Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2012, 2 (5):1497-1502

ISSN: 2248 –9215
CODEN (USA): EJEBAU

The Prevalence and Antimicrobial Susceptibility pattern of Bacterial Uropathogens Isolated from pregnant women

Getachew Ferede[1], Gizachew Yismaw[1], Yitayih Wondimeneh[1], Zufan Sisay[2]

[1]*School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Ethiopia*
[2]*Department of Medical Microbiology, Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia*

ABSTRACT

A cross-sectional study was conducted on a total of 200 pregnant women to determine the prevalence and antimicrobial susceptibility of bacterial uropathogens from January to May, 2011 at Gondar University Hospital antenatal clinic, Northwest Ethiopia. Clean catch mid-stream urine specimens were collected from each study subjects. Urine culture, identification and sensitivity tests were done using standard microbiologic procedure. In this study, the overall prevalence of urinary tract infection was 12%. Of this bacteriological screening of mid-stream urine specimens showed that 10.2% and 15.9%, had significant bacteriuria in asymptomatic and symptomatic group respectively. Prevalence of urinary tract infection was a statistically significant association with previous history of catheterization and urinary tract infection ($p<0.05$). Escherichia coli was the most frequently isolated organism (41.5%) followed by coagulase negative staphylococcus (25%). Gram negative and positive bacteria accounted for (58.3%) and (41.7%) respectively. Rate of susceptibility for all isolated bacteria showed, ceftriaxon and gentamicin (87.5%) for each, amoxicillin–clavulanic acid (83.3%), ciprofloxacine (75%), and norfloxacilin (70.8%). However, most resistance was to ampicillin (91.7%), amoxicillin (79.2%), tetracycline (58.3%), cotrimoxazol (50%), and chloramphinicol (33.3%). Multi drug resistance (resistance two or more drugs) of 91.7% (22/24) was observed among the isolated bacterial uropathogens.

Key words: UTIs, pregnant women, bacteriuria, antimicrobial drug resistance.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infectious diseases and serious ailment in humans due to the frequency, recurrence and difficulty in eradication poses stiff challenge to the medical professionals. It is important because it may involve kidneys, ureters, bladder and urethra [1]. It has been reported in all age groups and in both sexes [2]. However, women are more susceptible than men, due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with fecal flora [3]. Additionally, the physiological increase in plasma volume during pregnancy decreases urine concentration and up to 70% pregnant women develop glucosurea, which encourages bacterial growth in the urine [4].

But the main factors predisposing women to bacteriuria are pregnancy and sexual intercourse [5]. Sexual activity increases the chances of bacterial contamination of female urethra. Sexual intercourse may also cause bacteria to be pushed into the urethra. This anatomical relationship of the female urethra to the vagina makes it liable to trauma during sexual intercourse as well as bacteria being massaged up the urethra into the bladder during pregnancy or child birth [6, 7]. Using a diaphragm can also lead to UTIs because diaphragms push against the urethra and make it harder to completely empty the bladder and the urine that stays in the bladder is more likely to grow bacteria and cause infections [8].

In female, the urinary tract has an important association with the reproductive organs because of its proximity. In the non-pregnant state, the uterus lies just behind and partly over the bladder while in the pregnant state; the enlarging uterus affects all the tissues of the urinary tract at various times [9]. Pregnant women are at increased risk for UTIs. Beginning in week 6 and peaking during weeks 22 to 24, approximately 90 percent of pregnant women develop urethral dilatation, which will remain until delivery. Increased bladder volume and decreased bladder tone, along with decreased urethral tone, contribute to increased urinary stasis and ureterovesical reflux [10].

UTIs are common complications of pregnancy and it may manifest as asymptomatic bacteriuria (ASB) or symptomatic bacteriuria (SB). Asymptomatic bacteriuria, in which urine culture reveals a significant growth of pathogens, that is greater than 10^5 bacteria/ml but without the patient showing symptoms of UTI, can be found in both pregnant and non pregnant women and also bacteriuria associated with pregnancy has a direct bearing not only on the health of the woman, but also on the pregnancy [11]. Pregnancy enhances the progression from asymptomatic to symptomatic bacteriuria which could lead to pyelonephritis and adverse obstetric outcomes such as prematurity, low-birth weight, and higher fetal mortality rates [12].

