Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

BACTERIAL PROFILE AND ANTIBIOGRAM PATTERN OF UTI IN PREGNANT WOMEN AT TERTIARY CARE TEACHING HOSPITAL

NITHYALAKSHMI J.* AND VIJAYALAKSHMI

Dept. of Microbiology, Sri Muthukumaran Medical College and research institute, Mangadu Chennai, Tamilnadu, INDIA.

ABSTRACT

Urinary Tract Infection is a common medical complication of pregnancy. UTI is of great clinical concern in this group, as it has adverse maternal and obstetric outcomes. The changing pattern of antimicrobial resistance of uropathogens is a growing problem. Knowledge about the current trends of bacterial profile and their antibiogram pattern in pregnant women may help the clinician to choose the appropriate regime.The aims of this study were to assess bacterial profile that causes UTI among pregnant women and to determine the resistance patterns of urinary isolates in our region.Three hundred and three pregnant women with asymptomatic and symptomatic UTI were included in this study from March 2012 to march 2013. Samples were processed and isolates were identified as per the CLSI guidelines. Antibiotic susceptibility was done by kirby bauer disk diffusion method.The overall prevalence of UTI in pregnant women was 14.19%. The age range of the study group was 20 to 42 years. Screening for uropathogens by the gold standard culture technique revealed that 14.77% and 10.25% of the total midstream urine samples had significant bacteriuria in asymptomatic and symptomatic group. The predominant bacterial pathogens were *E. coli* (65.11%) followed by *Klebsiella pneumonia* (11.62%), *Staphylococcus aureus* (11.62%) and *Proteus mirabilis* (4.65%). The gram positive and gram-negative bacteria accounted for 18.6% and 81.39% respectively. The susceptibility pattern of gram negative bacteria showed that most of the isolates (>70% of the isolates) were sensitive to Norfloxacin, Ceftriaxone and Augmentin. Resistance to amoxicillin and cotrimoxazole was significant in all isolates. In this study the gram negative isolates showed a low degree of susceptibility to Gentamicin (22.85%) and Amikacin (25.71%). Among the gram positives, more than 75% of the isolates were sensitive to Ampicillin, Ciprofloxacin, Norfloxacin, Gentamicin, Amikacin , Ceftriaxone and Augmentin.Significant bacteriuria was observed both in symptomatic and asymptomatic pregnant women. Antimicrobial sensitivity pattern of isolated uropathogens showed a changing pattern,so we recommend periodic monitoring of resistance pattern and development of specific guidelines based on local susceptibility patterns, as this would prevent the spread and further development of resistant strains eventually.

KEYWORDS: UTI, pregnancy, E.coli, Asymptomatic bacteriuria, Significant pyuria, Antibiotic resistance.

NITHYALAKSHMI J.
Dept. of Microbiology, Sri Muthukumaran Medical College and research institute, Mangadu Chennai, Tamilnadu, INDIA

