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Symptomatic and Asymptomatic Urinary Tract Infection by *Escherichia coli* Among Pregnant Women Attending Out Patient Clinic of Obstetrics and Gynecology

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The present study was carried out to determine the prevalence of symptomatic and asymptomatic UTI among pregnant women, to evaluate the diagnostic efficacy of screening tests among pregnant women and to document antibiotic sensitivity pattern of predominant urinary pathogen, *Escherichia coli*. The study population consisted of consecutive 656 pregnant women attending out patient department of Obstetrics and Gynecology at Navodaya Medical College Hospital and Research Laboratory, Raichur. Clean -catch mid stream urine samples collected in sterile disposable container were screened for bacteriuria and pyuria, and cultured by semi quantitative standard loop culture technique. The prevalence of asymptomatic and symptomatic UTI was found to be 16.3 % and 48.3 % respectively. The Gram stain of uncentrifuged urine was found to be the most useful single test with good sensitivity (82.17%), specificity (95.86%), and Negative Predictive Value (94.41%). The predominant organism isolated was *Escherichia coli* 142 (82.5 %) which exhibited high degree of resistance to ampicillin (58.4%) followed by gentamicin (40.6%), cephalexin (37.3%), and co-trimoxazole (28.9%). Out of 180 *Escherichia coli* isolated from pregnant women, 148 (82.2%) were resistant to three or more antibiotics. In our study, the positive correlation in antibiotics susceptibility pattern between asymptomatic and symptomatic UTI groups was established by Spearman's rank correlation ($r=0.78$, $p<0.01$).

To conclude, high rate of symptomatic bacteriuria in the present study is worrying. Increasing antibiotic resistance to ampicillin and cephalexin among *Escherichia coli* complicates empirical regimen given in pregnancy. Even asymptomatic bacteriuria is higher compared to other studies. This indicates proper microbiological screening and sensitivity for selection of antibiotics. Gram stain of uncentrifuged urine was useful screening method to be adapted for screening all antenatal cases.

Key words: Pregnancy, Urinary tract infection, *Escherichia coli*; Antimicrobial resistance.

Urinary tract infections (UTI) are the most commonly encountered infections in obstetric patients due to the morphological and physiological changes that take place in the genito-urinary tract during pregnancy[1, 2].

Acute 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[3]. Asymptomatic bacteriuria is a condition characterized by bactriuria without classical symptoms attributable to the urinary tract[3, 4]. Asymptomatic bactreiuria is a major risk factor for the development of UTI in pregnant women[5]. Untreated UTIs can lead to

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complications such as pyelonephritis, low-birth weight of infants, premature delivery and occasionally still birth[6]. Asymptomatic bacteriuria is defined as equal to more than 100,000 colonies of a single bacterial species per ml of urine in two consecutive mid stream urine samples[4]. Symptomatic UTI occurs in 1 to 2% percent of all pregnancies, while asymptomatic bacteriuria has been reported in 2 to 13 % of all pregnant women[7]. Pregnancy does not increase the incidence of asymptomatic bacteriuria; however, pyelonephritis develops in 20-40 % of pregnant women with untreated asymptomatic bacteriuria[8]. Results of multiple studies have shown that women with asymptomatic bacteriuria have a higher preterm delivery rate than women without bacteriuria[9]. Approximately 40-80 % of the pregnancy complications caused by acute pyelonephritis could be prevented by treating asymptomatic bacteriuria[10]. *Escherichia coli* accounts for the majority of the urinary tract infection in pregnant women[11].

Despite universal guidelines recommending screening and treatment of asymptomatic bacteriuria in pregnancy, there is an ongoing debate in the literature regarding the role of asymptomatic bacteriuria in perinatal outcomes. Similarly controversy exists as to whether antibiotic treated pyelonephritis leads to adverse pregnancy outcome[12].

There is no sufficient local data available on symptomatic and asymptomatic urinary tract infection among pregnant women in Raichur district, north-interior part of Karnataka, India. The aim of the present study was to determine the prevalence of asymptomatic and symptomatic UTI among pregnant women, to evaluate the diagnostic efficacy of screening tests among pregnant women and to document antibiotic susceptibility pattern of *Escherichia coli*, a major urinary pathogen.

MATERIAL AND METHODS

Subjects

The study population consisted of consecutive 656 pregnant women attending out patient department of Obstetrics and Gynecology at Navodaya Medical College Hospital and Research Laboratory, Raichur. The study and data collections were carried out with the approval from institutional ethical committee and informed consent were taken from all the subjects willing to participate.

