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Prevalence And Antibiotics Susceptibility Pattern Of Common Bacterial Uropathogens Isolated From Pregnant Women Attending Antenatal Care Clinic At St. Paul Hospital Millennium Medical College And Selam Health Center, Addis Ababa, Ethiopia.

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ABSTRACT:The objective of this study was to determine the prevalence and antibiotics susceptibility patterns of common bacterial uropathogens, and identify associated risk factors of UTIs from pregnant women attending antenatal care clinic of St.Paul Hospital Millennium Medical College and Selam Health Center, Addis Ababa, Ethiopia. Cross sectional study was conducted among pregnant women attending antenatal care clinic at St. Paul Hospital Millennium Medical College and Selam Health Center from September to November, 2013. Urine samples (5-10ml) were collected from a total of 320 pregnant women. Data were cleaned with EPI info version 3.5.1 and transferred to SPSS version 21 for analysis. Frequencies and percentage for each variable were calculated. Binary logistic regression was used to identify association between variables. Tables and figures were used for data presentation. Bacteriological screening of urine samples revealed significant growth of bacteria in 28.8% and 23.5% for symptomatic and asymptomatic pregnant women respectively with overall prevalence of 25.9%. The most common isolates detected were E.coli (27.7%), S.saprophyticus (26.5%) and S.aureus (21.6%). Both gram positive (GP) and gram negative (GN) bacteria showed high sensitivity against Nitrofurantoin with a rate of 89.28% and 81.48%, respectively. Among the risk factors assessed in this study, educational status was significantly associated with urinary tract infections (P=0.029). The magnitude of urinary tract infections with isolation of bacterial pathogens both from symptomatic and asymptomatic pregnant women, with increased resistant to the commonly prescribed antibiotics calls for an early screening of all pregnant women to UTIs and periodic monitoring any changes in the antibiotics susceptibility patterns of urinary tract pathogens.

Key words: UTIs, Pregnant women, Prevalence, Uropathogens, Antibiotics resistance.

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INTRODUCTION

Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year and commonly occur next to respiratory tract infections [1]. It is the most common bacterial infection during pregnancy and constitutes a significant cause of maternal and perinatal morbidity and mortality [2]. Approximately, 50%-70% of women have UTIs during their lifetimes and 20%-30% of them have recurrent episodes [3].

In low-income countries, antibiotics resistance provide further complication by lack of enough data on bacteria causing UTIs during pregnancy, cost of antibiotics and relative inaccessibility to information on safety and efficacy of new antibiotics. Under these circumstances, it is possible that antibiotics are being used inappropriately to treat UTIs. Inappropriate antibiotics use can lead to inadequate therapy and contribute to further antibiotics resistance among the species of UTIs pathogens [1]. Multi-Drug resistance of 91.7% observed among the isolated bacterial uropathogens in Gondar [4].

Productions of β-lactamases, the enzymes that degrade beta-lactam antibiotics are most widespread and threatening mechanism of antibiotic resistance [5]. Detection of β-lactamase producing bacteria is of importance for infection control and epidemiological surveillance [6].

In Ethiopia, different studies tried to indicate the magnitude of UTI among pregnant women and it ranges from 9.2% -12% but it reached to 25.6% in case of both women and men of suspected cases. Increase antibiotic resistance from time to time is recommended by various studies to screen bacterial profile and antibiotic susceptibility pattern among all pregnant women during their antenatal care follow-up. As Addis Ababa is the largest and capital city of Ethiopia, various referred patients will attend their medical follow-up in federal hospitals such as St. Paul Hospital Millennium Medical College. Antenatal care follow-up is one of the most and major task in hospitals and health centers of Addis Ababa. There is, however information gap on prevalence of urinary tract infection, associated risk factors and antibiotic susceptibility pattern in the study area. Therefore the objective of this study was to determine prevalence, associated risk factors and antibiotic susceptibility pattern of common uropathogens.

MATERIALS AND METHOD

Study Area and Period: The study was conducted at St. Paul Hospital Millennium Medical College and Selam Health Center from September 21 to November 30, 2013. St. Paul Hospital is found in Addis Ababa, Ethiopia. It is teaching and referral Hospital located western part of Addis Ababa, Gulele sub-city, Wereda 9, House No 461. The hospital is built by Emperor Haileselassie in 1969 E.C with the help of the German Evangelican church aimed to serve the poor. A medical college was started in 2007 G.C. Now it provides service as referral hospital for those people in Addis Ababa and referred from other places and teaching center for Medicine. The Hospital Serves an average of 700 Patients daily including private wing. The Hospital has 340 beds. Many patients referred from different parts of the country. The hospital provides different services for those referred patients. Selam Health Center is also one of the most popular health center found in Gulele Sub-City.

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The health center is built in 1996 E.C. It has 106 staff and provides service for about 37,813 people of the catchment area. The health center provides different services for under five children and out-patient for an average of 200 Patients daily.

Study Design: Health facility based cross sectional study was conducted at St. Paul Hospital Millennium Medical College and Selam Health Center.

Source Population: All pregnant women who were at ANC clinic of St. Paul Hospital Millennium Medical College and Selam Health Center with and without signs and symptoms of urinary tract infections during the study period.

Study Population: Those pregnant women who were attending ANC clinic at St. Paul Hospital Millennium Medical College and Selam Health Center with and without sign and symptoms of urinary tract infection during the study period.