The incidence of these complications can be decreased by treating promptly ASB and SB during pregnancy [10]. Due to the potential adverse sequelae of UTI in pregnancy, most clinics perform routine urinalysis of midstream urine specimen during one or more antenatal clinic (ANC) visits [13]. However, culture and antimicrobial drug susceptibility testing are needed for surveillance purposes to guide the clinicians on the proper management and prevent empirical treatment of pregnant women with ASB and SB.

The organisms that cause UTIs during pregnancy are the same as those found in non-pregnant patients and the leading causes of acute and uncomplicated UTI have been reported to be due to *Escherichia coli* (80%–90%) and the rest are caused by, *Staphylococcus aureus, Proteus spp, Klebsiella spp* and *Pseudomonas aeruginosa* [14, 15]. Data on the current distribution and antimicrobial drug susceptibility patterns among urinary bacterial isolates from pregnant women in Ethiopia; particularly in the study area is limited. Therefore, this study aimed to determine the prevalence and antimicrobial susceptibility of uropathogens causing urinary tract infections among pregnant women attending ANC in Gondar University Hospital, Gondar, Ethiopia.

MATERIALS AND METHODS

A cross-sectional study was conducted from January to May, 2011 on pregnant women attending the antenatal clinic of Gondar University Hospital. Verbal informed consent was obtained from each pregnant woman prior to sample collection. Information on age, previous history of catheterization, clinical sign, and period of gestation, parity, and number of pregnancies was collected by face-to-face interview with the pregnant women. Ethical approval was obtained from Gondar University Hospital.

Clean-catch midstream urine was collected from each patient into a sterile test tube container and samples were cultured on cysteine lactose electrolyte deficient agar (CLED), using a calibrated drop delivering 0.002ml of urine. Streaked culture plates were incubated at 37^oc overnight. On the next day, the bacterial growth on the respective media was looked, and total colony count was done. A single colony was picked from culture plates with significant bacteriuria (10^5 colony forming units per ml urine of one or two isolate (s) and was suspended in nutrient broth, and then sub cultured onto blood agar and MacConkey agar and finally incubated at 37^oc for further purification. Pure isolates of bacterial pathogen were preliminary characterized by colony morphology, gram-stain, and catalase test. A standard biochemical procedure was used for full identification of gram- positive and gram negative bacteria. Antimicrobial susceptibility testing was performed for bacterial isolates by using agar diffusion method described by Bauer *et al*., 1966 on Mueller-Hinton agar (oxoide) [16].

After a pure culture was obtained, a loop full of bacteria was taken from a colony and was transferred to a tube containing 5 ml sterile normal saline (0.85 % NaCl) and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the optical density of a Mc far land 0.5 tubes measured at 500 nm absorbance in order to standardize the inoculums size.

A sterile cotton swab was then dipped into the suspension and the excess was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria suspension evenly over the entire surface of Mueller-Hinton plates. The inoculate plates were left at room temperature to dry for 3-5 minutes while the Petridis lids was in place. By using sterile forceps, appropriate antimicrobial discs were evenly distributed on the inoculated plates.

Gram-positive and gram-negative bacteria isolates were tested against the following antimicrobials: ampicillin (AMP) (10µg), amoxicillin-clavulanic acid (AMC) (30µg), ceftraixone (CRO) (30 µg), chloramphenicol (C) (30µg), ciprofloxacin (CIP) (5 µg), gentamicine (CN) (10µg), trimethoprim-sulphamethoxazole (SXT) (25µg) and tetracycline (TTC) (30 µg). The plates were then incubated at 37^{o}C for 18-24 hours.

Diameter of the zone of inhibition around the disc was measured to the nearest millimeter using a metal caliper and the isolate were classified as sensitive, intermediate and resistant according to National Committee for Clinical Laboratory Standards (NCCLS (2002)). Standard strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *Pseudomonas aeruogenosa* (ATCC 27853), which were sensitive to all antibacterial drugs were used routinely in this study as control.