The subjects were further divided in to two groups:

i. Symptomatic group comprising of 356 cases and
ii. Asymptomatic group comprising of 300 cases

Pregnant women consulting for their first prenatal checkup, who were willing to participate, were included in the asymptomatic study group.

Inclusion criteria followed for symptomatic group were -

a) Patients with history of fever
b) Patients with any two of the following genito-urinary complaints: dysuria, urinary hesitancy, urgency, slow stream, incontinence, frequency, incomplete voiding and burning micturition.

Patients with any intake of antibiotics for any indication of UTI during the current pregnancy were excluded.

Inclusion criteria for asymptomatic group were –

a). Two consecutive mid stream urine samples showing ³ 100,000colony forming units of a single bacterial uropathogen per mL of urine and
b). The absence of symptoms attributable to UTI.

Methods

Clean -catch mid stream urine samples collected in (uricol, Hi-Media) sterile disposable container were immediately transported to the service laboratory of Microbiology department and processed within one hour.

Screening methods

Gram staining of uncentrifuged urine

A drop of uncentrifuged well mixed urine was taken on clean grease free glass slide, air dried and stained by Gram staining method and examined under oil immersion field of microscope (examining 20 fields). Presence of ≥ 01 bacteria per oil immersion field correlates with significant bacteriuria[13].

Wet examination for leucocytes count

Well mixed 0.05 ml of uncentrifuged urine was transferred on to the slide. A cover slip having 22 x 22 mm dimension was placed on the slide avoiding trapped bubbles and observed with the high power (40x) field dry objective of the

microscope. Finding of 01 leukocyte per 7 high power fields corresponds with 10^4 leucocytes per ml and the finding of clearly larger number than this indicates significant pyuria[13].

Culture and sensitivity

Semi quantitative calibrated loop technique was employed to culture urine on blood agar and Mac Conkey agar. The inoculated plates were incubated over night at 37°C. Significant isolates (colony count ≥ 10^5 CFU/ml) were identified by standard procedures[14]. Antibiotic sensitivity test was done by Kirby Bauer disc diffusion method[15] using following antibiotics: ampicillin (10mcg), amoxyclav (20/10mcg), co-trimoxazole (1.25/23.75 mcg), gentamicin (10mcg), cefotaxime (30mcg), cephalexin (30mcg), ceftazidime (30mcg), norfloxacin (10mcg), nitrofurantoin (300mcg) and imipenem (10mcg).

Statistical analysis

Results were analyzed with descriptive statistics wherever appropriate. The uncorrelated Chi square test was applied for categorical variables; Ninety five percent confidence intervals were used for proportions. The correlation analysis was done by spearman's rank correlation. All the statistical tests were two tailed and considered *p*-value of < 0.05 to be statistically significant.

RESULTS

A total of 656 pregnant women were included in this prospective study. The age group of pregnant women with symptomatic UTI was 24.15 ± 4.06 (range 17- 38) years. The age group among asymptomatic pregnant women was 24.40± 4.56 (range 18-35) years. Among pregnant women, 48.3% (172/356) symptomatic urinary tract infection and 16.3% (49/300) asymptomatic urinary tract infections were identified.

Culture was taken as Golden standard and against which the screening tests were compared. The maximum numbers of true positives were identified by Gram staining of uncentrifuged urine in both symptomatic (125/142) and asymptomatic (29/38) UTI, leading to the increased sensitivity. Least number of true positives were identified by pus cell count in symptomatic (82/142) and asymptomatic (14/38) UTI cases, there by decreasing the sensitivity [Table 1]. The sensitivity (88.03%), specificity (94.39%) and Negative Predictive Value (92.24%) of Gram stain in symptomatic UTI cases and sensitivity (76.32%), specificity (97.33%) and Negative Predictive Value (96.59%) of Gram stain in asymptomatic UTI cases were found to be much superior to other method.

The most predominant organism isolated from urine samples of pregnant women with symptomatic and asymptomatic UTI was *Escherichia coli*. The urine from 142 (82.5%) symptomatic and 38 (77.5%) asymptomatic UTI cases showed the growth of *Escherichia coli*. Other organisms isolated in significant number in symptomatic cases were *klebsiella pneumoniae* 19(11%), CONS 6(3.5%), *Proteus mirabilis* 2(1.7%), *Pseudomonas aeruginosa* 2(1.7%), and *Staphylococcus aureus* 1(0.6%). *Klebsiella pneumoniae* 8(16.3 %) was second commonest organism isolated from asymptomatic UTI cases followed by CONS 2(4.1%) and *Staphylococcus aureus* 1(2.6 %).

