

Bacteriological profile and antibiogram of uropathogens among antenatal cases in a Tertiary Care Hospital

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Abstract

Introduction: Urinary tract infection (UTI) is one of the commonest complications occurring during the antenatal period. It can result in adverse outcomes like abortion, premature delivery, low birth weight. Screening of antenatal patients help in early diagnosis of UTI and thus to prevent maternal and fetal morbidity and mortality.

Aims: The present study was carried out:

1. To determine the prevalence of UTI in pregnant women.
2. To study the bacteriological profile and antimicrobial susceptibility pattern of the uropathogens.

Materials and Method: 269 antenatal patients who attended the antenatal clinic during the study period January 2015 – December 2016 were included in this study. Mid stream urine sample was collected from these patients. Samples were processed and isolates were identified. Antibiotic susceptibility was recorded as per CLSI guidelines.

Results: The prevalence of UTI among pregnant women was 16.9%. Majority of the culture positive patients belonged to the age group of 25-29 yrs. 91.3% were Gram negative isolates and 8.7% were Gram positive organisms. E.coli was the most common uropathogen. Ampicillin, amoxicillin clavulanic acid, cotrimoxazole were found to be resistant to most of the Gram negative uropathogens. Most of the Gram negative isolates were sensitive to amikacin, nitrofurantoin, ceftriaxone, quinolones. For Gram positive uropathogens, ampicillin can still be safely prescribed as first line antibiotic.

Conclusion: This study reveals the importance of screening of pregnant women for UTI. Increasing resistance observed among the uropathogens emphasizes the need to rationalize the use of antibiotics which would eventually prevent the development of resistant strains.

Keywords: Urinary tract infections, Pregnancy, Uropathogens, Antibiotic resistance

Introduction

Urinary Tract Infections (UTIs) are one of the most common infectious diseases that we encounter in hospital settings. UTI in pregnancy can result in serious life threatening complications if left untreated. 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⁾ In pregnancy it may be symptomatic in the form of urethritis, cystitis, or pyelonephritis or it may remain asymptomatic.⁽³⁾ Some of the risk factors include increased age, high parity, poor perineal hygiene, history of recurrent UTI, diabetes mellitus, neurogenic bladder retention, anatomic or functional urinary tract abnormality, and increased frequency of sexual activity.^(5,6) Predisposing determinants of high prevalence of UTI in pregnancy include hormone induced ureteral dilatation, urinary stasis, reduced immune function, and presence of vesicoureteric reflux.^(4,5,6) Maternal complication includes overt pyelonephritis in 25%–40% of patients as pregnancy advances among those with asymptomatic bacteriuria, and in 1%–2% in those without asymptomatic bacteriuria.⁽⁷⁾ Other maternal

complications include maternal anemia, hypertension, pre-eclampsia, chronic pyelonephritis, and occasionally, renal failure.^(8,9) The fetus is at risk of prematurity, low birth weight, intrauterine growth restriction, and fetal death.^(9,10)

It is universally accepted that UTI can only be ascertained on the basis of microscopy and microbial culture.^(11,12) The dipstick/dip-slide method used in many centres serves only as a screening method but culture is needed for the final diagnosis.⁽¹³⁾ This study was done to know about the uropathogens prevalent in our hospital setting and to know about the sensitivity and resistance pattern of the causative organisms of UTI. Such studies would further help in laying down antibiotic policies, so as to prevent development of multidrug resistant pathogens.

Materials and Method

269 pregnant women attending antenatal clinic with or without clinical symptoms of UTI during the study period January 2015 – December 2016 were included in this study. This was an analysis of data generated from the records of urine samples received in the laboratory during study period. Clean-catch midstream urine (MSU) samples received in leak proof wide-mouthed sterile screw-capped container from the participants. Initially samples were examined

microscopically. Culture results were interpreted as significant and insignificant, based on standard Kass criteria. Significant bacteriuria is defined as a urine sample containing more than 10^5 colonies/ml of urine in pure culture using a standard calibrated bacteriological loop.⁽¹⁴⁾ Cultures with more than three types of colonies were discarded as contaminants.

All samples were inoculated onto blood agar, nutrient agar and Mac conkey agar plates using a calibrated loop [0.001ml] and were incubated overnight at 37°C and examined next day. Colony counts yielding bacterial growth of 10^5 CFU/ml were considered significant as per recommendations.