Table 1: Prevalence of UTI vs. socio-demographics, obstetrics and clinical variables of pregnant women at Gondar University Hospital, Northwest Ethiopia, 2011

Variables	Non –significant bacteriuria N0. (%)	Significant bacteriuria N0. (%)	Total	Significance N0.
Age(years)				
16-20	38(88.4)	5(11.6)	43	X^2= 1.519
21-25	65(90.3)	7(9.7)	72	P= 0.911
26-30	54(87.5)	10(12.5)	64	
31-35	10(90.9)	1(9.1)	11	
36-40	7(87.5)	1(12.5)	8	
41-45	2(100)	0(0)	2	
Gravidity				
1-3	148(88.1)	20(11.9)	168	X^2= 0.036
4-6	22(88)	3(12)	25	P= 0.982
7-9	6(85.7)	1(14.3)	7	
Parity				
Nullipara	66(90.4)	7(9.6)	73	X^2= 1.166
One	42(84)	8(16)	50	P= 0.558
Multipara	68(88.3)	9(11.7)	77	
Trimester				
1st	19(100)	0(0)	19	X^2= 3.504
2nd	85(85)	15(15)	100	0.173
3rd	72(88.9)	9(11.1)	81	
current symptom of patient				
Yes	53(84.1)	10(15.9)	63	X^2= 1.306
No	123(89.8)	14(10.2)	137	P= 0.253
His. Catheterization				
Yes	7(63.6)	4((36.4)	11	X^2= 6.543
No	169(89.4)	20(10.6)	189	P= 0.011
His. of UTI				
Yes	29(72.5)	11(27.5)	40	X^2= 11.375
No	147(92)	13(8)	160	P= 0.001

RESULTS

Out of 200 pregnant women examined for UTI, 24 were positive for significant bacteriuria, giving the overall prevalence of 12%. Of this bacteriological screening of mid-stream urine specimens showed that 14/137(10.2%) and

10/63(15.9%), had significant bacteriuria in asymptomatic and symptomatic group respectively. Of all the bacteria isolated, gram-negative bacteria were more prevalent, 58.3%, than gram-positive bacteria, 41.7%. The most commonly isolated bacteria were *Escherichia coli* 10 (41.7%), followed by coagulase negative *Staphylococcus* 6 (25%).

Antimicrobial susceptibility of isolated bacteria showed that: ceftriaxon and gentamicin (87.5%), amoxicillin–clavulanic acid (83.3%), ciprofloxacine (75%), and norfloxacilin (70.8%), chloramphinicol (66.7%), cotrimoxazol (50%), tetracycline (47.7%), amoxicillin (20.8%), ampicillin (8.3%). Multi drug resistance (resistance two or more drugs) of 91.7% (22/24) was observed among the isolated bacterial uropathogens.

Table 2: Etiology and frequency of bacterial uropathogens isolated from asymptomatic and symptomatic pregnant women at Gondar University Hospital, Northwest Ethiopia, 2011.

Bacterial isolates	Symptomatic (n=63)	Asymptomatic (n=137)	Total (n= 200)
	No. (%)	No. (%)	No. (%)
E. coli	5(50)	5(35.7)	10(41.7)
Enterobacter aerogenosa	1(10)	1(7.1)	2(8.3)
Klebsiella pneumonia	0(0)	2(14.3)	2(8.3)
CNS	4(40)	2(14.3)	6(25)
S.aureus	0(0)	4(28.6)	4(16.7)
Total	**10(41.7)**	**14(58.3)**	**24(100)**

**CNS= coagulase negative Staphylococcus*

Table 3: Antimicrobial susceptibility pattern of bacteria isolated from urine culture of pregnant women at Gondar University Hospital, Northwest Ethiopia, 2011.