Table 1. Screening tests to detect symptomatic and asymptomatic UTI in pregnant women

Tests	True positives	True Negatives	False positives	False Negatives
Symptomatic UTI (n=356)				
Gram's stain	125	202	12	17
Pus cell count	82	180	34	60
Combined Gram stain & Pus cell count test	96	186	28	46
Asymptomatic UTI (n=300)				
Gram's stain	29	255	7	9
Pus cell count	14	249	13	24
Combined Gram stain & Pus cell count test	19	249	10	22

Antibiotic sensitivity testing of *Escherichia coli* isolated from pregnant women in significant number revealed high prevalence of resistance to ampicillin (58.4%), gentamicin (40.6 %), and cephalexin (37.3%). Imipenem (98.3%) was found to be most sensitive antibiotic for *Escherichia coli* followed by nitrofurantoin (76.6%), cefotaxime (75.0%), co-trimoxazole (71.1 %) and amoxy-clav (69.4%). [Table 3]

Table 2. Sensitivity, Specificity and Predictive Values of screening tests

Tests	% Sensitivity	% Specificity	% Positive Predictive Value	% Negative Predictive Value
Symptomatic UTI (n=356)				
Gram's stain	88.03	94.39	91.24	92.24
Pus cell count	57.75	84.11	70.69	75
Combined Gram stain & Pus cell count test	67.61	86.92	77.42	80.17
Asymptomatic UTI (n=300)				
Gram's stain	76.32	97.33	80.56	96.59
Pus cell count	36.84	95.04	51.85	91.21
Combined Gram stain & Pus cell count test	46.34	96.14	65.52	91.88

Table 3. Antibiotic susceptibility pattern of *Escherichia coli* isolated from

Antibiotics	Pregnant women			
	Symptomatic UTI, n=142 (I)	Asymptomatic UTI, n=38 (II)	Significant Bacteriuria, n=180 (III)	Chi-square test *p*-value I vs. II
Ampicillin	56(39.4)	19 (50)	75 (41.6)	0.32
Amoxy-clav	96 (67.6)	29 (76.3)	125 (69.4)	0.33
Co-trimoxazole	102 (71.8)	26 (68.4)	128 (71.1)	0.69
Genatmicin	81(57.0)	26 (68.4)	107 (59.4)	0.26
Cefotaxime	104 (73.2)	31(81.5)	135 (75.0)	0.39
Cephalexin	85 (59.1)	28 (73.6)	113 (62.7)	0.14
Ceftazidime	89 (62.6)	24 (63.1)	113 (62.7)	1
Norfloxacin	87 (61.2)	30 (78.9)	117 (65.0)	0.05 *
Nitrofurantoin	106 (74.0)	32(84.2)	138 (76.6)	0.28
Imipenem	139 (97.8)	38 (100)	177 (98.3)	1

Figures in the parenthesis indicate percentage value; * indicate significant difference ($p<0.05$)

Table 4. Antibiotic profile of uropathogenic *Escherichia coli*

UTI in Pregnancy	No. of isolates	Sensitive to all antibiotics	Resistance to two antibiotics	Resistance to 3 or more antibiotics
Symptomatic UTI	142	5 (3.5)	18 (12.6)	119 (83.8)
Asymptomatic UTI	38	2 (5.2)	7 (18.4)	29 (76.3)
Total	180	7 (3.8)	25 (13.8)	148 (82.2)

Figures in parenthesis indicate percentage value

In our study, no significant difference (p>0.05) in antibiotics sensitivity pattern between asymptomatic and symptomatic UTI was found except for sensitivity of *Escherichia coli* (p<0.05) to norfloxacin. Overall a positive correlation between asymptomatic and symptomatic UTI was found (Fig. 1) with respect to antibiotic sensitivity pattern (Spearman's rank correlation r=0.78, p<0.01).

Out of 180 *Escherichia coli* isolates, 7 (3.8%) were sensitive to all antibiotics, 25 (13.8 %) were resistant to two antibiotics and 148 (82.2%) were resistant to three or more antibiotics Table 4.

DISCUSSION

In the present study, 221 out of 656 (33.6%) urine samples have shown significant bacteriuria in pregnant women. Incidence of asymptomatic UTI was found to be high (16.3%) when compared to a prospective study on asymptomatic bacteriuria (7.4%) carried out by Jayalakshmi and jayaram[16]. The prevalence of bacteriuria during pregnancy depends on a number of factors, including age and parity, and the figures ranged from 2.5% to 15% [17, 18].