Bacterial pathogens were identified by gram reactions, motility and biochemical characteristics as per standard microbiological techniques. The antibiotic susceptibility pattern of the isolates was determined by the Kirby-Bauer disk diffusion method as per Clinical Laboratory Standards Institute (CLSI) guidelines.

Gram negative bacilli (GNB) were tested against Ampicillin (AMP) (10 µg), Amoxicillin clavulanic acid (AMC) (30µg), Cefazolin (CZ) (30µg), Ceftriaxone (CTR) (30µg), Cefepime (CPM) (30µg), Amikacin(AK) (30µg), Co- trimoxazole (COT) (1.25/23.75µg), Nitrofurantoin (300µg), Ciprofloxacin (CIP) (5µg), Norfloxacin (10µg), Piperacillin tazobactam (PIT) (100/10µg), Imipenem (IPM) (10µg).

For Gram positive organisms, Ampicillin (AMP) (10µg), Amoxicillin clavulanic acid (AMC) (30µg), Ciprofloxacin (CIP) (5µg), Norfloxacin (10µg), Erythromycin (E) (15µg), High Level Gentamicin (120µg), Cefoxitin (30µg) Cloxacillin (CLOX) (30µg), Tetracycline (TET) (30µg), Linezolid (LZ) (15µg), Vancomycin (30 µg), Teicoplanin (30 µg) were used.

Second line antibiotics were tested only for organisms resistant to all 1st line antimicrobials. These included Imipenem, Cefepime, Ofloxacin and Piperacillin-Tazobactam for Gram negative organisms and Vancomycin, Linezolid for Gram positive organisms.

For quality control of the Gram positive and Gram negative panel of antibiotics, the discs were tested with ATCC Staphylococcus aureus 25923 and ATCC E.coli 25922 respectively. Quality control was said to have been passed, when the zone sizes were as per the CLSI criteria.⁽¹⁵⁾

The detection of Extended Spectrum Beta-Lactamase (ESBL) production in GNB and Methicillin resistance in Staphylococcus was carried out using Cefoxitin (30 µg) disc according to CLSI guidelines.⁽¹⁵⁾ Test for ESBL production was performed on Muller-Hinton agar via the disc diffusion method using third generation Cephalosporin, Ceftazidime (30 µg) and Cefotaxime (30µg) discs alone and in combination with clavulanic acid. The organism was considered ESBL producing if the zone diameter was ≥ 5 mm for either antimicrobial tested in combination with clavulanic acid

versus its zone diameter when tested without clavulanic acid.

Results

A total of 272 MSU samples from pregnant women received during January 2015 to December 2016 were included in this study. The prevalence of UTI among pregnant women is shown in Table 1. Out of the 272 samples, 46 (16.9%) showed significant growth and the remaining 226 (83.1%) samples showed insignificant growth. None of the samples yielded more than one isolate.

Table 1: UTI prevalence among pregnant women

Total no of urine samples screened	No. of samples showing significant growth	% of UTI prevalence in pregnant women
272	46	16.9%

Out of the 46 culture positive pregnant women, 18 (39.1%) were primi gravida and 28 (60.9%) were multi gravid and based on the trimester, 32(69.6%), 8(17.4%) and 6(13%) were in first, second, third trimester respectively.

Majority of the culture positive patients belonged to the age group of 25-29 yrs. 34.78% were in the age group 20-24yrs, 4.35% were in the age group 30-34yrs and 10.87% were in the age group >35yrs. (Table 2)

Table 2: Age distribution among pregnant women with UTI

Age group	No of cases positive for culture	Prevalence of UTI in% (n = 46)
20-24	16	34.78
25-29	23	50
30-34	2	4.35
>35	5	10.87

Among the total 272 antenatal patients included in the study, 210 patients were asymptomatic and the remaining 62 patients were symptomatic. Of the 210 asymptomatic patients, 40 (19.05%) were found to be culture positive and of the 62 symptomatic patients, 6 (9.68%) were found to be culture positive. (Table 3)

Table 3: Prevalence of UTI among pregnant women with reference to clinical symptoms

	Total no of cases	Culture positives	Percentage
Asymptomatic cases	210	40	19.05%
Symptomatic cases	62	6	9.68%

Of the total 46 culture positives, the total number of Gram negative organisms isolated were 42 (91.3%) and Gram positive organisms were 4 (8.7%).

E.coli (32.6%) was the most common uropathogen isolated, followed by Acinetobacter sp (28.3%), Klebsiella pneumoniae (21.7%), Enterobacter sp (4.4%), Pseudomonas aeruginosa (4.4%), Enterococcus sp (4.4%), Staphylococcus saprophyticus (2.3%) and Streptococcus sp (2.3%).

