
ORIGINAL ARTICLE**Asymptomatic Bacteriuria in Pregnancy***Smita Mangalgi^{*}, Annapurna Sajjan¹**¹Department of Microbiology, Shri B. M. Medical College, BDLE (Deemed to be University),
Vijayapura-586103, (Karnataka) India***Abstract:**

Background: The risk of urinary tract infection is higher in pregnancy. Due to lack of symptoms in asymptomatic bacteriuria, patients seldom seek any treatment. If left untreated, it may lead to complications like acute pyelonephritis, preterm labor, low birth weight etc. Adequate and early treatment reduces the incidence of these complications. **Aim and Objectives:** The objectives of this study were to determine the prevalence of Asymptomatic Bacteriuria (ASB) among pregnant women, evaluate the accuracy of rapid tests in assessing ASB in pregnancy, to know the common causative organisms and their sensitivity pattern and to correlate the demographic data. **Material and Methods:** One thousand four hundred and ten pregnant women were included in the study. Mid-stream urine specimen was subjected to semi quantitative culture method and also for urine analysis by microscopy and reagent strip method. **Results:** Of the 1410 pregnant women, 117(8.3%) had ASB. Sensitivity, specificity, positive and negative predictive value of reagent strip test was good enough when both leukocyte esterase and nitrate test results together or separately were considered positive. *E. coli* was the commonest organism 65.8 % followed by *Klebsiella* species 30.7%. Amongst the *E. coli*, sensitivity to nitrofurantoin, ceftazidime/clavulanic acid and imipenem was found to be 94.1%, 88.2%, and 88.2% respectively. **Conclusion:** Due to lack of symptoms and harmful consequences in pregnancy, ASB needs special considerations. All the pregnant females should be screened for ASB during pregnancy to prevent maternal and perinatal morbidity. Rapid strip test could

be used as an OPD diagnostic test to screen ASB among pregnant women and reserve urine culture for those who turnout positive in screening tests. Wide variation in antibiotic sensitivity pattern of the bacteria causing ASB emphasizes the need of antimicrobial susceptibility testing rather than empirical treatment which is a common practice.

Keywords: Asymptomatic Bacteriuria, strip method, Klebsiella

Introduction:

Urinary Tract Infection (UTI) is more common in females due to shorter length of urethra and proximity to the anus. In pregnancy, additional factors like increased bladder volume, decreased urethral tone cause urinary stasis thus increasing the risk of infection. Asymptomatic Bacteriuria (ASB), acute cystitis and acute pyelonephritis are the three major types of UTI seen in pregnancy [1, 2].

ASB is defined as the presence of actively multiplying bacteria, greater than 10^5 /ml of urine, without classical symptoms attributable to the urinary tract.

If untreated ASB progresses to symptomatic bacteriuria in 40-60% of cases, which further may lead to acute pyelonephritis and adverse obstetric outcomes such as premature labor, low birth weight, postpartum hypertensive disease, anemia and higher fetal mortality rates [1, 3-5]. These consequences can be avoided if screened and

treated early. Although there are numerous methods of screening for bacteriuria, culture still remains the gold standard. However, culture is expensive, takes about 48 hours and requires skilled personnel; hence it is generally not performed in many laboratories [6, 7]. Reagent strip test that analyzes several urinary properties like pH, presence of glucose, ketones, blood, protein, bilirubin, urobilinogen, nitrate and leukocyte esterase is being performed in many laboratories. The test has the advantage of being cost effective, rapid, easy to perform and interpret, can be carried out in smaller laboratories with no culture facilities. Due to immediate availability of the results, compliance of the patient is better. Though evaluation of this method has been conducted in different studies, this type of study has not been undertaken in the Northern part of Karnataka in and around Vijayapur [8-12].

The objectives of this study were to determine the prevalence of ASB among pregnant women, evaluate the accuracy of rapid tests in assessing asymptomatic bacteriuria in pregnancy, to know the common causative organisms and their sensitivity pattern and to correlate the demographic data.

