ORIGINAL ARTICLE

URINARY BACTERIAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN AMONG PREGNANT WOMEN IN NORTH WEST ETHIOPIA

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ABSTRACT

BACKGROUND: Urinary tract infection in pregnancy is associated with significant morbidity for both the mother and the baby. The aim of this study was to determine the bacterial profile and antibiotic resistance pattern of the urinary pathogens isolated from pregnant women at Felege Hiwot Referral Hospital Bahirdar, Ethiopia.

METHODS: A total of 367 pregnant women with and without symptoms of urinary tract infection were enrolled as a study subject from October 2010 to January 2011. Organisms were identified from mid-stream clean catch urine samples and antibiotic susceptibility was performed using bacteriological standard tests. Data were collected using structured questionnaires and were processed and analyzed using SPSS for Windows version 16.

RESULTS: Out of 367 pregnant women, 37 were symptomatic and the rest 330 asymptomatic. Bacteriological screening of urine samples revealed growth of bacteria in 8.5% (7/37) and 18.9% (28/330) for symptomatic and asymptomatic pregnant women respectively with overall prevalence of 9.5%. The most common isolates detected were E.coli (45.7%) followed by coagulase negative Staphylococcus (17.1%) and S.aureus (8.6%). Gram-negative bacteria showed resistance rates in the range of 56.5% -82.6 % against trimethoprim/sulfamethoxazole, tetracycline, amoxicillin & ampicillin. Gram positive isolates showed resistant rate ranging from 50-100% against tetracycline, trimethoprim-sulphamethoxazole, amoxicillin and penicillin-G. Both Gram positive and gram negative bacteria showed high sensitivity against Nitrofurantoin with a rate of 82.3% and 87%, respectively. All isolated Gram positive bacterial uropathogens were sensitive for Amoxicillin-clauvlanic acid.

CONCLUSIONS: The isolation of bacterial pathogens both from symptomatic and asymptomatic pregnant women that are resistance to the commonly prescribed drug calls for an early screening of all pregnant women to urinary tract infection.

KEYWORDS: Bacterial profile, antibiotic resistance, pregnancy, Bahirdar

INTRODUCTION

Urinary tract infection (UTI) is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract. It is usually due to bacteria from the digestive tract which climb the opening of the urethra and begin to multiply to cause infection (1, 2). In contrast to men, women are more susceptible to UTI, and this is mainly due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora (3).

Urinary tract infection in pregnancy is associated with significant morbidity for both

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mother and baby. The combination of mechanical, hormonal and physiologic changes during pregnancy contributes to significant changes in the urinary tract, which has a profound impact on the acquisition and natural history of bacteriuria during pregnancy (4).

Urinary tract infection can be either symptomatic or asymptomatic. Patients with significant bacteriuria who have symptoms referable to the urinary tract are said to have symptomatic bacteriuria. Asymptomatic bacteriuria (ABU) is a condition characterized by presence of bacteria in two consecutive clear-voided urine specimens both yielding positive cultures ($\geq 10^5$cfu/ml) of the same uropathogen, in a patient without classical symptoms. *E.coli* is the major etiologic agent in causing UTI, which accounts for up to 90% of cases. *P.mirabilis*, *Klebsiella* species, *P.aeruginosa* and *Enterobacte*r species are less frequent offenders. Less commonly, Enterococci, *G. vaginalis* and *U.urealyticum* are also known agents in UTIs. Gram-positive organisms are even less common in which Group B Streptococcus, *S. aureus, S. saprophyticus* and *S. haemolyticus* are recognized organisms (5).

Current management of UTIs are usually empirical, without the use of a urine culture or susceptibility testing to guide therapy. However, as with many community acquired infections, antimicrobial resistance among the pathogens that cause UTIs is increasing and is a major health problem in the treatment of UTI (6, 7). There is growing concern regarding antimicrobial resistance worldwide, particularly to *E.coli* which is the dominant causative agent of UTI in pregnant women (8).

Urinary tract infections in pregnancy may lead to unfavorable pregnancy outcomes and complications like preterm delivery, low birth weight, pre-eclamptic toxaemia and anemia, so it must always be screened and treated timely (9). In most developing countries including Ethiopia, screening for UTI in pregnancy is not considered as an essential part of antenatal care. Therefore, this study is designed to determine the bacterial profile and antibiotic susceptibility pattern of uropathogen among pregnant women in Felege Hiwot Referral Hospital, Bahirdar, North West Ethiopia, that will give area based prevalence and antibiotic sensitivity pattern for empirical therapy.