INTRODUCTION

Urinary Tract Infections (UTIs) are one of the most common infectious diseases in clinical practice both in community and hospital settings. Due to its frequency and recurrence, UTI poses a real challenge to the medical professionals. Urinary tract infections are more common in women than men, nearly 10% of women will experience a UTI during their lifetime [1]. Higher incidence of urinary tract infection in women can be attributed to factors like shortness of female urethra, absence of prostatic secretions, easy contamination of urinary tract with fecal flora and pregnancy [2]. Pregnancy has complex physiological and morphological effects. This includes ureteral dilatation, decreased urine concentration and urethral tone leading to urinary stasis, which is the single most important factor that can initiate the proliferation of microorganisms All these factors increases the susceptibility of pregnant women to UTI [3].UTI has been reported among 20% of the pregnant women. UTI in pregnancy contributes significantly to maternal and perinatal morbidity and mortality. These infections may manifest as asymptomatic or symptomatic bacteriuria, accounting for 17.9% and 13.0% among pregnant women, respectively [4].Pregnancy can enhance the progression of asymptomatic to symptomatic bacteriuria, which could lead to cystitis [40%] and pyelonephritis [20-30%]. Pyelonephritis in pregnancy has been attributed to be associated with adverse obstetric outcomes like prematurity, low birth weight and high fetal mortality rates. Other maternal complications include maternal anemia, hypertension, pre-eclampsia, chronic pyelonephritis, and occasionally, renal failure [5].Organisms that cause UTI are those from the normal vaginal, perineal, and fecal flora. They include *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Klebsiella species,* and *Streptococcus species* amongst others. *E.Coli,* the predominant isolate in various reports accounts for 80% - 90% infections in pregnancy[6] However, recent studies in Karnataka [7] and Nigeria [8] show an increasing involvement of *Klebsiella* Spp. in urinary tract infection in pregnancy. Thus, the microbial profile of UTI has never been constant; keep changing with variations in the geographical location, so it is always better to know the organisms responsible for UTI in our settings to guide the empirical treatment.Antimicrobial agents including selective ß-Lactams, Nitrofurantoin, Quinolones And Co-Trimoxazole can be considered during pregnancy [9]. However, resistance of urinary tract pathogens has been known to increase worldwide, especially to commonly used antimicrobials and antibiogram pattern of isolates may vary over short periods and depend on environmental conditions [10].In developing countries, especially in low resource settings to prevent or reduce maternal and fetal morbidities, antibiotics are usually given empirically before the urine culture reports are available. To ensure appropriate therapy, current knowledge of the organism that causes the UTIs and their antibiogram pattern is mandatory. The aim of this study is to therefore determine the prevalence of UTI in pregnancy and the common causative microbes, as well as their antibiotic sensitivity patterns.

MATERIALS AND METHODS

Consecutive 303 pregnant women attending antenatal clinic with or without clinical symptoms of UTI during the study period Mar 2012 –MAR 2013 was included in this study. A proper instruction regarding aseptic collection of urine samples was briefed to all the subjects. Early morning clean-catch midstream urine was collected from each pregnant woman into a leak proof wide-mouthed sterile screw- capped container.Initially samples were examined microscopically. 10 ml of the urine sample was poured into a test tube and centrifuged at 2500rpm for 5 minutes. The supernatant was discarded while a drop of the deposit was placed on a grease free slide and covered with a cover slip. It was then examined under the microscope at 40X magnification. The urine specimen was also examined for pus cells. Pus cells >5/HPF were considered significant for infection [11].

The urine samples were also immediately cultured on Nutrient and Macconkey agar. All samples were processed within one hour of collection, delayed samples were refrigerated at 4ºc and processed within 4 - 6 hours.Using a sterile standard wire loop that delivers 0.001 ml of urine, a loopful of urine sample was streaked on to a Nutrient and Macconkey agar to obtain discrete colonies. The plates were examined after overnight incubation aerobically at 37°C. Identification of bacterial pathogens was made on the basis of Gram reaction, morphology and biochemical characteristics. Only colony count, yielding a bacterial growth of 10^5 cfu/ml or more was taken as significant in both symptomatic and asymptomatic pregnant women. The culture isolates were identified by standard microbiological methods. All culture media were procured from HiMedia Laboratories, Mumbai, India.[12]The standardized Kirby-Bauer disc diffusion test of the Clinical and Laboratory Standards Institute (formerly NCCLS) was used for antibiotic susceptibility testing were carried out. The panel of antibiotics tested against gram positive and gram negative isolates were Ampicillin, Amoxicillin, Amikacin, Norfloxacin, Ciprofloxacin, Nitrofurantion, Gentamicin, Ceftriaxone and Augmentin, and results were interpreted according to CLSI guidelines[13]

RESULTS

Table 1
Prevalence of uti among pregnant women

Total no. Of urine samples Screened	No. Of samples showed significant growth in culture	%age of UTI prevalence in pregnant women
303	43	14.19

A total of 303 Samples received from ANC during Dec 2011 and Dec 2012 were screened in this study .Out of 303 samples , significant growth was observed in 43,remaining samples either had no growth(251) or insignificant growth(9). almost in all the positive culture, single type of organism had grown. The overall prevalence of UTI was 14.19%[TABLE 1].