Material and Methods:

This cross-sectional study was conducted from April 2016 to May 2017. The study was approved by the Institutional Ethical Committee. After explaining the relevance and the objectives of the study, an informed consent was obtained from the study participants before collecting the sample. Inclusion criteria: Pregnant women who were registered at any gestational age without urinary complaints and volunteered to participate in the study were included. Exclusion criteria: Pregnant

women who had fever/genitourinary symptoms and who were not willing to participate were excluded. All the participants were given well-structured questionnaire in local language regarding socio-demographic data such as age, residential area, number of children, gestational age, educational level, occupation and monthly family income. The participants were given soap strips and instructed about the method of collection. Clean catch mid-stream urine samples were collected into a wide-mouthed sterile screw capped containers. The samples were immediately transported and processed within 1-2 hours of collection.

Urine sample was first processed for culture and then analyzed by microscopy and strip test. For cultural study, samples were inoculated by semi-quantitative method on Mac-Conkey's and Cystine Lactose Electrolyte Deficient agar by standard procedure. The plates were incubated at 37°C for 24 hours. The culture was interpreted as sterile if no growth was seen, significant if 10^5 Colony Forming Units (CFU)/ml were noted. Insignificant growth was reported if colony count obtained corresponded to less than 10^5 CFUs/ml of urine except in case of gram positive cocci where even 10^2 CFUs/ml was considered significant. Specimens were labeled as contaminated, when mixed bacterial growth was observed. The isolates were identified by standard protocol as per Cowan and Steel's manual [13]. The antibiotic sensitivity testing was performed according to NCCLS guidelines [14].

Urine analysis by reagent strip method was carried out using Multistix^R 10 SG strips by Simens Health Care Private Limited that detected ten parameters. The procedure and interpretation

of the results were done according to the manufacturer's instructions. The strips included reagent pads for semi-quantitative assessment of protein, blood, pH, specific gravity, bilirubin, urobilinogen, leukocyte esterase, nitrate, glucose and ketone.

For Gram's staining a drop of uncentrifuged well mixed urine was smeared on a clean grease-free slide and stained by Gram's method and examined under oil immersion objective (20 fields). Presence of ≥ 1 bacteria per oil immersion field correlates with significant bacteriuria of $\geq 10^5$ CFU/mL of urine.

Urine specimen was centrifuged for 5 minutes at 1500 rpm and supernatant was poured off leaving 0.5 ml of urine to re-suspend the sediment. A drop of this was placed on a glass slide and covered with a cover slip and was examined immediately under low power and high power objective. Pus cells of >10 /HPF or presence of clumps was considered suggestive of urinary tract infection.

Results:

Of the 1410 urine samples from pregnant women processed for culture, 117(8.3%) yielded significant growth (Table 1). The rapid strip test result showed 74 of the pregnant women positive for leukocyte esterase and 85 for nitrate (Table 2). Commonest bacterium isolated was *Escherichia coli* (65.81%) followed by *Klebsiella* (Fig. 1). Antimicrobial susceptibility pattern of the uropathogens is depicted in Table 3. Most of the *E. coli* isolates were sensitive to nitrofurantoin, amikacin, ceftazidime/clavulanic acid and imipenem. About 59% sensitivity was documented for gentamicin and piperacillin/tazobactam. Other gram negative bacilli though showed 100% sensitivity to ceftazidime/clavulanic acid and imipenem, were resistant to nitrofurantoin. Socio-demographic features of women having asymptomatic bacteriuria are shown in Table 4. High frequency of infection was noted in the age range of 18-25 years, in the third trimester and in multiparous women.

Table 1: Cultural Results of 1410 Urine Specimens of Study Subjects

Culture results	Number of cases (%)
Significant bacteriuria	117 (8.3)
Insignificant bacteriuria	68 (4.8)
Contamination	27 (1.9)
Sterile	1198 (85)
Total	1410 (100)

Table 2: Urine Strip Test for Leukocyte Esterase and Nitrate and Urine Microscopy for Pus Cells and Gram's Stain in Relation to Culture