SUBJECTS AND METHODS

A hospital based prospective study was conducted at Felege Hiwot Referral Hospital (FHRH) from October 2010 to January 2011 to determine the profile of bacteria and their susceptibility pattern isolated from pregnant women with and without symptoms of urinary tract infection (UTI). The sample size was determined using the standard proportion method with 95% confidence and 5% precision taking as reference a study finding 11.6% in Addis Ababa (10). A study participant who fulfilled the entry criteria were enrolled conveniently until the required sample size achieved. Pregnant women who have taken antibiotics within seven days at the time of recruitment and who were not willing to participate were excluded from this study.

A pre-designed and structured questionnaire was used for the collection of data on socio-demographic characteristics. Collection of information on sign and symptoms of UTI were done by Gynaecologists and senior nurses.

Mid-stream urine samples were collected using sterile, wide mouthed glass bottles with screw cap tops. On the urine sample bottles, patient's name, age, and time of urine collection were indicated. Study participants were informed to clean their hands with water and their genital area with swab soaked in normal saline before collection of the clean catch mid stream urine samples. Urine specimens were processed in the laboratory within 2 hours of collection and specimens that were not processed within 2 hours were kept refrigerated at 4 °C until it was processed.

A calibrated sterile platinum wire loop that has a 4.0 mm diameter designed to deliver 0.01 ml was used for the semi-quantitative method and plating . A loopful of the well mixed urine sample was inoculated MacConkey, Manitol Salt Agar and Blood Agar (Oxoid, Ltd, England). All plates were then incubated at 37^oC aerobically for 24h. The plates were then examined macroscopically for bacterial growth. The bacterial colonies were counted and multiplied by 100 to give an estimate of the number of bacteria present per milliliter of urine. A significant

bacterial count was taken for specimens that produced $\geq 10^5$ colonies but specimen that produced $< 10^5$ colonies considered insignificant or due to contamination (2, 11, 12).

Isolation and identification of cultured isolates was done according to the standard bacteriological techniques. Colony morphology, hemolytic pattern, Gram reaction and microscopic features were used as primarily identification criteria. Biochemical tests, namely indole, citrate, oxidase, catalase, H_2S production, lysine decarboxylase, lactose fermentation, urea hydrolysis and gas production were performed for identification for Gram negative. Catalase, coagulase test and haemolysis pattern on blood agar were used for identification of Gram positive bacteria. Lancefield grouping test was also done to determine the Lancefield grouping of Streptococcus (13, 14). The sterility of culture media were checked by incubating 3-5 % of the batch at 35 – 37^{o}C for overnight and observed for bacterial growth. Those media which show growth were discarded.

Antimicrobial susceptibilities of the bacterial isolates were performed according to the criteria of Clinical and Laboratory Standards Institute (CLSI, 2005) using the Kirby–Bauer disc diffusion method on Muller-Hinton Agar (Oxoid, Ltd, England) (15). A loop full of bacteria was taken from a pure culture colony and transferred to a tube containing 5ml of phosphate buffer saline and mixed gently 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 medium. The following antimicrobials were used with their respective concentration: Amoxicillin (AML,25µg), Tetracycline (TET,30µg), Ciprofloxacin(CIP, 5µg), Nitrofurantoin (F,300µg), Trimethoprim-sulfamethoxazole (SXT, 1.25/23.75µg), Ampicillin (AMP,10µg), Amoxicillin-clauvlanic acid (AMC, 20/10µg), Gentamicin (CN,10µg), Kanamycin (K, 30µg), Nalidixic acid (NA, 30µg), Erythromycin (E,15µg), Chloramphenicol (C, 30µg), Norfloxacin (NOR,10µg), Penicillin-G (P,10IU). All the antimicrobials used for the study were obtained from Oxoid Ltd and Bashingstore Hampaire, UK. These antimicrobial drug discs were selected based on Clinical and Laboratory Standards Institute (CLSI) and also by considering the availability of these drugs in the study area. After that the antibiotic discs were placed on Muller Hinton Agar and incubated at 37°C for about 18 to 24 hours and the zones of inhibition were measured using metal calliper. The interpretation of the results of the antimicrobial susceptibility tests were based on the national committee for Clinical and Laboratory Standards institute (CLSI, 2005) criteria as sensitive, intermediate and resistant(15). The standard reference strains, *Staphylococcus aureus* (ATCC25923), *Escherichia* coli (ATCC25922 and *P. aeruginosa* (ATCC 27853) were used to assure testing performance of the potency of antibiotic discs. Isolates was considered as multidrug resistance (MDR) if isolate resistant for more than two antibiotics.