Table 4: Uropathogens isolated from pregnant women

Organism	No. of isolates (n =46)	Percentage (%)
E.coli	15	32.6
K.pneumoniae	10	21.7
Enterobacter sp	2	4.4
Pseudomonas aeruginosa	2	4.4

Acinetobacter sp	13	28.3
Staphylococcus saprophyticus	1	2.3
Streptococcus sp	1	2.3
Enterococcus sp	2	4.4

The resistance profile of Gram negative and Gram positive organisms is shown in Table 5 and 6 respectively. Gram negative organisms showed a high resistance to ampicillin (95.2%), amoxycylav (81%). Most of the Gram negative isolates were found to be sensitive to Amikacin. Of the 42 Gram negative organisms isolated, 3 were found to be ESBL producers (1 E.coli, 1 Klebsiella pneumonia and 1 Acinetobacter sp).

Most of the Gram positive organisms were susceptible to the antibiotics tested. One isolate of Staphylococcus saprophyticus isolated was found to be oxacillin resistant. None of the Gram positive isolates were vancomycin resistant.

Table 5: Resistance profile of Gram negative uropathogens

Organism	No.(%) resistant to											
	AMP	AMC	CZ	CTX	CPM	AK	COT	NIT	CIP	NOR	PIT	IPM
E.coli (n = 15)	13 (86.7)	13 (86.7)	10 (66.7)	6 (40)	4 (26.7)	2 (13.3)	7 (46.7)	2 (13.3)	3 (20)	2 (13.3)	1 (6.7)	5 (33.3)
K.pneumoniae (n = 10)	10 (100)	5 (50)	6 (60)	4 (40)	1 (10)	0 (0)	1 (10)	3 (30)	2 (20)	1 (10)	1 (10)	0 (0)
Enterobacter sp (n = 2)	2 (100)	1 (50)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
P.aeruginosa (n = 2)	2 (100)	2 (100)	1 (50)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Acinetobacter sp (n = 13)	13 (100)	13 (100)	13 (100)	8 (61.5)	7 (53.8)	3 (23.1)	13 (100)	13 (100)	9 (69.2)	7 (53.8)	8 (61.5)	2 (15.4)
Total (42)	40 (95.2)	34 (81)	32 (76.2)	19 (45.2)	13 (31)	5 (12)	23 (54.8)	18 (42.9)	14 (33.3)	10 (23.8)	10 (23.8)	7 (16.7)

Table 6: Resistance profile of Gram positive uropathogens

Organism	No.(%) resistant to										
	AMP	AMC	CIP	NOR	E	HLG	CLOX	LZ	TET	TEI	VA
Staphylococcus saprophyticus (n = 1)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Streptococcus sp (n = 1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
Enterococcus sp (n = 2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
Total (4)	1 (25)	1 (25)	1 (25)	1 (25)	0 (0)	0 (0)	1 (25)	0 (0)	2 (50)	0 (0)	0 (0)

Discussion

The antimicrobial susceptibility pattern keeps changing over years. Hence it is essential to update our antibiotic policies in order to meet with the current resistant strains. This study gives us valuable data regarding the prevalence of UTI among antenatal patients and also their antibiogram pattern. This data regarding the predominant pathogens in a hospital setting will be very useful for choosing the appropriate antibiotic for empirical treatment of pregnant women with UTI.

In our study the prevalence of UTI among pregnant women is 16.9%. This is almost similar to the global prevalence of UTI reported by Masinde et al, (14.6%)⁽¹⁶⁾ and Olsen et al, (16.4%)⁽¹⁷⁾ from Tanzania. A study in Nigeria (12.4%), Karnataka (10.4%) and Tamilnadu (16.3%) also reported similar findings.⁽¹⁸⁾

In our study, the prevalence of asymptomatic and symptomatic bacteriuria was observed to be 19.05% and 9.68% respectively which is in accordance with the findings. This was slightly higher than the prevalence (17.77% and 10.25% respectively) reported by T. Jeyaselan et al.⁽¹⁹⁾ and Balamurgan et al.⁽²⁰⁾

Our study also showed that the infection rate was high in the age group 25-29 yrs (50%), followed by 20 – 24yrs (34.78%), > 35yrs (10.87%) and 30-34yrs (4.35%). The high prevalence of UTI in the age group of 25-29 yrs was probably due to decreased glycogen deposition, reduced lactobacilli related to aging, bacterial adherence and invasion.⁽¹⁹⁾ The high prevalence of UTI among pregnant women of >35yrs than that compared to 30-34yrs was probably because there were more number of multiparous women belonged to this age group.