Table 2
Prevalence of UTI among pregnant women with reference to clinical symptoms

Total cases	No. Of cases screened	Culture Positive	Prevalence %
Asymptomatic cases	264	39	14.77
Symptomatic cases	39	4	10.25

Among the 39 pregnant women who presented with the clinical symptoms, mid stream urine samples of 4(10.25%) were found to be positive for culture. Similarly, out of 264 asymptomatic cases, significant bacteriuria was in 39(14.77%).

Table 3
Age distribution among pregnant women with UTI

Age group	No of cases positive for culture	Prevalence of uti (n=43) %
20-24	14	32.5
25-29	19	44.2
30-34	8	18.6
>35	2	4.65

The prevalence of UTI was more in the age group of 25 -29 yrs[44.18%], followed by 20 -24yrs [32.5%] and 30 -34[18.6%]. Least prevalence observed in the age group above >35 yrs [4.65%].

Table 4
Comparison of wet mount microscopy and culture of urine samples

Pus cells >5cells/hpf	Culture		Total
	Positive	Negative	
Present	41	32	73
Absent	2	228	230

Wet mount examination of 303 samples revealed significant pyuria in 73 ,but in culture only 43 isolates were grown significantly [TABLE 2]. Pus cells were found in 95.34% (41/43) of culture positive cases whereas among the culture negative specimens 12.3% (32/260) were positive for pus cells. Specificity 87.69% and Sensitivity 95.34%.

Table 5
Frequency of gram positive and gram-negative uropathogens isolated

	Gram positive uropathogens	Gram negative uropathogens
Total no of isolates	8	35
%age of isolates	18.6%	81.39%

Among 43 isolates obtained from 303 samples, majority of isolates were gram negative bacteria [81.39%]. Gram-positive organisms were responsible only for 18.6% shown in the table 5.

Table 6
Distribution of isolated bacterial uropathogens

Isolated and identified organism	No of isolates (n=43)	% of UTI isolates
E.coli	28	65.11%
Klebsiella pneumonia	5	11.62%
Proteus mirabilis	2	4.65%
Staphylococcus aureus	5	11.62%
CONS	3	6.97%

Spectrum of five different uropathogens was identified. E.coli(65.11%) was the most prevalent uropathogen isolated followed by Klebsiella pneumonia and staphylococcus aureus, accounted 11.62% for each. The least prevalent bacteria isolated was Proteus mirabilis(4.65%)

Table 7
Antibiogram of GNB isolated from UTI

Antibiotics Tested	E.coli (n=28) S(%)	R(%)	K.pneumoniae (n=5) S(%)	R(%)	P.mirabilis (n=2) S(%)	R(%)	Total Resistance %
Ampicillin	12 (42.8)	16 (57.14)	2 (40)	3 (60)	1 (50)	1 (50)	57.1
Amoxicillin	24 (85.7)	4 (14.28)	1 (20)	4 (80)	0	2 (100)	28.6
Augmentin	27 (96.4)	1 (3.57)	4 (80)	1 (20)	2 (100)	0	5.7
Nitrofurantoin	19 (67.8)	9 (32.14)	4 (80)	1 (20)	0	2 (100)	34.3
Ciprofloxacin	21 (75)	7 (25)	3 (60)	2 (40)	1 (50)	1 (50)	28.6
Norfloxacin	26 (92.8)	2 (7.14)	4 (80)	1 (20)	2 (100)	0	8.6
Cotrimoxazole	7 (25)	21 (75)	2 (40)	3 (60)	0	2 (100)	74.3
Gentamicin	2 (7.14)	26 (92.85)	4 (80)	1 (20)	2 (100)	0	77.1
Amikacin	6 (21.4)	22 (78.57)	2 (40)	3 (60)	1 (50)	1 (50)	74.3
Ceftriaxone	25 (89.2)	3 (10.71)	4 (80)	1 (20)	2 (100)	0	11.4

Out Of 28 E.coli isolates, 27(96.42%) are sensitive to Augmentin, 26(92.85%) to Norfloxacin, 25 (89.28%) to Ceftriaxone, 24(85.71%) to Amoxicillin and 21(75%) to Ciprofloxacin. E.coli, the predominant pathogen showed highest percentage of resistance to Gentamicin(92.85%), Amikacin(78.57%), and Cotrimoxazole(75%). The sensitive antibiotics to klebsiella pneumonia isolates are Augmentin(80%), Nitrofurantoin(80%), Norfloxacin(80%), Gentamicin(80%) andCeftriaxone(80%).