Ethical clearance was obtained from Jimma university Public Health and Medical Sciences Ethical Review Committee. Only participants who gave their consent before the sample were included on the study Those who were positive for the test were referred to the physician for management of the infection.

RESULTS

A total of 367 pregnant women with and without symptoms of UTI were investigated during the study period. The age of the pregnant women ranged from 17 - 39 years, with mean of 25.8 years. Majority (43.9%) of the study participants were in the age group of 20-24 years. Educational status of participants varied from illiterate to postgraduate studies. Sixty six(18.0%) of the respondents were unable to read and write while 25.1% of the participants had their higher educational level (Table 1).

A total of 35 (28 from asymptomatic and 7 from symptomatic pregnant women) bacterial uropathogens were identified (Table 2). From the 35 isolates, 23 were Gram negative while 12 were Gram positive bacteria. *E.coli* was the most common bacteria accounting for 16 (45.7%) isolates followed by Coagulase negative Staphylococcus (CNS) and *S.aureus* with isolation rate of 17.1% and 8.6% respectively. Out of 330 asymptomatic pregnant women a total of 28 bacterial isolates were identified of which *E. coli* (44.4%) was the most common isolate

followed by CNS 5(17.2%). Gram negative and Gram positive bacteria accounted for 64.3% and 35.7% of the ABU infections, respectively. From 37 symptomatic pregnant women, 7 bacterial isolates were identified of which *E. coli* was the most common accounting for 57.1%. All in all, the Gram negative bacteria accounted for 71.4% of all isolates in symptomatic women (Table 3).

Table 1. Socio-demographic characteristics of study participants in FHRH, Bahirdar, October 2010-January 2011.

Variables	Number	Percent
Age		
15-24	200	54.5
25-34	159	43.3
35-44	8	2.2
Marital status		
Married	334	99.2
Others	3	0.8
Occupation		
Governmental worker	76	20.7
Nongov. Worker	34	9.3
Farmer	9	2.5
Merchant	45	12.3
Student	10	2.7
Unemployed	193	52.6
Residence		
Urban	354	96.5
Rural	13	3.5
Religion		
Orthodox	321	87.5
Muslim	33	9
Protestant	13	3.5
Ethnicity		
Amhara	357	97.3
Oromo	5	1.4
Tigray	3	0.8
Other	2	0.5
Education		
Illiterate	66	18
Read and write	12	3.3
Elementary	67	18.3
High school	130	35.4
Higher education	92	25.1
Monthly Income		
<500	53	14.4
501-1000	96	27.5
1001-1500	63	17.4
1501-2000	55	15.0
>2000	100	28.3

Table 2 Rate of UTI among asymptomatic and symptomatic pregnant women in FHRH, Bahirdar, October 2010-January 2011.

	Significant bacteriuria		
Type of UTI	Positives No (%)	Negative No (%)	Total No (%)
ABU	28(8.5%)	302(91.5%)	330(89.9%)
Symptomatic UTI	7(18.9%)	30(81.1%)	37(10.1%)
Overall UTI	35(9.5%)	332(90.5%)	367(100%)

In general Gram-negative isolates showed resistance rate of 82.6% to ampicillin, 78.3% to amoxicillin and 69.6% to tetracycline. Their resistance against trimethoprim-sulphamethoxazole, chloramphenicol, nalidixic acid, amoxicillin-clavulnic acid, ciprofloxacin, gentamycin, norfloxacin and kanamycin ranged from 21.7- 56.2%. However, all Gram negative bacterial isolates revealed least resistance against nitrofurantoin (13%). *E.coli* which constituted for 45.7% of the Gram negative bacteria showed that 56.8%, 68.8%, 75%, and 81.7 % resistance against trimethoprim-sulphamethoxazole, tetracycline amoxicillin and ampicillin, respectively. Nitrofurantoin was the most effective drug against *E.coli* with sensitivity rate of 93.7%. *P. aeruginosa* which accounting for 8.3% of the Gram negative isolates was fully sensitive to nitrofurantoin and ciprofloxacin. However, it was relatively resistant to trimethoprim-sulphamethoxazole (50%), nalidxic acid (50%), kanamycin (50%), amoxicillin-clavulnic acid (50%), amoxicillin (50%), tetracycline (50%) and gentamycin (50%) and fully (100%) resistant to norfloxacin, chloramphenicol and ampicillin (Table 4).