Bacterial isolate	Total	S/R	Antimicrobial agents tested									
			AMP	AMOX	CRO	C	CIP	GN	NOR	SXT	TTC	AMC
			No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No.(%)	No. (%)	No (%)
E. coli	10	S	0(0)	0(0)	10(100)	10(100)	10(100)	9(90)	10(100)	9(90)	7(70)	9(90)
		R	10(100)	10(100)	0(0)	0(0)	0(0)	1(10)	0(0)	1(10)	3(30)	1(10)
Enterobacter aerogenosa	2	S	0(0)	0(0)	2(100)	2(100)	2(100)	2(100)	2(100)	0(0)	1(50)	1(50)
		R	2(100)	2(100)	0(0)	0(0)	0()	0(0)	0(0)	2(100)	1(50)	1(50)
Klebsiela pneumonia	2	S	0(0)	0(0)	1(50)	2(100)	1(50)	1(50)	1(50)	0(0)	0(0)	1(50)
		R	2(100)	2(100)	1(50)	0(0)	1(50)	1(50)	1(50)	2(100)	2(100)	1(50)
CNS	6	S	1(16.7)	3(50)	4(66.7)	0(0)	2(33.3)	5(83.3)	2(33.3)	1(16.7)	1(16.7)	5(83.3)
		R	5(83.3)	3(50)	2(33.3)	6(100)	4(66.7)	1(16.7)	4(66.7)	5(83.7)	5(83.)	1(16.7)
S.aureus	4	S	1(25)	2(50)	4(100)	2(50)	3(75)	4(100)	2(50)	2(50)	1(25)	4(100)
		R	3(75)	2(50)	0(0)	2(50)	1(25)	0(0)	2(50)	2(50)	3(75)	0(0)
Total	**24**	**S**	**2(8.3)**	**5(20.8)**	**21(87.5)**	**16(66.7)**	**18(75)**	**21(87.5)**	**17(70.8)**	**12(50)**	**10(41.7)**	**20(83.3)**
		R	**22(91.7)**	**19(79.2)**	**3(12.5)**	**8(33.3)**	**6(25)**	**3(12.5)**	**7(29.2)**	**12(50)**	**14(58.3)**	**4(16.7)**

AMP= ampicillin *C= chloramphinicol* *NOR= norfloxacin* *AMC= amoxicillin +clavulinic acid*
AMOX= amoxicillin *CIP= ciprofloxacin* *SXT= co-trimoxazole*
CRO= ceftriaxon *GN= gentamicin* *TTC= tetracycline*

DISCUSSION

Proper investigation and prompt treatment are needed to prevent serious life threatening condition and morbidity due to UTI that can occur in pregnant women [17]. In this study, the overall prevalence of urinary tract infection in pregnant women was 12%. Of these the prevalence of bacteriuria among symptomatic and asymptomatic pregnant women was 15.9% and 10.2% respectively. This was in agreement with similar study reported in, Addis Ababa Ethiopia (11.6%); of these 20% in symptomatic and 10.6% in asymptomatic pregnant women and Similarly in Tanzania [18, 19], asymptomatic pregnant women of UTI in Gondar Ethiopia 9.8% [20], in Nigeria 10.7% [21] was reported. However, lower prevalence of asymptomatic UTI compared to the present study was reported: Iran 3.3% [22], United Arab Emirates 4.8% [23] and Ghana 7.3% [24].

Symptoms did not associate with the prevalence of symptomatic urinary tract infection in this study (p=0.253). From 63 pregnant women who complain to have symptoms that suggests symptomatic urinary tract infection, only 10/63(15.9%) was found to have culture confirmed urinary tract infection. Similar findings were also reported in Addis Ababa Ethiopia and Tanzania [18, 19]. Symptomatic patients whose urine culture didn't show appreciable growth might be due to several different microorganisms can cause UTIs, including protozoan parasites, fungi and viruses, even though bacteria are the major causative organisms [25].