Test	Parameter		Culture			Sensitivity	Specificity	PPV	NPV
			Positive	Negative	Total				
Strip test	LE	Positive	74	43	96	69.16	96.7	63.25	97.45
		Negative	33	1260	1314				
		Total	117	1293	1410				
	NT	Positive	85	51	114	72.65	96.06	62.50	97.49
		Negative	32	1242	1296				
		Total	117	1293	1410				
	LE/NT	Positive	113	26	139	96.58	97.99	81.29	99.69
		Negative	04	1267	1271				
		Total	117	1293	1410				
Microscopy	PC	Positive	65	28	89	55.56	98.14	73.03	96.06
		Negative	52	1269	1321				
		Total	117	1293	1410				
	Gr	Positive	61	19	80	52.14	98.53	76.25	95.79
		Negative	56	1274	1330				
		Total	117	1293	1410				
	GR/PC	Positive	78	41	119	66.96	96.22	61.11	97.04
		Negative	39	1252	1291				
		Total	117	1293	1410				

LE – Leukocyte Esterase, NT- Nitrate, PC-Pus Cells, Gr.- Gram's Stain, PPV-Positive Predictive Value, NPV- Negative Predictive Value

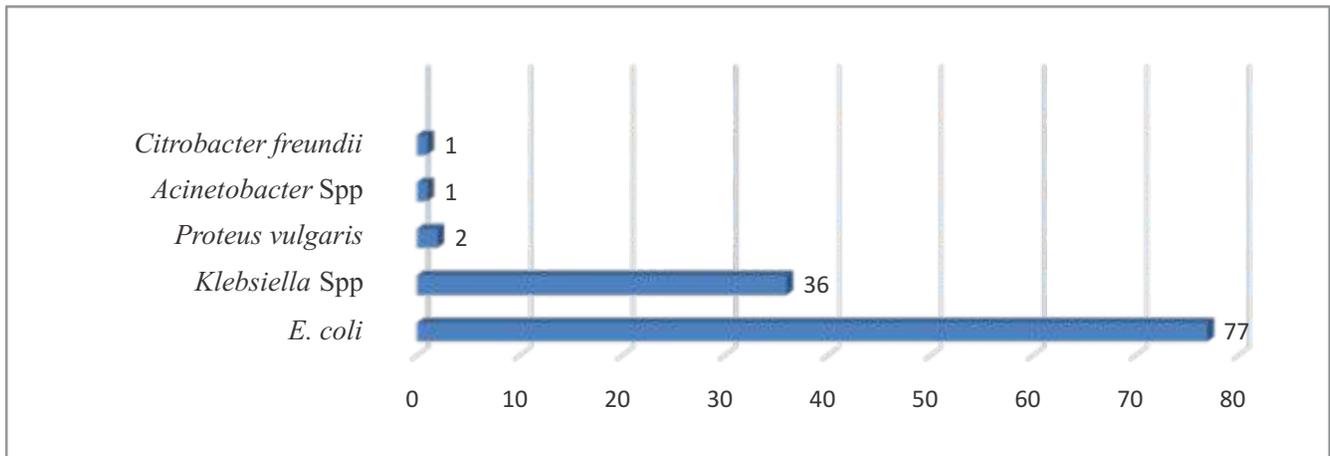


Fig. 1: Bacteriological Profile of Culture Positive ASB Cases

Table 3: Antimicrobial Susceptibility Pattern of the Uropathogens

Bacteria	Antimicrobial susceptibility (%)												
	A	COT	NX	CIP	GEN	NA	AMC	AK	CS	PIT	CAC	NIT	IMI
<i>E. coli</i>	71.1	54.2	8.4	47.4	59.3	15.25	17.6	88.4	88.2	58.8	88.2	93.2	88.2
<i>Klebsiella Spp</i>	34.8	91.3	78.3	91.3	100	47.8	26.0	100	91.3	91.3	100	16.66	100
<i>P. vulgaris</i>	100	100	00	00	50	50	0	100	100	50	100	00	100

A - Ampicillin, COT - Co-trimoxazole, NX – Norfloxacin, CIP - Ciprofloxacin, GEN- Gentamicin, NA - Nalidixic Acid, AMC - Amoxyclav, AK - Amikacin, CS-Cefoperazone/Sulbactam, PIT - Piperacillin/Tazobactam, CAC-Ceftazidime/Clavulanic Acid, NIT- Nitrofurantoin, IMI-Imipenem