In contrast to E.coli, 80% of the isolates had shown resistance to Amoxicillin.

Table 8
Antibiogram of gram positive bacteria isolated from urine culture of pregnant women

Antibiotics Tested	S.aureus (n =5)		CONS (n=3)		Total Resistance %
	S (%)	R (%)	S (%)	R(%)	
Ampicillin	4(80)	1(20)	2(66.6)	1(33.3)	25
Amoxicillin	3(60)	2(40)	2(66.6)	1(33.3)	37.5
Augmentin	4(80)	1(20)	3(100)	0	12.5
Nitrofurantoin	2(40)	3(60)	2(66.6)	1(33.3)	50
Ciprofloxacin	4(80)	1(20)	2(66.6)	1(33.3)	25
Norfloxacin	5(100)	0(0)	3(100)	0	0
Cotrimoxazole	0(0)	5(100)	2(66.6)	1(33.3)	75
Gentamicin	4(80)	1(20)	3(100)	0	12.5
Amikacin	5(100)	0(0)	3(100)	0	0
Ceftriaxone	5(100)	0(0)	3(100)	0	0

As indicated on table 8, the rate of susceptibility of gram-positive range from 20% - 100%. Among the gram positives, 7(87.5%) and 5(62.5%) of the isolates were sensitive to Augmentin and Amoxicillin. Norfloxacin, Amikacin and Ceftriaxone were found to be effective against all the 8(100%) isolates.

DISCUSSION

This study provides valuable laboratory data to know the prevalence of UTI among antenatal women, to determine the trends in the antibiogram pattern of uropathogens in our region and to suggest appropriate treatment for empirical treatment. Another important aspect of this study is, it also allows comparison of the situation in tamilnadu state with other regions within and outside India, as very limited data are available. The overall prevalence of UTI among pregnant women is 14.9%. This is similar to the global prevalence of UTI reported by Masinde et al.., (14.6%) [1] and Olsen et al.., (16.4%) [14] from Tanzania. A study in Nigeria (12.4%),karnataka (10.4%) and Tamilnadu (16.3%) also reported similar findings [15].However, the finding of this study is lesser than the high prevalence rate of 49.4% reported from Karnataka.[2] Geographical location and varied distribution of microorganisms may be the reason for this wide difference in prevalence.In this study pus cells were found in 95% of culture positive and 12.3% of culture negative cases. This shows that considering pus cells alone as an indicator of UTI, would expose 12.3% of false positive cases to unnecessary anibiotics. 5% of infected cases were negative for pus cells, thus this study found to have a low false negative. It may, therefore, be unreliable in patients with low pyuria [17].The sensitivity of direct microscopy for pus cells is 95% and specificity was 88%.To confirm the diagnosis culture is therefore essential. The prevalence of symptomatic and asymptomatic bacteriuria was observed to be 10.25% and 14.77%, respectively. This is in accordance with the findings reported by jeyaselan *et a*l,chiteralekha *et al* [18],[19].In our study, the highest percentage of infection (44.18%) was seen in the pregnant women of age group 26-29 . Our results regarding the age of infection concur with various reports across our country.[3,6,20].A possible explanation could be,a pregnant woman of this age group are likely to be multiparous and it has been well recognized that multiparity is a risk factor for significant bacteriuria in pregnancy. Another reason would be due to the aging process which is associated with decreased glycogen deposition and reduced lactobacilli, the bacterial adherence and invasion is enhanced[18].81.39% of isolates were Gram negative bacteria belonging to *Enterobacteriaceae* followed by gram positive organisms which accounted for 18.61%. This finding is similar to other reports, which suggest GNB are the predominant isolates[10].Spectrum of isolates have been changing from time to time, from place to place. In our study, *E. coli* strains were the most common isolate followed *by Klebsiella sp, Proteus sp, Staphylococcus aureus and CONS*. Various studies from different places around the world showed that E.coli strains are still commonest pathogens