Sample Size Determination: The sample size is determined using double population proportion method with 95% confidence interval and 5% precision taking as reference a study finding 10.6%& 20% for asymptomatic and symptomatic bacteriuria in Addis Ababa, Ethiopia by Assefa *et al* [36=7] as follows;

$$N= \{Z_1-\alpha_{/2}\sqrt{[2P^-(1-P^-)]} + Z_{1-\beta}\sqrt{[P_1(1-P_1) + P_2(1-P_2)]}\}^2$$

$$(P_1-P_2)^2$$

Where: Values of Z for 95% power = 1.96, for 80% power = 0.82, d = total allowable error =5 %. Proportion, $P_{1=}$ 10.6% and P_2=20%, P^- = average of sample proportions, 0.106+0.2/ 2= 0.153 was used. There by N = 1.96 √ [2x0.153 (1-0.153)] + 0.82 √ [0.106(1-0.106) + 0.2 (1-0.2)/ $(0.106-0.2)^2$ =160. Hence a total of 320 pregnant women that is 160 asymptomatic and 160 symptomatic was included in the study.

Sampling Technique: Consecutive sampling technique was used.

Inclusion Criteria: All pregnant women at ANC clinic who were not at antibiotics therapy within the past two weeks prior to sample collection and Those pregnant women willing to participate in the study.

Exclusion Criteria: Those pregnant women who have complication and unable to provide sample.

Socio-demographic Data: Socio-demographic variables (Age, Marital status, Educational level, Occupation, Address, Parity, Gravidity, Trimester, Sexual experience, Hygienic experience and other relevant clinical data such as; History of Catheterization, History of UTIs, History of Diaphragm use, Diabetes Mellitus) were obtained using a pre-designed structured questionnaire by nurses and principal investigator.

Biological Data (Specimen collection): Clean catch midstream urine (the recommended type of specimen for microbiological culture and antibiotics susceptibility testing by CLSI) specimens were collected from all study participants who were willing to participate in the study. Written consent form was obtained from each study participant prior to sample collection. Good and clear orientation on how to collect the sample was given for study participants. Front to back wiping with sterile gauze to make it dry before urination, remove the first little urine in to the toilet to flush urethral flora and then collect the required amount of urine in the container. Finally the remaining urine must be voided in the toilet. From a total of 320 pregnant women, 5-10 ml of clean catch midstream urine specimens were collected using sterile, graduated, wide mouthed,

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dry, leak proof plastic containers. On the urine colleting container, participants identification number, date and time of collection was labeled. Urine samples were transported with in cold box transportation system (2-8^{o}C). All of the specimens were analyzed in Bacteriology and Mycology Research Laboratory of Ethiopian Health and Nutrition Research Institute by using well organized standard operating procedures.

Specimen Processing: Specimen processing held within 6 hours of collection. Culturing of bacteria were mediated by using calibrated wire loop of 0.001 ml (1µl) volume and inoculated on MacConkey Agar (Defico, France) and Blood Agar (Oxoid, ltd) plates and incubated aerobically at 37°C for 24-48 hours. The plates were examined macroscopically for morphological appearance as presumptive identification. Colony counts were done for presence of significant bacterial growth ($\geq 10^3$CFU/ml and $\geq 10^5$CFU/ml for symptomatic and asymptomatic pregnant women respectively). Total colony counts were multiplied by $10^3$to report per milliliter of urine because we were using 1µl loop) after overnight incubation.

Isolation and identification of cultured isolates were done according to standard microbiological techniques. Hemolytic reaction (on Blood Agar plate), pigment production or color changes surrounding carbohydrate fermenting colonies (on MacConkey Agar plate), colony characteristics (white, golden yellow, pink, and grey in color or mucoid in appearance), gram reaction of the organisms and microscopic appearance (direct wet mount) were the presumptive identification criteria. Biochemical tests such as; Indole, Citrate utilization, H_2S production, Urea hydrolysis, Lysine decarboxylation, Lactose fermentation and Motility were used for identification of gram negative bacteria. Coagulase, Catalase, DNase, Novobiocin (NOV, 5µg) antibiotic disk, Serological test (Pastorex^{TM} Meningitis, Code 61613; Antigen detection by using homologous antibody) were used for identification of gram positive bacteria.

Antibiotics Susceptibility Testing: Antibiotics susceptibilities of the bacterial isolates were performed according to the criteria of Clinical Laboratory Standards Institute (CLSI,2012) using the Kirby-Bauer disc diffusion method on Muller-Hinton Agar (Oxoid, Ltd, England) for gram negative isolates and *Staphylococcus spp*. Muller-Hinton Agar supplemented with 5% Sheep blood was also used for fastidious bacteria such as *S.agalactae*. A loop full of bacteria (approximately 4-5 colony) was taken from a pure culture colony of same morphology and transferred to a tube containing 5ml of Nutrient Broth and mixed gently by using vortex until it forms a homogenous suspension. The turbidity of the suspension was adjusted to the turbidity of McFarland 0.5 standard in a tube and swabbed on Muller-Hinton Agar uniformly by rotating at 60^{o} for at least three times. The plates were incubated at 35-37^{o}C overnight. The zones of inhibition around the antibiotic disks were measured by using caliper.

Amoxicillin/Clavulinicacid(AMC,20/10µg),Ampicillin(AMP,10µg),Nitrofurantoin(F,300µg),Gentamicin(CN,10µg),Oxacillin(OX,1µg),Sulfamethoxazole/Trimethoprim(SXT,1.25/23.75µg),Chloramphenicol(C,30µg),Ciprofloxacin(CIP,5µg),Tetracycline(TTC,30µg),Clindamycin(Cln,2µg),Penicillin(P,10units),Erythromycin(E,15µg),Norfloxacin(NOR,10µg),Vancomycin(Van,30µg),Cefoxitin(FOX,30µg),Ceftazidime(CAZ,30µg),Cefuroxime/So

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dium(CXM,30µg),Cephazoline(KZ,30µg),Cefotaxime(CTX,30µg) were used in the study with their respective concentration.