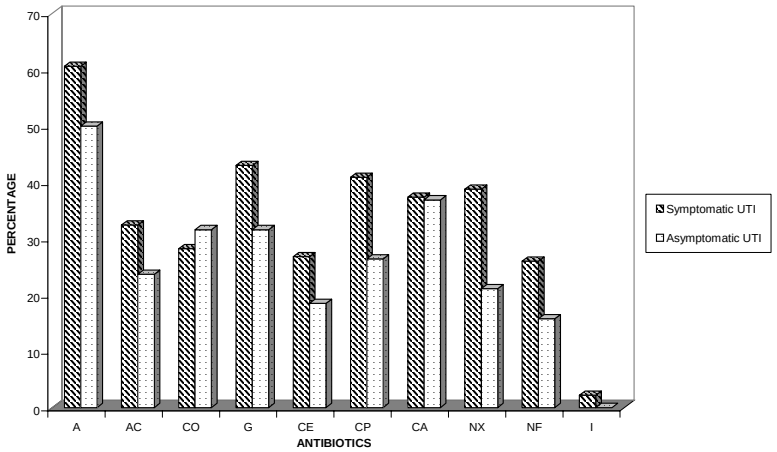

Fig. 1. Antibiotic resistance pattern of *E. coli* isolated from urine of pregnant women

Gaymans *et al.*,[19] noted that 30% of 58 women who were initially diagnosed with asymptomatic bacteriuria later became symptomatic and required antimicrobial treatment for their symptoms. Thus, it appears that asymptomatic and symptomatic UTI are not distinct entities.

An ideal screening test should be simple, rapid and must identify all positive cases, thus a sensitive test with a high Negative Predictive Value and specificity is desirable. The Gram stain of uncentrifuged urine was found to be the most useful single test with high sensitivity (82.17%), specificity(95.86%), Negative Predictive Value (94.41%) and statistically highly significant (p < 0.0001), when only those urine containing a pure culture of a potential pathogen of e" 10^5 CFU/ml was considered positive.

The predominant organism isolated from urine sample of pregnant women was *Escherichia coli* 142 (82.5 %) and this is comparable with the results of previous reports[19-22]. *Escherichia coli* isolated from pregnant women exhibited high degree of resistance to ampicillin (58.4%) followed by gentamicin (40.6%), cephalexin (37.3%), co-trimoxazole (28.9%) and nitrofurantoin (23.4%).

James et al[23] reported high incidence of resistance in the organisms to ampicillin and suggested to avoid ampicillin monotherapy given empirically for UTI in pregnancy. Enayat et al [24] found still higher prevalence of antibiotic resistance in pregnant women for nalidixic acid (73.4%), co-trimoxazole (79.74%) and nitrofurantoin (70.88%). A study by Gupta et al revealed that the urinary pathogens resistant to ampicillin was increased from 29% to 38% from 1992 to 1996, while resistance to co-trimoxazole

increased from 8% to 16 % during the same period[25]. Drug resistance for three or more antibiotics was found to be 83.8% in symptomatic UTI and 76.3% in asymptomatic UTI cases in the present study. Kumar et al[26] reported 53.9 % resistance for 3 or more antibiotics for *E. coli* in asymptomatic bacteriuria among school going girls.

In this investigation, we used *in vitro* antibiotic sensitivity test on uropathogenic *Escherichia coli* as the primary outcome, but more than this clinical response to therapy is important. However present study confirms earlier studies, which demonstrated the resistance rate of uropathogens to ampicillin is high enough to prevent this drug being acceptable for empiric treatment for UTI in pregnancy. Our study adds to growing evidence towards the proportion of uropathogenic *Escherichia coli* becoming resistant to Co-trimoxazole, Nitrofurantoin, Norfloxacin and Cefotaxime.

To conclude, high rate of symptomatic bacteriuria in the present study is worrying. Even asymptomatic bacteriuria is higher compared to other studies. This indicates proper microbiological screening and sensitivity for selection of antibiotics. Gram stain of uncentrifuged urine was useful screening method to be adapted for screening all antenatal cases. An early detection and treatment of asymptomatic UTI may be of considerable importance not only to forestall acute pyelonephritis and chronic renal failure in mother, but also to reduce prematurity and fetal mortality. Pregnant women diagnosed with asymptomatic or symptomatic urinary tract infections are often treated empirically with short courses of ampicillin and cepahlexin due to potential adverse effect of therapy on the fetus. Increasing antibiotic resistance to ampicillin and cephalexin among *Escherichia coli* complicates empirical regimen. Further prospective studies with follow up of pregnant women during different trimesters and post pregnancy is crucial to understand the true picture of magnitude of urinary tract infection in pregnancy.

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