The prevalence of urinary tract infection was not associated with age, parity and trimester in this study. This was in agreement with studies in Tanzania and Sudan [19, 26]. However, maternal age and parity have been previously observed as risk factors for UTI among pregnant women [27]. This may be due to changes in physiology with increasing age and the immune system also becomes weaker with age increase. Likewise in this study trimester was not associated with UTI among these women. It might be due to most of study participants were within 2nd trimester. However, it has been reported that, UTI developed in third trimester [28]. Perhaps the susceptibility of UTI during this period is due to urethral dilatation which started as early as 6 week and reaching the maximum during 22-24 weeks [10].

Urinary tract infection in pregnant women with previous history of urinary tract infection was significantly higher than those without previous history of urinary tract infection (p=0.001). This had similarity with study in Saudi Arabia and Pakistan [29, 30]. Urinary tract infection in pregnant women who had previous history of catheterization was significantly higher than those without history of previous catheterization (p=0.011). This was in agreement with Seung reports [31]. This might be due to catheterization could induce urethral mucosa injury and might induce haematogenous bacterial spread in a urinary tract infection.

In this study, gram-negative bacteria were more prevalent (58.3%) than gram-positive bacteria which constituted 41.7%. Similar findings have been reported in Tanzania and Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia [17, 18]. *E. coli* was the most predominant bacteria isolated. Similar findings have been reported in Nigeria, Sudan, and Yemen [8, 26, 32] respectively. *E. coli* is considered uropathogenic due to a number of virulence factors specific for colonisation and invasion of the urinary epithelium, such as the P-fimbria and S-fimbria adhesions [33]. Coagulase-negative *Staphylococci* and *S. aureus* were the second and third most predominant pathogen isolated.

In current study, 87.5% isolates were susceptible for both, ceftriaxon and gentamicin and 83.3% for amoxicillin-clavulanic acid. This finding is comparable to other reports [18, 34]. However, there was a high prevalence of resistant bacteria to a number of antimicrobials tested in this study. A large number of the isolates were resistant to ampicillin 91.7%, amoxicillin 79.2% and tetracycline 58.3%. Similar findings have been reported in Iran and Aligarh [22, 35]. This observed resistance to these drugs is a probable indication of earlier exposure of the isolates to these drugs, which may have enhanced resistant development. These drugs are very common due to low cost and often purchased without prescription in different areas.

An irrational and unnecessary use of antibacterial agents can result in the emergence of bacterial strains that exhibit multidrug resistance [36]. In the present study most of the isolated pathogens showed multiple drug resistance of two and more antibacterial agents tested. Similarly, multidrug resistances in bacterial uropathogens were reported [34, 35, 37]. In conclusion**,** significant bacteriuria in both asymptomatic and symptomatic pregnant women and high level of multidrug resistance was observed. This suggests a need for continuous monitoring of uropathogens in all pregnant women, and antibiotic susceptibility testing before antibiotic prescription in order to ensure adequate treatment of urinary tract infection.

Acknowledgments
We would like to thank all laboratory staffs in the unit of Bacteriology, Deprthement of Medical Microbiology and nurses working in ANC of Gondar University Hospital

REFERENCES

[1] Al-Dujiaily A, *Medical Journal of Tikrit*, **2000**, 6, 220–4.
[2] Hooton T, Winter C, Tiu F, and Stamm W, *Journal of American Medical Association*, **1995**, 273, 41-5.
[3] Awaness A, Al-Saadi MG, Aadoas S, *Kufa medical journal*, **2000**, 3, 159.