All isolates were screened for production of β-lactamase enzyme by using MAST ID™ Intralactam Strip (Code ETO/1, 5.7cm by 0.6cm, which are printed to identify the Test, Positive control and Negative control areas). The strips are impregnated with Benzyl Penicillin and Bromocresol Purple at appropriate concentrations. It was equilibrated to room temperature before opening the aluminum foil. The strip was moistening with physiological saline and then rubbed the area with negative control, sample to be tested and positive control respectively. The colonies were from fresh media and from non-fermentable media (Blood Agar) because any acid they produce may give false positive result. Interpretation of result was according to the manufacturer's instruction observation of any color change (yellow color and no color change for positive and negative results respectively) within 10 minutes.

Data management and Quality assurance: Data quality was ensured through use of standardized data collection materials, pre-testing of the questionnaires, proper data collection, supervision and processing of activities by the principal investigator. For laboratory analysis quality control samples were run to assess the functionality of the instrument and user manual of the laboratory for each test, reagents and equipment was strictly followed. In addition, well-trained and experienced laboratory professionals were participate in the laboratory analysis procedure.

Sterility and performance of culture media were tested prior to using it. The sterility of culture media was checked by incubating 3-5% of the batch at 35-37^{o}C for overnight and observed for bacterial or any growth. Those media which show growth were discarded. Quality control strains of bacteria such as; standard strains of *E.coli ATCC 25922* and *S.aureus ATCC 25923* were used to check performance test during culture and antibiotics susceptibility testing. *S.aureus ATCC11632* and *E.coli ATCC 25922* were used as positive and negative control strains respectively for MAST ID™ Intralactam strip tests. Reagents for biochemical tests and cartridges were refrigerated at 2-8^{o}C and equilibration were achieved by placing it at room temperature for few minutes. All inoculation and identification procedure were performed aseptically in BSC level 2 safety cabinet for inoculation and using a Bunsen burner for identification techniques and other procedures.

Data processing and analysis: Data were cleaned out in to EPI info version 3.5.1 and transferred to SPSS version 21 for analysis. Quantitative variables were described by mean and standard deviation. Binary logistic regression was used to determine presence of an association of several key variables with prevalence of Urinary tract infections. Odds ratio was calculated to show strength of association between variables. The statistical tests were considered significant for $P<0.05$. Frequencies and percentage of each variable were calculated. Tables and figures were used for data presentation.

Ethical consideration: Ethical clearance was obtained from Departmental Research and Ethics Review Committee of Addis Ababa University, College of Heath Science, School of Allied Health

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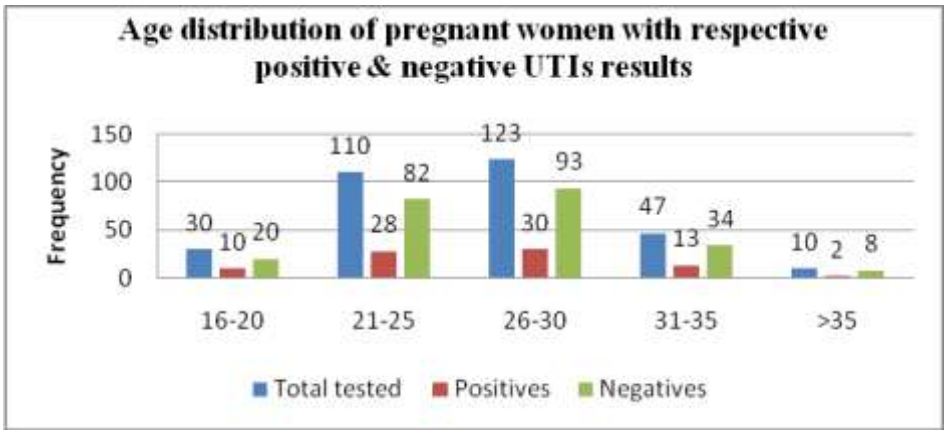
Science and Department of Medical Laboratory Science and Institutional Review Board (IRB) of St. Paul Hospital Millennium Medical College. The study participants were informed about the study objective and then written consent form was obtained prior to data collection. Every patient identity were coded and was only be used for the study purpose. Patients who were found to be culture positive were properly treated by communicating with their physicians.

Results: A total of 324 study subjects socio-demography were seen during the study period. About 320(98.76%) pregnant women accepted, interviewed and participated for study related tests in addition to routine antenatal and baseline assessments. However, 4(1.23%) of the recruited pregnant women did not submit their urine specimen and were excluded from analysis. Those 320(98.76%) pregnant women with complete data were used for the analysis.

Pregnant women enrolled in this study were with the age ranges of 16-39 years with mean age of 26(±4.5) years. Among the study subjects, 309(96.6%) were married. Two hundred eighty seven (89.7%) had educational level of primary and above with 33(10.3%) were illiterate. 307(95.9%) were urban dwellers. Based on their parity, 263(82.2%) were nulli-parous and mono-parous but 57(17.8 %) were multi-parous. Whereas, 289(90.3%) were in their 2nd and 3rd trimester of pregnancy. Fifty one (15.9%), eighty five (26.6%) and five (1.6%) of study subjects had history of catheterization, urinary tract infections and diaphragm, respectively. Three hundred twelve (97.5%) of the study subjects had experience of frequent sexual intercourse.

Figure 1:- Age distribution of pregnant women (N=320) investigated for Urinary tract infection at St. Paul Hospital Millennium Medical College and Selam Health Center from September to November, 2013.



