organisms from the perineum, urethra, or vagina. The patient should be given detailed instructions for proper specimen collection.

Quality control

- Process the specimen as soon after receipt as possible. If there is a delay in processing, place the specimen in the refrigerator.
- Verify that the patient name and identifiers on the specimen match with the requisition form.
- Ensure that all media and supplies used have passed the required QC and are used before

Method

1. Bring the culture medias to room temperature and label with appropriate information (Patient ID, Specimen type and Date of inoculation)
2. Mix the specimen. Dip a 0.001 ml (1 ul) sterile loop into the specimen until the top of the loop circle just enters the specimen.
3. Put the specimen in a single line down the middle of the plate. Cross-streak the line of material with a series of very close streak lines such that the entire plate surface is utilized
4. Incubate plates for 18- 24 hours at 35 – 37 ^{o}C aerobically
5. If there is no growth after 18-24 hours of incubation, discard the plates and issue final report
6. If there is growth after 18-24 hours of incubation, count the number of colonies of each colony type present. Each colony counted represents 1000 CFU in the original specimen.
7. If ≤ 2 pathogens, perform identification & susceptibility tests on each If >2 pathogens, report mixed flora.

   Result interpretation

Determine colony count of each bacteria colony. With a 0.001 ml loop (1µl), one colony equals 1,000 CFU/ml and 0.01ml loop (10 µl) equals 10,000 CFU/ml.

Interpretation is based on method of collection and clinical condition:

- Asymptomatic patient; clean-catch or indwelling catheter specimen: report if growth is ≥ 100,000 colony forming unit (CFU)/ml of potential pathogen.
- Symptomatic ambulatory patient; clean-catch specimen: report if growth is ≥ 10,000 CFU/ml with one to two species of a potential pathogen. If >two species, urine is considered to be "contaminated," report as "mixed flora."
- All patient types, for specimens obtained by surgery or bladder aspiration: report growth any colony count of potential pathogens.
- All patient and specimen types: report any isolate of yeast.

Antimicrobial susceptibility test

Principle

Disk diffusion testing is one of several phenotypic assays which can be utilised to determine the antimicrobial resistance profile (antibiogramme) of an organism. Agar plates are inoculated with a standardised inoculum of the bacteria and an antimicrobial disk is placed on the inoculated agar plate. The disks used for a disk diffusion assay contain a standardised known amount of an antimicrobial agent, which diffuses into the agar when in contact with the agar surface. The plate is incubated under standardised conditions following CLSI guidelines. During incubation, the antimicrobial agent diffuses into the agar and inhibits growth of the bacteria, producing a "zone of inhibition" around the disk. Following incubation, the diameter of this zone is measured and the results are interpreted as resistant, intermediate, or susceptible using standard guidelines. The size of the inhibition zone indicates the degree of resistance, and might also give important information about the resistance mechanism and the resistance genes involved.

Quality assurance of the susceptibility testing of Enterobacteriaceae includes testing of *E. coli*, Non-Enterobacteriaceae includes *P.aerugenosa*, Gram Positive *S.aureus*.

Method

1. Take 4 to 5 well isolated colonies.
2. Transfer the growth to the tube of saline
3. Emulsify the inoculum
4. Adjust the inoculum standard to a 0.5 McFarland
5. Ensure plates are: free of visible contamination, poured to a uniform depth of approximately 4mm, not excessively wet, not cracked or dry.
6. Dip a sterile cotton swab into the inoculum. Rotate the swab several times and press firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab.
7. Streak the swab over the entire surface of the Mueller Hinton agar plate.
8. Rotate the plate approximately 60^{o} then repeat streaking motion.(3x)
9. Complete inoculation by running the swab around the rim of the agar.

10. Dispense disks to the agar surface with sterile forceps (Disk dispenser). As per the CLSI guideline

    NB: Avoid using over-heated forceps, Do not relocate any disks after contact with the agar.
11. After application, insure that the disk has made complete contact with the agar surface by touching the top of the disk with forceps.
12. Incubate plates inverted at 36±1^{o}C for 16 to 18 hours in ambient air/ under Incubation time becomes 24hr in case of S.aureus.
13. After incubation with appropriate time, check that zones are round; not oval, deformed or have jagged edges.
14. Measure the diameter of inhibition zones.
15. If no inhibition is present (confluent growth is present up to the border of the disk), the diameter of the disk should be recorded (6mm).
16. For interpretation of results refer CLSI M100 guideline for each type of organism and antibiotics

Annex **V**

Delivery register Log book

Identification				Labor & maternal outcome			Newborn birth outcome				Preventive service					
											Newborn			Maternal HIV + care & followup		
Personal inf					Mode of delivery		Apgar Score 1'/5'	Weight in gram	Live/ Dead	Still birth	MRY NEW Born's	Vit K	BCG	HIV testing	HIV delivery link to PMTCT	New born NVP
S. N	MRN	Name	Age	Delivery date	SVD	CS										
1																
2																
3																

The actual log book contains 65 columned table, and hence only data relevant to the study are included in this annex.