Gram positive isolates showed resistance rate ranging from 50-75% to tetracycline, trimethoprim-sulphamethoxazole, amoxicillin and penicillin-G and <50.0% to chloramphenicol (41.7%), ciprofloxacin (25.0%), norfloxacin (25.0%), kanamycin (25.0%) and erythromycin (25.0%). Gram positive bacteria had relatively high sensitivity to gentamicin (83.3%), nitrofurantoin (83.3%) and amoxicillin-clavulnic

Table 3 Distribution of the causative agents of ABU and symptomatic UTI among pregnant women in FHRH, Bahirdar, October 2010-January 2011.

Isolated bacteria	ABU(n=330) No (%)	Symptomatic UTI(=37) No (%)	Total (n=367) No (%)
E.coli	12(42.9)	4(57.1)	16(45.7)
CNS	5(17.9)	1(14.3)	6(17.1)
S.aureus	3(10.7)	0(0)	3(8.6)
C.freundii	2(7.1)	0(0)	2(5.7)
P.aeruginosa	2(7.1)	0(0)	2(5.7)
P.mirabilis	1(3.6)	1(14.3)	2(5.7)
Group B Streptococci	1(3.6)	1(14.3)	2(5.7)
K.pneumoniae	1(3.6)	0(0)	1(2.9)
Group D Streptococci	1(3.6)	0(0)	1(2.9)
Total	28(80)	7(20)	35(100)

acid (100%). Coagulase negative staphylococcus (CNS) was the common isolates comprising 50.0% of the Gram positive bacteria and they have shown different level of resistance for tested antibiotics. However, all isolates CNS were susceptible to Amoxicillin-clavulnic acid. Similarly, *S. aureus* was also have shown resistance to most antibiotic but sensitive to ciprofloxacin (100%), kanamycin (100%), gentamycin (100%), norfloxacin (100%) and amoxicillin-clavulnic acid (100%) (Table 5). All the Gram positive isolates and 93.8% of the Gram negatives were multi drug resistant.

DISCUSSION

Pregnant women are at increased risk of developing UTI mainly due to a shift in the position of the urinary tract and hormonal changes during pregnancy make it easier for bacteria to travel up the urethra to the kidney and lead to the development of bacteriuria both symptomatic and asymptomatic UTIs (8, 2). Unless intervention is made on time, UTI will cause serious problem on both woman as well as on the fetus life, therefore early screening and antimicrobial treatment is the best preferred interventions (9, 16, 17).

The reported prevalence of symptomatic UTI in this study is in agreement with previous study conducted from Addis Ababa, Ethiopia (10), The Sudan (18) and Tanzania (19) but higher than a study conducted in Pakistan (3). The variation from the latter study may be due to the small number of symptomatic pregnant women included in our study. On the other hand the 8.5% prevalence of ABU is in line with previous local reports from Ethiopia (10, 20, 21) and elsewhere in the world such as in Ghana (17) Qatar (22), Iran (23) but lower than others finding(24) and higher than in the one from Iran (25). The differences in methodologies and study populations might affect comparison of prevalence in different surveys. The low incidence rate of symptomatic UTI reported in this study as compared to the latter study may also be due to the extensive health education given regularly in health facilities and public awareness among pregnant women about antenatal care service follow up during pregnancy in the study area.

The most prevalent organism identified in our study was *E .coli* which is similar with previous works in Ethiopia and other countries (2, 10, 20, 21). The major contributing factor for isolating higher rate of *E.coli* is due to urine stasis in pregnancy which favors for *E.coli* strain colonization (24, 25, 26). 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. In our finding the second common isolate was CNS for both ABU and symptomatic UTI which is comparable with others report (21, 23, 27).

Table 4 Antimicrobial resistance pattern of Gram negative bacteria isolated from asymptomatic and symptomatic UTI in FHRH, Bahir Dar from October 2010-January 2011.