Prevalence and Bacterial isolates of Urinary tract infections: The overall prevalence of UTIs was 25.9% with 28.8% and 23.1% were pointed out for symptomatic and asymptomatic study subjects respectively. Among seven bacterial species identified, three were gram negatives and four were gram positives with a total number of 83(37 from asymptomatic and 46 from symptomatic) isolates. Gram positive bacteria were more prevalent 56(67.4%) than gram negative bacteria 27(32.5%). The most commonly isolated bacteria were *E.coli* 23(27.7%),

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*S.saprophyticus*22 (26.5%), *S.aureus* 18(21.7%), *S.agalactae* 9(10.8%), *S.epidermidis* 7(8.4%), *P.vulgaris* 3(3.6%) and *P.mirabilis* 1(1.2%). (Table 1)

Among all gram positive bacterial isolates, 26(46.4%) and 30(53.6%) were isolated from symptomatic and asymptomatic pregnant women respectively. In gram negative isolates, the highest 20(74.1%) were isolated from symptomatic women. The highest bacterial isolates among symptomatic and asymptomatic pregnant women were reported as *E.coli* 17(73.9%) and *S.saprophyticus*16 (72.7%) respectively. Bacterial isolates were not significantly associated (P=0.252) with clinical feature or being symptomatic and asymptomatic of the study participants.

Table 1:- Prevalence of isolates among symptomatic and asymptomatic pregnant women at St. Paul Hospital Millennium Medical College and Selam Health Center, Addis Ababa, September to November, 2013.

Bacterial isolates	Symptomatic (N=160) No (%)	Asymptomatic (N=160) No (%)	Total (N=320) No (%)
E. coli	17(73.9)	6(26.1)	23(27.7)
S. saprophyticus	6(27.3)	16(72.7)	22(26.5)
S. aureus	11(61.1)	7(38.9)	18(21.7)
S. agalactae	6(66.7)	3(33.3)	9(10.8)
S. epidermidis	3(42.9)	4(57.1)	7(8.4)
P. vulgaris	2(66.7)	1(33.3)	3(3.6)
P. mirabilis	1(100)	0(0)	1(1.2)
Total	46(55.43%)	37(44.57%)	83(100%)

Risk factors associated with Urinary tract infections: Among all study participants, 95.9% were urban participants. The frequency of history of urinary tract infections reported as 26.6%. History of catheterization had reported in 15.9% of study participants. According to their hygienic experience, use of water and napkin for cleaning purpose after toweling or sexual intercourse were reported as 98.1%. Among all considered risk factors educational status of pregnant women was statistically significant with UTIs (P=0.029), illiterate women were at increased risk for UTIs than those with primary education and above. (Table 2)

Table 2:- Association of selected risk factors with UTIs among pregnant women (N=320) at St. Paul Hospital Millennium Medical College and Selam Health Center from September to November, 2013.

Characteristics	Tested No.(%)	Negative No. (%)	Positive No. (%)	COR (95%CI)	P-value
Address					
Urban	307(95.9)	229(74.6)	78(25.4)	1	
Rural	13(4.1)	8(61.5)	5(38.5)	1.84(0.58-5.78)	0.299

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Marital status					
Married	309(96.6)	231(74.8)	78(25.2)	2.47(0.73-8.31)	0.145
Single	11(3.4)	6(55.6)	5(44.4)	1	
Occupation					
Employee	73(22.8)	52(71.2)	21(28.8)	1	
Un-employee	247(77.2)	185(74.9)	62(25.1)	0.83(0.46-1.48)	0.530
Educational status					
Illiterate	33(10.3)	29(90.6)	4(9.4)	3.86(1.15-13.02)	0.029
≥Primary education	287(89.7)	207(72.1)	80(27.9)	1	
Symptoms of UTI					
Yes	160(50.0)	114(71.2)	46(28.8)	0.75(0.45-1.23)	0.252
No	160(50.0)	123(74.9)	37(23.1)	1	
Gravidity					
First	129(40.3)	94(72.9)	35(27.1)	1	
≥2times	191(59.7)	142(74.7)	48(25.3)	0.90(0.54-1.49)	0.689
Parity					
0-1	263(82.2)	191(72.6)	72(27.4)	1	
≥2	57(17.8)	45(80.6)	11(19.6)	0.63(0.31-1.29)	0.210
Trimester					
1st	31(9.7)	20(64.5)	11(35.5)	1	
2nd&3rd	289(90.3)	216(74.7)	73(25.3)	0.63(0.27-1.32)	0.210
History of catheterization					
Yes	51(15.9)	39(76.5)	12(23.5)	1.16(0.58-2.35)	0.669
No	269(84.1)	198(73.6)	71(26.4)	1	
History of UTIs					
Yes	85(26.6)	58(68.2)	27(31.8)	0.67(0.39-1.16)	0.154
No	235(73.4)	179(76.2)	56(23.8)	1	
History of diaphragm					
Yes	5(1.6)	3(60.0)	2(40.0)	0.52(0.08-3.16)	0.477
No	315(98.4)	234(74.3)	81(25.7)	1	
Diabetes mellitus					
Yes	23(7.1)	19(82.6)	4(17.4)	1.72(0.57-5.22)	0.337
No	297(92.8)	218(73.4)	79(26.6)	1	
Sexual experience					
Frequently(daily)	312(97.5)	231(74.0)	81(26.0)	0.96(0.19-4.81)	0.951
Rarely	8(2.5)	6(75.0)	2(25.0)	1	
Hygienic experience					
Napkin only	6(1.9)	5(83.3)	1(16.7)	1.77(0.20-15.35)	0.606
Water &Napkin	314(98.1)	232(73.9)	82(26.1)	1	