in UTI [2,19,21]. This pattern could be due to the fact that urinary stasis is common in pregnancy and since most Escherichia coli strains prefer that environment, they cause UTI. Another reason could be due to their ability to colonize the urogenital mucosa readily with adhesin, pili, fimbriae and P-1 blood group phenotype receptor.Although recent reports have shown that organisms such as *Staphylococcus spp, Klebiella* spp and *Proteus spp* are overtaking the position of *Escherichia sp* in UTI.In our study *Staphylococcus spp* was noticed only in 11.62% of bacteriuric pregnant women which is in agreement with earlier studies[22].Antimicrobial resistance among uropathogens to the commonly used antibiotics have been increasing, this scenario leaves very limited therapeutic options for treatment. In this study susceptibility pattern of gram negative isolates showed that most of the isolates were sensitive to Augmentin(94.28%), Ceftriaxone(88.57%)andNorfloxacin(91.42%). we observed increasing resistant pattern for Gram negative isolates to Cotrimoxazole (74.28%), Amikacin (74.28%) and Gentamicin (77.14%). In contrary, a similar study by Vercaigne et al [23] reported high sensitivity of 80% to Cotrimoxazole and 90% to Amikacin and Gentamicin by Alemu et al[10]. The resistant pattern for other commonly used antibiotics were Ampicillin [57.14%], Amoxicillin [31.2%], Nitrofurantoin [34.28%] and ciprofloxacin[28.57%]. Surprisingly, these are the com- monly available and cheap drugs. This trend of resistance might be due to improper and indiscriminate use of antibiotics as these drugs are easily available over the counter [24].Among the gram positive bacteria tested, all isolates have retained susceptibility of 100% to Norfloxacin, Amikacin and Ceftriaxone. It is interesting to note that even among gram positive isolates, only 25% of the total were sensitive to the most commonly prescribed drug co-trimoxazole.

CONCLUSION

The present study showed high rate of prevalence of UTI among pregnant women. We observed that use of Ampicillin, Amoxicillin and Cotrimoxazole, which were used as a single agent for empirical treatment in pregnant women, could no longer be effective in our settings. Most of the bacterial isolates were sensitive to Norfloxacin, Ceftriaxone and Augmentin. We recommend periodic monitoring of resistance pattern and development of specific guidelines based on local susceptibility patterns, as this would prevent the spread and further development of resistant strains eventually.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. A. Masinde[1*], B. Gumodoka[1], A. Kilonzo[1] and S.E. Mshana[2]. Prevalence of urinary tract infection among pregnant women at Bugando Medical Centre, Mwanza, Tanzania Journal of Health Research, Vol. 11, No. 3, July, 2009, pp. 154-161
2. Manjula N. G.1, Girish C. Math.1, Shripad A. Patil2, Incidence of Urinary Tract Infections and Its Aetiological Agents among Pregnant Women in Karnataka Region. *Advances in Microbiology*, 2013, 3, 473-478.
3. Paul Erhunmwunse Imade, Patience Emiolu Izekor, Nosakhare Odeh Eghafona, Asymptomatic bacteriuria among pregnant women, N Am J Med Sci. Jun 2010; 2(6): 263-266.
4. Dr. Kawser Parveen, Dr.Afroza Momen, Dr. Arzumath Ara Begum, Dr. Monowara Begum. Prevalence Of Urinary Tract Infection During Pregnancy. National Med. Coll. Hos. 2011; 17 (02): 8-12
5. Asymptomatic bacteriuria and obstetric outcome following treatment in early versus late pregnancy in north indian women.
6. Fatima N, Ishrat S. Frequency and risk factors of asymptomatic bacteriuria during pregnancy. J Coll Physicians Surg Pak 2006;16:273-5.
7. Nawaz Umar*, Syeda Nazla KUlSUm

and IlyaKhaT all spectrum of Urinary Tract Infections in Pregnant Women *Biomedical & Pharmacology Journal* Vol. **6**(2), 349-353 (2013)