[4] Lucas M, Cunningharm F, *Clinical obstetrics and gynecology*, **1993**, 36, 855– 68.
[5] National Institutes of Health (NIH), *National Institutes of Health Publication*, **2004**, 4, 4807.
[6] Ebie M, Kandakai-Olukemi Y, Ayanbadejo J, Tanyigna KB, *Nigeria Journal of Microbiology*, **2001,** 15, 31-37.
[7] Kolawole A, Kolawole O, Kandaki-Olukemi Y, Babatunde S, Durowade K, and Kolawole C, *International Journal of Medicine and Medical Science*, **2009**, 1, 163-167.
[8] Okonko I, Ijandipe L, Ilusanya A, Donbraye-Emmanuel O, Ejembi J, Udeze A, Egun O, Fowotade A, and Nkang A, *Malaysian Journal of Microbiology*, **2010**, 6, 16-24.
[9] Warren J, Tenney J, Hoopes J, Munere H, Anthony W, *Journal of Infectious Diseas*, **1982**, 146, 719-723.
[10] Delzell J, Lefevre M, *American Family Physician*, **2000**, 61,713 21.
[11] Gilbert D, Moellering R, Eliopoulos G, Sande N, Sanford guide to Antimicrobial Therapy. 32nd. ed Hyde Park, Vermont, Antimicrobial Therapy, **2005**, 22–23.
[12] Connolly A, Thorp J, *Urologic clinic of North America*, **1999**, 26, 776-787.
[13] Smaill, F, *Best Practice* & *Research*, **2007,** 21, 439–450.
[14] Barr J, *British journal of obstetrics and gynecology*, **1985**, 92, 506–10.
[15] Foxman B, *American Journal of Public Health*, **1990**, 80, 331-3.
[16] Bauer A, Kirby W, Sherris J, Turk M, *American Medical Journal of Clinical Pathology*, **1966**, 45 493-496.
[17] Sabrina J, Said A, Mabula K, and Samuel Y, *Journal of Health Research*, **2010**, 12, 4.
[18] Assefa A, Asrat D, Woldeamanuel Y, G/Hiwot Y, Abdella A, Melesse T, *Ethiop Medical Journal* **2008**, 46, 227-35.
[19] Masinde A, Gumodoka B, Kilonzo A,& Mshana S, *Tanzania Journal of Health Research*, 2009, 11, 154-159.
[20] Tadesse A, Negash M, Ketema L, *Ethiopian Medical J*ournal, **2007**, 45, 143-9.
[21] Awonuga D, Fawole A, Dada-Adegbola H, Olola F, Awonuga O , *Nigeria Journal of Medical*, **2010** 19:188-93.
[22] Ali Jazayeri, Gholamreza Irajian, *Iranian Journal of Pathology*, **2009**, 4, 105 -108.
[23] Abdullah A and Al-Moslih M, *Eastern Mediterranean health journal*, **2005**, 11, 5 – 6
[24] Turpin C, Bridget M, Danso K, and Frimpong E, *Ghana Medical Journal*, **2007**, 41, 26–29.
[25] Bonadio M, Meini M, Spitaler P, and. Gigli C, *Europian journal of Urology*, **2001**, 40, 439-445.
[26] Hamdan Z, Abdel Haliem M Ziad, Salah K Ali and Ishag Adam, *Annals of Clinical Microbiology and Antimicrobials*, **2011**, 10, 2.
[27] Haider G, Zehra N, Munir AA, Haider A , *Journal of Pakistan Medical Association*, **2010**, 3, 213-6.
[28] Tugrul S, Oral O, Kumru P, Köse D, Alkan A, Yildirim G , *Clinical Exp Obstetric Gynecology*, **2005**, 4, 237-40.
[29] Al-Sibai M, Saha A, Rasheed P, **1989**, 103,113-21.
[30] Sheik M, Khan A., Khatoon and Arain G, *Eastern Mediterranean Health Journal*, **2000**, 6, 265-271.
[31] Seung-Eun Lee, Kyoung-Tae Kim, Yong-Sook Park, Young-Baeg Kim, *Journal of Korean Neurosurgery Soc*, **2010**, 47, 265-270.
[32] Al-Haddad A, *Eastern Mediterranean Health Journal*, **2005**, 11, 505.
[33] Sheffield, J. & Cunningham, F, *Obstetrics* & *Gynecology*, **2005**, 106, 1085–1092.
[34] Moges A, Genetu A, *East African Medical Journal*, **2002**, 79, 140-2.
[35] Asad U and Mohd S, *Biomedical Research*, **2006**, 17, 179-181.
[36] Wolday D, Erge W, *Ethiop Med J* , **1997**, 35, 127–135.
[37] Fantahun B, Bayeh A, *Ethiopian Journal of Health Development*, **2009**, 23, 236-238.