COR=Crude odds ratio, **CI**=Confidence Interval

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Antibiotics susceptibility pattern of bacterial uropathogens: The result of antibiotics susceptibility pattern of the isolates are shown on Tables 3 and 4 below. Nitrofurantoin, Clindamycin and Ciprofloxacin were the most effective antibiotics for gram positive isolates with less efficacy of Sulfamethoxazole/Trimethoprim for these bacteria. Gentamicin, Amoxacillin/Clavulanicacid, Norfloxacin and Ciprofloxacin were the antibiotics of choice for gram negative isolates. Among gram negative isolates, *P.mirabilis* were 100% resistant to Ampicillin, Ceftazidime, Cephazoline, Cefotaxime, Chloramphenicol and Nitrofurantoin. All isolates of *S.agalactae* were 100%sensitive for Chloramphenicol, Vancomycin, Penicillin, Oxacillinand Nitrofurantoin. The predominant isolate *E.coli*, demonstrated high sensitivity for Chloramphenicol, 22(95.7%), Cephazoline 21(91.3%), Gentamicin, Nitrofurantoin and Ceftazidime 20(86.9%) each. Its Intermediate response was reported for Norfloxacin, Amoxacillin/Clavulanicacid and Cefuroxime/Sodium. Clindamycin 20(90.9%), Nitrofurantoin 19(86.4%) and Ciprofloxacin 18(81.8%) were antibiotics with greater potency to *S.saprophyticus*, the predominant isolates from Gram-positives. But its resistance pattern were found to be 18(81.8%) for Sulfamethoxazole/Trimethoprim and Penicillin each. Relatively increased number of intermediate response of *S.saprophyticus* were reported as 3(13.6%) for Chloramphenicol and Sulfamethoxazole/Trimethoprim each.

With the exception of *S.agalactae* all isolates were screened for β-lactamase production and being able to produce β-lactamase enzyme were detected in 37(50.0%) of isolates. Higher β-lactamase production from screening test by gram positive isolates 29(61.7%) than gram negative isolates 8(29.6%) were reported. The predominant isolate *E.coli* showed5 (21.7%) positive for β-lactamase screening test. Screening of *S.agalactae* for production is not necessary for clinical purpose and not done routinely because production of β-lactamase has not been recognized in *S.agalactae* strains [8].

Table 3:-Antibiotics susceptibility pattern of Gram-negative bacteria isolated from urine culture of pregnant women (N = 27) at St. Paul Hospital Millennium Medical College and Selam Health Center, September to November, 2013.

Antibiotics agent	Bacterial isolate								
	E.coli			*P.vulgaris*			*P.mirabilis*		
	S No (%)	I No (%)	R No (%)	S No (%)	I No (%)	R No (%)	S No (%)	I No (%)	R No (%)
TTC	16(69.6)	0(0)	7(30.4)	1(33.3)	0(0)	2(66.7)	1(100)	0(0)	0(0)
NOR	17(73.9)	1(4.3)	5(21.7)	2(66.7)	0(0)	1(33.3)	1(100)	0(0)	0(0)
CIP	16(69.6)	0(0)	7(30.4)	2(66.7)	0(0)	1(33.3)	1(100)	0(0)	0(0)
AMP	14(60.9)	0(0)	9(39.1)	1(33.3)	1(33.3)	1(33.3)	0(0)	0(0)	1(100)
AMC	17(73.9)	3(13.0)	3(13.0)	2(66.7)	0(0)	1(33.3)	1(100)	0(0)	0(0)

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F	20(86.9)	0(0)	3(13.1)	1(33.3)	1(33.3)	1(33.3)	0(0)	0(0)	1(100)
CTX	18(78.3)	0(0)	5(21.7)	1(33.3)	0(0)	2(66.7)	0(0)	0(0)	1(100)
CAZ	20(86.9)	0(0)	3(13.1)	1(33.3)	1(33.3)	1(33.3)	0(0)	0(0)	1(100)
KZ	21(91.3)	0(0)	2(8.7)	1(33.3)	0(0)	2(66.7)	0(0)	0(0)	1(100)
CXM	14(60.9)	3(13.)	6(26.1)	1(33.3)	0(0)	2(66.7)	0(0)	1(100)	0(0)
C	22(95.7)	0(0)	1(4.3)	1(33.3)	0(0)	2(66.7)	0(0)	0(0)	1(100)
CN	20(86.9)	0(0)	3(13.1)	2(66.7)	0(0)	1(33.3)	1(100)	0(0)	0(0)

S=Sensitive, **I**=Intermediate, **R**=Resistant, **TTC**=Tetracycline, **NOR**= Norfloxacin, **CIP**=Ciprofloxacin, **AMP**= Ampicillin,**AMC**=Amoxacillin/Clavulanicacid,**F**=Nitrofurantoin,**CTX**=Cefotaxime,**CAZ**=Ceftazidime,**KZ**=Cephazoline, **CXM**= Cefuroxime-Sodium, **C**= Chloramphenicol, **CN**= Gentamicin

Table 4:-Antibiotics susceptibility pattern of Gram-positive bacteria isolated from urine culture of pregnant women (N = 56) at St. Paul Hospital Millennium Medical College and Selam Health Center, September to November, 2013.

Antibiotics agent	Bacterial isolates											
	S.saprophyticus			S.aureus			S.agalactae			S.epidermidis		
	S No (%)	I No (%)	R No (%)	S No (%)	I No (%)	R No (%)	S No (%)	I No (%)	R No (%)	S No (%)	I No (%)	R No (%)
TTC	9 (40.9)	1 (4.5)	12 (54.5)	8 (44.4)	0 (0)	10 (55.6)	3 (33.3)	0 (0)	6 (66.7)	4 (57.1)	0 (0)	3 (42.9)
E	17 (77.3)	1 (4.5)	4 (18.2)	11 (61.1)	0 (0)	7 (38.9)	5 (55.6)	0 (0)	4 (44.4)	6 (85.7)	0 (0)	1 (14.3)
SXT	1 (4.5)	3 (13.6)	18 (81.8)	6 (33.3)	0 (0)	12 (66.7)	6 (66.7)	0 (0)	3 (33.3)	3 (42.9)	0 (0)	4 (57.1)
P	4 (18.2)	-	18 (81.8)	3 (16.7)	-	15 (83.3)	9 (100)	-	0 (0)	2 (28.6)	-	5 (71.4)
CIP	18 (81.8)	0 (0)	4 (18.2)	15 (83.3)	0 (0)	3 (16.7)	7 (77.8)	0 (0)	2 (22.2)	5 (71.4)	1 (14.3)	1 (14.3)
NOR	14 (63.6)	2 (9.1)	6 (27.3)	15 (83.3)	1 (5.6)	2 (11.1)	7 (77.8)	0 (0)	2 (22.2)	6 (85.7)	0 (0)	1 (14.3)
C	16 (72.2)	3 (13.6)	3 (13.6)	17 (94.4)	0 (0)	1 (5.6)	9 (100)	0 (0)	0 (0)	7 (100)	0 (0)	0 (0)
OX	7 (31.8)	-	15 (68.2)	6 (33.3)	-	12 (66.7)	9 (100)	-	0 (0)	3 (42.9)	-	4 (57.1)
CLN	20 (90.9)	2 (9.1)	0 (0)	15 (85.3)	1 (5.6)	2 (11.1)	6 (66.6)	0 (0)	3 (33.3)	6 (85.7)	0 (0)	1 (14.3)