Organism (no)	Number of Bacterial species (%) Resistant to Antibiotics tested											
	AMP	AML	CIP	C	SXT	CN	F	NOR	NA	TET	K	AMC
E.coli (n=16)	13(81.7)	12(75)	3(18.8)	6(37.5)	9(56.2)	5(31.2)	1(6.3)	4(25)	7(43.8)	11(68.8)	3(18.8)	6 (37.5)
P.aeruginosa (n=2)	2(100)	1(50)	0(0)	2(100)	1(50)	1(50)	0(0)	2(100)	1(50)	1(50)	1(50)	1(50)
P.mirabilis (n=2)	1(50)	2(100)	0(0)	1(50)	1(50)	0(0)	2(100)	0(0)	0(0)	2(100)	1(50)	1(50)
C.freundii (n=2)	2(100)	2(100)	2(100)	2(100)	1(50)	0(0)	0(0)	0(0)	1(50)	1(50)	0(0)	0(0)
K.pneumoniae (n=1)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)
Total (n=23)	19(82.6)	18(78.3)	5(27.7)	11(47.8)	13(56.5)	6(26.1)	3(13)	6(26.1)	9(39.1)	16(69.6)	5(21.7)	8(34.8)

AML: Amoxicillin, CIP: Ciprofloxacin, C: Chloramphenicol, E: Erythromycin, SXT: trimethoprim/sulfamethoxazole, CN: Gentamicin, F: Nitrofurantoin, NOR: Norfloxacin , P: Penicillin G, TET: Tetracycline, K: Kanamycin, AMC: Amoxicillin-clavulanic acid ,

Table 5 - Antimicrobial resistance pattern of Gram positive bacteria isolated from asymptomatic and symptomatic UTI in FHRH, Bahir Dar, from October 2010-January 2011.

Organism (no)	Number of bacterial species (%) resistant to											
	AMP	AML	CIP	C	SXT	CN	F	NOR	NA	TET	K	AMC
CNS (n=6)	3(50)	2(33.3)	3(50)	1(16.7)	4(66.7)	1(16.7)	1(16.7)	2(33.3)	5(83.3)	3(50)	2(33.3)	0(0)
S.aureus (n=3)	2(66.7)	0(0)	1(33.3)	1(33.3)	1(33.3)	0(0)	1(33.3)	0(0)	2(66.7)	2(66.7)	0(0)	0(0)
GBS (n=2)	1(50)	1(50)	1(50)	1(50)	1(50)	1(50)	0(0)	1(50)	1(50)	1(50)	1(50)	0(0)
GDS (n=1)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(100)	0(0)	0(0)
Total (12)	7(58.3)	3(25)	5(41.7)	3(25)	6(50)	2(16.7)	2(16.7)	3(25)	9(75)	6(50)	3(25)	0(0)

AML: Amoxicillin, CIP: Ciprofloxacin, C: Chloramphenicol, E: Erythromycin, SXT: trimethoprim/sulfamethoxazole, CN: Gentamicin, F: Nitrofurantoin, NOR: Norfloxacin , P: Penicillin G, TET: Tetracycline, K: Kanamycin, AMC: Amoxicillin-clavulanic acid , GBS: group B Streptococcus, GDS: group D Streptococcus, CNS: Coagulase Negative Staphylococcus

When we compare the rate of isolates of Gram negative and Gram positive bacteria, Gram negative bacteria were the dominant causative agent of UIT which is in line with others report (2,10,21,23). In general the uropathogens identified in our study are similar to those of many other studies conducted in different countries (1, 10, 17, 20, 21, 22, 28, 29).

Higher rate of antibiotic resistance was identified for Gram negative bacterial isolates which is comparable with the study done in Gonder(20). Among Gram negative bacteria *E.coli* isolates showed greater resistance to most antibiotics which is in conformity to study done in Bahir Dar and Gonder (20,30).

Among Gram positive bacteria, CNS showed higher rate of resistance for ciprofloxacin, norfloxacin, kanamycin, gentamicin, nitrofurantoin, erythromycin, amoxicillin-clavulnic acid which is similar to with previous studies in Bahir Dar(30), and in Addis Ababa(10). The resistance of Gram negative and Gram positive bacteria to nitrofurantoin was relatively low in our study. The reason could be due to the less frequent use of nitrofurantoin in the study area. The multi drug resistance result of our study is comparable with the local retrospective study of drug resistance (30) but relatively higher than the study done in Addis Ababa (10).

In conclusion, the prevalence of UTI in pregnant women at FHRH is comparable with other previous studies in Ethiopia and other developing countries elsewhere in the world. However, our study findings shown the isolation of bacterial uropathogens which are resistance for commonly used antimicrobial agents and an increase in multidrug resistance rate. Therefore, early screening of pregnant woman for UTI causing bacterial uropathogens and determining their antibiotic susceptibility pattern is an important intervention to prevent complications that may endanger the life of both the pregnant women and the fetus.

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