8. Rc Onoh1 OUJ Umeora1 Ve egwuatu2 Antibiotic sensitivity pattern of uropathogens from pregnant women with urinary tract infection in abakaliki, Nigeria. Infections and drug resistance 2013 ;vol 6;225-233.
9. Colgan R, Nicolle LE, McGlone A, Hooton TM. Asymptomatic bacteriuria in adults. Am Fam Physician 2006; 74:985-90.
10. Agersew Alemu, Feleke Moges, Yitayal Shiferaw. Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at University of Gondar Teaching Hospital, Northwest Ethiopia, BMC Res Notes, 2012; 5: 197
11. Kass EH. Bacteriuria and the diagnosis of infections of the urinary tract; with observations on the use of methionine as a urinary antiseptic. AMA Arch Intern Med 1957;100:709-14.
12. Collee JG, Miles RS, Watt B. Tests for identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14 th ed. Singapore: Churchill Livingstone; 2006. p. 131 -49.
13. Clinical Laboratories Standards Institute. Performance of standards for antimicrobial disk susceptibility tests; approved standards. 10th ed. M02-A10. Vol.29 Wayne, PA: CLSI; 2009.
14. Olsen, B.E., Hinderaker, S.G., Lie, R.T., Gasheka, P., Baerheim, A., Bergsjo, P. & Kvale, G. (2000) The diagnosis of urinary tract infections among pregnant women in rural Tanzania; Prevalences and correspondence between different diagnostic methods.*Acta Obstetrica Gynecology Scandinavia* 79, 729–736.
15. (Onyemelukwe, NF, Obi SN, Ozumba BC. Significant Bacteriuria in pregnancy in Enuhun, Nigeria. Journal of College of Medicine.2003; 8 (2): 20 – 22.
16. EMahesh, D. Ramesh, V. A. Indumathi *et al*., "Compli- cated Urinary Tract Infection in a Tertiary Care Center in South India," *Al Ameen Journal of Medical Science*, Vol. 3, No. 2, 2010, pp. 120-127.
17. Eigbefoh, J. O., Isabu, P., Okpere, E. & Abebe, J. (2008) The diagnostic accuracy of the rapid dipstick test to predict asymptomatic urinary tract infection of pregnancy. *Journal of Obstetrics and Gynaecology* 28, 490 — 495
18. T. Jayaseelan Senthinath, P. Chitra Rajalakshmi, R. Keerthana, R.S. Vigneshwari, P. Revathi, N. Prabhu, A.R. Susethira, International Journal of Current Microbiology and Applied Sciences, ISSN: 2319-7706 Vol.2 No.1, 2013: 80-85.
19. Chitralekha S, Lakshmipriya R, Illamani V, Kiran M, Menezes GA. Prevalence of aerobic bacterial organism causing asymptomatic bacteriuria during second trimester pregnancy. J Pharm Biomed Sci 2013;26:273 -277
20. Girishbabu R.J., R. Srikrishna, Ramesh, S.T. 2011. Asymptomatic bacteriuria in pregnancy, Int. J. Biol. Med. Res. 2(3): 740-742.
21. R Marahatta, B Acharya Dhungel, P Pradhan, SK Rai, D Roy Choudhury, Asymptomatic bacteriurea among pregnant women visiting Nepal Medical College Teaching Hospital, Kathmandu, Nepal, Nepal Med Coll J 2011; 13(2): 107-110.
22. Bolaji, R.O1*; Onaolapo, J.A1, Ibrahim, Y.K.E1 And Igwe, J.C incidence of uropathogens from asymptomatic bacteriuric pregnant women in zaria, nigeria nig. journ. pharm. sci., march 2013, vol. 12
23. vercaine ,Zhanel Recommended treatment for urinary tract infection in pregnancy.Ann Pharmacother.1994.Feb 28 (2)
24. Stapleton AE. Approach to the patient with urinary tract infection. In: Gorbach SL, Bartlett JG, Blacklow NR, editors. Infectious Diseases. 2nd ed. Philadelphia (USA): WB Saunders Company; 1998. P.943-954.