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FOX	10 (45.5)	-	12 (54.5)	11 (61.1)	-	7 (38.9)	7 (77.8)	-	2 (22.2)	5 (71.4)	-	2 (28.6)
VAN	-	-	-	-	-	-	9 (100)	0 (0)	0 (0)	-	-	-
F	19 (86.4)	2 (9.1)	1 (4.5)	16 (88.9)	1 (5.6)	1 (5.6)	9 (100)	0 (0)	0 (0)	6 (85.7)	0 (0)	1 (14.3)

(-)=for antibiotics with no intermediate response, those species without that particular antibiotics test
E=Erythromycin,**SXT**=Sulfamethoxazole/Trimethoprim,**OX**=Oxacillin,**CLN**=Clindamycin,**FOX**=Cefoxitin, **VAN**=Vancomycin, **P**=Penicillin.

The highest sensitivity of isolates from symptomatic pregnant women was reported as 12(80.0%) for Ampicillin, and Cefuroxime/Sodium each and 16(80.0%) for Amoxicillin/Clavulanic acid but the lowest was for Oxacillin 6(40.0%).Resistance pattern for isolates of symptomatic were higher than asymptomatic pregnant women and reported as 5(100%) and 4(80.0%) for Cephazoline and Ceftazidime respectively. The lowest resistance were reported in isolates of symptomatic than asymptomatic pregnant women for Ciprofloxacin 7(38.9%).Among intermediate response of antibiotics to isolates from symptomatic participants, 4(100%) for Cefuroxime/Sodium and 1(100%) were reported to Ampicillin and Cephazoline each. In case of isolates from asymptomatic pregnant women, intermediate response were reported as 3(100%) for Nitrofurantoin, 1(100%) for Tetracycline, Ciprofloxacin and Erythromycin each. There is no significant association between each antibiotics and bacterial isolates of symptomatic & asymptomatic pregnant women. (Table 5)

Table5:- Overall susceptibility pattern of bacterial isolates from symptomatic and asymptomatic pregnant women for antibiotic agents tested (N=19) at St. Paul Hospital Millennium Medical College and Selam Health Center, September to November, 2013.

Antibiotics agent	Clinical feature						
	Symptomatic			Asymptomatic			P-value
	S No(%)	I No (%)	R No (%)	S No (%)	I No(%)	R No(%)	
TTC	26(61.9)	0(0)	20(50.0)	16(38.1)	1(100)	20(50.0)	0.2962
NOR	37(59.7)	2(50.0)	7(41.2)	25(40.3)	2(50.0)	10(58.8)	0.3870
CIP	39(60.9)	0(0)	7(38.9)	25(39.1)	1(100)	11(61.1)	0.1338
AMP	12(80.0)	1(100)	7(63.6)	3(20.0)	0(0)	4(36.4)	0.5357
AMC	16(80.0)	2(66.7)	2(50.0)	4(20.0)	1(33.3)	2(50.0)	0.4364
F	40(55.6)	0(0)	6(75.0)	32(44.4)	3(100)	2(25.0)	0.0833
CTX	14(73.7)	0(0)	6(75.0)	5(26.3)	0(0)	2(25.0)	0.4844
CAZ	15(71.4)	1(100)	4(80.0)	6(28.6)	0(0)	1(20.0)	0.7718
KZ	15(68.2)	0(0)	5(100)	7(31.8)	0(0)	0(0)	0.0960

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CXM	12(80.0)	4(100)	4(50.0)	3(20.0)	0(0)	4(50.0)	0.1295
C	41(56.9)	0(0)	5(62.5)	31(43.1)	3(100)	3(37.5)	0.1381
CN	18(78.3)	0(0)	2(50.0)	5(21.7)	0(0)	2(50.0)	0.1556
E	16(41.0)	0(0)	10(62.5)	23(59.0)	1(100)	6(37.5)	0.2247
SXT	8(50.0)	2(66.7)	16(43.2)	8(50.0)	1(33.3)	21(56.8)	0.6952
P	8(44.4)	-	18(47.4)	10(55.6)	-	20(52.6)	0.1826
OX	6(40.0)	-	20(48.8)	9(60.0)	-	21(51.2)	0.1725
CLN	19(42.2)	1(33.3)	6(75.0)	26(57.8)	2(66.7)	2(25.0)	0.2067
FOX	19(46.3)	-	7(46.7)	22(53.7)	-	8(53.3)	0.1206
VAN	6(66.7)	-	-	3(33.3)	-	-	-

Note :-(-) = Antibiotic with no intermediate& resistant strains reported

Multiple antibiotics resistance patterns of the isolates: Among the total isolates (N=83) Multi-Drug resistance (resistance to ≥ 2 antibiotics) was reported in 66(79.5%) of isolates. Gram positive and Gram negative bacterial uropathogens reported as Multi-Drug resistance of 48(85.7%) and 18(66.6%) of respectively. *E.coli*; the predominant Gram negative isolate showed multi-drug resistance of 15(65.2%) isolates. (Table 6,7).