In our study, 91.3% of the organisms isolated were Gram negative bacilli (GNB) and 8.7% were Gram positive cocci (GPC). This data is similar to other report which suggest GNB are predominant isolates.⁽²¹⁾

The percentage of uropathogens isolated are listed in the table 4. Of the 46 culture positives, E.coli (32.6%) was the most common organism. This was followed by Acinetobacter (28.3%), K.pneumoniae (21.7%). The frequency of the remaining isolates were: Enterobacter sp (4.4%), P.aeruginosa (4.4%), Enetrococcus sp (4.4%), Streptococcus sp (2.3%), Staphylococcus saprophyticus (2.3%).

Table 5 and table 6 shows the overall resistance profile of Gram negative and Gram positive bacteria isolated from antenatal patients. 86.7% of isolates of E.coli were found to be resistant to ampicillin, followed by amoxicillin-clavulanic acid (86.7%) and cefazolin (66.7%). Resistance was low (13.3%) for amikacin, nitrofurantoin and norfloxacin. The next predominant organism was Acinetobacter sp. Most of the first line drugs were found to be resistant. However resistance was low for amikacin (23.1%) and imipenem (15.4%). The next common pathogen was K.pneumoniae. None of the isolates were resistant to amikacin. Low level of resistance was noted for Cotrimoxazole (10%), Norfloxacin (10%) and Nitrofurantoin (30%).

Of the total 42 Gram negative isolates, 8 isolates were found to be ESBL producers by phenotypic methods. This included 5 isolates of E.coli, 2 isolates of K.pneumoniae and 1 isolate of Acinetobacter sp.

Over all, among the Gram negative uropathogens, high resistance pattern was noted for ampicillin, amoxicillin clavulanic acid, cefazolin. This might be due to the indiscriminate use of these antibiotics. In this study the most useful antibiotic for Gram negative uropathogens were amikacin, nitrofurantoin, quinolones, ceftriaxone.

Among the Gram positive organisms, Staphylococcus saprophyticus was found to be oxacillin resistant. Over all, among the GPC, resistance was

noted for tetracycline. Most of the isolates were sensitive to ampicillin, amoxicillin clavulanic acid, quinolones. Sensitivity to ampicillin is encouraging as it can be safely prescribed during pregnancy as first line antibiotic.

The antibiotic with overall high sensitivity was amikacin and norfloxacin. Norfloxacin was found to be sensitive for both Gram positive and Gram negative organisms. This is similar to other reports where quinolones were the most effective and sensitive antibiotics to the organisms causing UTI.^(22,23) All quinolones used in this study had good antibiotic sensitivity pattern. Quinolones have been associated with teratogenicity in first trimester and risk of auditory and vestibular toxicity in the fetus in later trimesters, and therefore contraindicated in pregnancy. However for recurrence and persistent UTI, quinolones could be used with caution in late pregnancy or postpartum after counselling, especially if it is the only sensitive drug, as it is also secreted in breast milk.⁽²⁴⁾

In this study, third generation cephalosporins had a relatively good sensitivity pattern. 54.8% of the Gram negative isolates were sensitive to ceftriaxone. Cephalosporins, although expensive are safe during pregnancy. Currently, most cephalosporins have both oral and parenteral combinations and have been noted to be the first line drug for pyelonephritis and the most commonly used antimicrobials for symptomatic UTI in hospital settings.^(25,26)

Conclusion

The present study showed the prevalence of UTI among pregnant women was 16.9%. Significant bacteriuria was observed even among asymptomatic women in this study. The commonest uropathogen was E.coli. It was also observed that drugs like ampicillin, amoxicillin clavulanic acid, cotrimoxazole, which were most often used for empirical treatment in pregnant women, could no longer be effective in our hospital setting for Gram negative uropathogens. Over all, most of the Gram negative isolates were sensitive to amikacin, nitrofurantoin, ceftriaxone, quinolones. where as for Gram positive uropathogens ampicillin can still be safely prescribed as first line antibiotic. This emphasizes the need for screening of antenatal patients for UTI. Urine for bacterial culture is still the gold standard method for confirming UTI. Hence it is recommended that pregnant women should undergo periodic screening for UTI, so as to monitor the resistance pattern of the uropathogens and for the development of specific antibiotic policies based on local susceptibility patterns.

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