Table 6:-Multi-Drug resistance pattern of Gram negative bacterial isolates of pregnant women (N = 27) at St. Paul Hospital Millennium Medical College and Selam Health Center, September to November, 2013.

Bacterial isolate	No (%)	Antibiotics Pattern					
		R0	R1	R2	R3	R4	≥R5
E.coli	23(27.7)	6(26.1)	2(8.7)	5(21.7)	4(17.4)	2(8.7)	4(17.4)
P.vulgaris	3(3.6)	0(0)	1(33.3)	0(0)	0(0)	0(0)	2(66.7)
P.mirabilis	1(1.2)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)

R0: no resistance, **R1**: resistance to one antibiotic, **R2**: resistance to two antibiotics, **R3**: resistance to three antibiotics, **R4**: resistance to four antibiotics, **≥R5**: resistance to five and above antibiotics.

Table 7:-Multi-Drug resistance pattern of Gram positive bacterial isolates of pregnant women (N = 56) at St. Paul Hospital Millennium Medical College and Selam Health Center, September to November, 2013.

Bacterial isolate	No (%)	Antibiotics pattern					
		R0	R1	R2	R3	R4	≥R5
S.aureus	18(21.68)	0(0)	1(5.6)	3(16.7)	5(27.8)	3(16.7)	6(33.3)
S.saprophyticus	22(26.5)	0(0)	2(9.1)	4(18.2)	6(27.3)	1(4.5)	9(40.9)
S.agalactae	9(10.8)	1(11.1)	1(11.1)	1(11.1)	1(11.1)	2(22.2)	3(33.3)

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S.epidermidis	7(8.4)	0(0)	3(42.9)	0(0)	1(14.3)	2(28.6)	1(14.3)

R0: no resistance, **R1**: resistance to one antibiotic, **R2**: resistance to two antibiotics, **R3**: resistance to three antibiotics, **R4**: resistance to four antibiotics, **≥R5**: resistance to five and above antibiotics.

Discussion: Special attention to the pregnant women is one of the most important points in health care system. Pregnancy enhances the progression from asymptomatic to symptomatic bacteriuria, which could lead to hypertension, septicemia, maternal death, pyelonephritis and adverse obstetric outcomes such as; prematurity, low birth weight, and higher foetal mortality rates [9]. The adverse effects of undiagnosed asymptomatic bacteriuria on mother and fetus have made us to suggest routine urine culture screening. Unless intervention made on time, UTIs will cause serious problem on the mother as well as the fetus life, therefore early screening and antibiotics treatment is the best preferred intervention [10].

The overall prevalence of UTIs in this study (25.9%) is comparable to study in Dhaka by Parveen.*et al*., (26%) [11], Tanzania by Moyo.*et al*., (21%) [12], Yemen by Al-Haddad.*et al*., (30%) [13] and Egypt by Dimetry.*et al*., (31.3%) [14] but it is higher than the study conducted in Gondar by Ferede.*et al*., Alemu.*et al*.,(12%, 10.4%) [4,15], Bahirdar by Demilie.*et al*.,(9.5%)[10], Jimma by Beyene.*et al*.,(9.2%) [16] and Addis Ababa by Assefa.*et al*., (11.6%) [7].The higher prevalence could be due to increased number of study participants with complaining of urinary tract infections and having clear sign and symptoms in our study.

The reported prevalence of urinary tract infections among symptomatic women in this study (28.8%) was in agreement with the study conducted in Iraq by Mohamed.*et al*.,(28%) [17], Pakistanby Rizyi.*et al*.,(25.2%) [18] and Addis Ababa, Ethiopia by Assefa. *et al*.,(20%)[15]with some deviation and this may be due to variation in sample size, example; the smaller number of symptomatic cases involved in Addis Ababa, Ethiopia study (N=45). Symptomatic patients whose urine culture didn't show appreciable growth might be due to several different microorganisms that can cause UTIs, including protozoan parasites, fungi and viruses, even though bacteria are the major causative organisms [4].

In case of report on asymptomatic bacteriuria of this study the result (23.1%) was in agreement with study conducted in Bahirdar, Ethiopia by Demilie.*et al*., (18.9%) [10], Egypt by El-Sakkary.*et al*., (23.5%) [19], Nepal by Neupane.*et al*., (26%) [20] and Iran by Rahimkhani.*et al*., (29.1%) [1] but it is much smaller than the study conducted in Nigeria by Imade.*et al*.,(45.3%) [21], Ghana by Boye.*et al*., (56.5%) [22] and Akwa by Obiogbolu.*et al*., (54%) [23].This variation may be due to environmental factors, social habit of the community and small sample size of asymptomatic cases in our study (N=160) than higher number in Nigeria's study (N=1,228), but this may not be the reason in case of Ghana and Akwa because they use only (N=200) and (N=100) asymptomatic subjects respectively.

In our study, higher prevalence of UTIs among symptomatic pregnant women than asymptomatic have been reported (28.8%>23.1%) this is in line with the study in Addis Ababa, Ethiopia by Assefa.*et al*., (20% >10.6%) [7].There were no statistically significant association between the two groups or being symptomatic or asymptomatic for urinary tract infections were not significantly associated with prevalence of UTIs

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(P=0.252) this is in agreement with the study conducted in Khartoum by Hamdan. *et al*., [24].

The most prevalent organism identified in our study both in symptomatic and asymptomatic study participants was *E.coli* which is similar with previous works in Ethiopia by Demilie.*et al*., Ferede.*et al*., Alemu. *et al*., Assefa.*et al*., and Beyene.*et al*.,[10,4,15,7,16]. The major contributing factor for isolating higher rate of *E.coli* is due to urine stasis in pregnancy which favors for environment suitable for colonization of such strain and cause UTIs [7,9]. Another reason could be due to poor genital hygienic practices by pregnant women who may find it difficult to clean their anus properly after defecating or clean their genital after passing urine during their pregnancy [21]. In our finding the second common isolate was *S.saprophyticus* which is comparable with other studies reported as *CONs* in Ethiopia by Demilie.*et al*., Ferede.*et al*., Alemu. *et al*., Shiferaw.*et al*., Assefa.*et al*., Beyene.*et al*.,[10,4,15,25,7,16] and other African countries by Obiogbolu.*et al*., Iweriebor.*et al*., Moyo.*et al*., El-Sokkary.*et al*., Ezechil.*et al*.,[23,26,12,19,2] and studies at different European countries by Saffar.*et al*., Imade.*et al*.,Enayat.*et al*.,Ullah.*et al*.,[30,21,31,3]. *S.agalactae*, the most important urinary pathogen was isolated as 10.8% of isolates this was in agreement with the study conducted in Saudi Arabia by Sibiani *et al*., [27].

Among all considered risk factors educational status of pregnant women was statistically significant with UTIs (P=0.029), illiterate women were at increased risk for UTIs than those with primary education and above, this in agreement with the study conducted in Egypt by Dimetry.*et al*., [14]. In this study, trimester, parity, history of UTIs and symptoms of UTIs in index pregnancy were not associated with bacteriuria which is in agreement with the study conducted in Khartoum byHamdan.*et al*., [24] Iraq by Hazhir.*et al*., [28] Yemen by Al-Haddad.*et al*., [13]. This contrasts the study in Gondar, Ethiopia by Alemu.*et al*., where previous history of UTIs and catheterization had significant association with UTIs [15] and study conducted in Dhaka byParveen.*et al*., [11] where parity, sexual experience, certain contraceptives (Diaphragm), previous history of urinary tract infections had increased the risk of acquiring UTIs. The variation in history of UTIs as risk factor may be due to false diagnosis of the infection without culture confirmation. In case of sexual experience variation of result may be due to decrease current sexual experience due to pregnancy. When we justify the case of diaphragm, the variation may be due to long history of being used it in our study participants, but more recent exposure may lead to risk for UTIs.

The antibiotics sensitivity and resistance patterns vary from community to community and from hospital to hospital. This is because of emergence of resistant strains as a result of indiscriminate use of antibiotics [9]. Higher rate of resistant were reported in antibiotics susceptibility for our study reported as Multi-Drug resistance (resistance to ≥ 2 antibiotics) were recorded in 66(79.5%) of isolates. This is comparable to study conducted in Addis Ababa, Ethiopia, by Assefa et al., (74%) [7]. But this is less than study conducted in Gondar by Ferede *et al*.,(91.7%)[4] and Alemu *et al*.,(95%)[15]. The variation may be due to epidemiological variation of bacterial isolates or increased awareness of the society within the past few years. *E.coli*, the predominant bacteria were highly sensitive for Chloramphenicol (95.7%), Cephazoline (91.3%), Ceftazidime, Nitrofurantoin

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and Gentamicin (87.0%) this is in agreement with study conducted in Gondar, Ethiopia by Ferede.*et al*.,[4] but its response to Nitrofurantoin decreases from previous study conducted in Nigeria byOkonko.*et al*., where 100% sensitivity were reported [29]. Chloramphenicol was the most effective antibiotics for *E.coli* whereas Clindamycin (90.9%, 85.7%), Ciprofloxacin (81.8%, 71.4%) and Norfloxacin (63.6%, 85.7 %,) were the most effective antibiotics for *S.saprophyticus* and *S.epidermidis* respectively. This finding is comparable to study conducted in Jimma, Ethiopia by Beyene.*et al*., [16].

The current study showed high level of β-lactamase production from screening test by gram positive isolates 29(61.7%) than gram negative isolates 8(29.6%). The predominant isolate *E.coli* shows 5(21.7%) positive for β-lactamase screening test that is smaller than previous study conducted in Pakistan by Rizvi *et al*., (47%) [18], the variation may be due to *E.coli* was accounting 41.9% of the urinary isolates in their study but only 27.7% in our report. Although, the result have been compared to resistance pattern to single β-lactam antibiotics (Cefotaxime and Ceftazidime for gram negatives and Penicillin for gram positives) almost all isolates with positive strip screening test have corresponding result to these antibiotics. Noteworthy to mention that Cefotaxime or Ceftazidime alone may not be enough as screening antibiotics for β-lactamase. However, this is an important finding, since it is likely to indicate that bacteria with certain strains prone to Multi-Drug resistance may be present. It is therefore important that routine screening of β-lactamase in urinary isolates is carried out to prevent widespread of resistant isolates.

Conclusion: Prevalence of urinary tract infections among pregnant women in this study is higher than previous studies in Ethiopia with an increased number of *S.agalactae*. This is worrisome because UTIs may causes renal failure, still birth or abortion, low birth weight that cost life of mother and the fetus. Relatively increased multi-drug resistance was reported in gram positives bacterial isolates than gram negatives. An increased resistance to commonly used antibiotic agents, enhanced Multi-Drug resistance rate as well as production of β-lactamase in all bacterial isolates was reported. It was found to be so serious and leads to Multi-Drug resistance in almost all antibiotics currently available. In addition to personal life, the highest rate of increased prevalence and Multi-Drug resistance, will affect the economy and health coverage of the country.

Early screening of pregnant woman for UTIs causing bacterial uropathogens and determining their antibiotic susceptibility patterns should be done as an intervention mechanism in our setting. *S.agalactae* is an important etiological agent of neonatal meningitides worldwide. In this study pregnant women were shown 10.8% carriage rate for *S.agalactae*. Which implies that there neonate contract this pathogen during the delivery time. So the finding of our setting alarm the concerning bodies to undertake national wise surveillance of these pathogens among pregnant women. Even though the result of screening test is good indicator for β-lactamase production, further confirmatory test is recommended in depth.

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