Table 4: Socio-demographic Data of the Participants

Variables	Number (%)
Age	
15-19	09(7.69)
20-24	21(17.94)
25-29	56(47.86)
30-34	27(23.07)
>35	04 (3.14)
Gestation stage	
First trimester	19(16.23)
Second trimester	36(30.76)
Third trimester	62(52.99)
Parity	
1	17(14.5)
2-3	41(35)
>3	59(50.4)
Occupation	
Employed	13(11.1)
Unemployed	104(88.8)
Residence	
Urban	68(58.1)
Rural	49(41.8)
Literacy	
Illiterate	Nil
Elementary	33(28.2)
High school	43(36.75)
Higher education	41(35.04)
Total income/month in rupees	
<10000	11(9.4)
1001-20000	49(41.8)
20000 – 30000	44(37.6)
>30000	13(11.1)

Discussion:

Women with asymptomatic bacteriuria during pregnancy are more likely to deliver premature or low-birth-weight infants and have a 20 to 30 fold increased risk of developing pyelonephritis during pregnancy, compared to women without bacteriuria [15]. Prevalence of ASB among pregnant women was 8.3%, which is closer to the results of other studies [9-12].

The present study gave a lower sensitivity of 69.16% and Negative Predictive Value (NPV) of 97.45% for Leucocyte Esterase (LE) test because of 33 false positive cases with sterile pyuria. False positive findings in LE test may be seen in cases with high levels of ascorbic acid, phenazopyridine and albumin in the urine. However, the LE test had the advantage of identifying ASB even when the pus cells were disintegrated in the specimen.

Among the screening tests evaluated, nitrate test was better followed by LE. Considering culture as the gold standard, the sensitivity and specificity for leukocyte esterase was 69.16 and 96.7 and for nitrate test was 72.65 and 96.06. Reckoning positive results for both leukocyte esterase and nitrate strip test result together with positive results in either of the test, the sensitivity increased to 96.58% rather than using either of the tests alone. In the same way sensitivity of microscopy with Gram's stain alone and pus cell detection was 55.56 and 52.14%, which increased to 66.96% when either of the tests or both were considered positive.

The sensitivity of nitrate test in our study was in accordance with the study by Jayalakshmi and Miami *et al.* whereas that of LE test was slightly higher [3,16]. When compared to the study by Demilie *et al.* sensitivity of both leukocyte

esterase and nitrate tests by urine strip method was quite high [12]. In this study the sensitivity of urine microscopy was found to be less when compared to the strip test.

The strip test had the advantage of being simple, rapid, not requiring any expertise or equipment and costing 1/10th of culture. Therefore, strip test could be used as an office diagnostic test to screen ASB among pregnant women and reserve routine culture only for screening test positives.

The bacteria responsible for asymptomatic bacteriuria are often of fecal origin which colonize the periurethral area. *E. coli* is the main culprit reported, which is true in our study too. Though the spectrum of agents causing UTI in pregnant women is relatively constant, their antimicrobial sensitivity pattern differs from community to community and from hospital to hospital. In this study, high percentage of *E. coli* strains were resistant to orally administered drugs such as cotrimoxazole, norfloxacin, ciprofloxacin, nalidixic acid and amoxyclav. Though least resistance was noted for nitrofurantoin in *E. coli*, a very high resistance was demonstrated in other gram negative enteric bacteria. The overall sensitivity was better to amikacin, imipenem, ceftazidime/clavulanic acid, cefoperazone/ sulbactam and piperacillin/tazobactam in that order. Both *Klebsiella* and *P. vulgaris* showed 100% sensitivity to imipenem and ceftazidime-clavulanic acid. Resistance was highest to nitrofurantoin.

Though no further follow-up study could be accomplished to find out the various complications,

many studies have reported toxemia, prematurity, IUGR, LBW babies, perinatal mortality [3-5, 17]. The cost of managing these complications is far higher than that of screening and treating asymptomatic bacteriuria. Therefore, it is better to screen for asymptomatic bacteriuria during pregnancy and provide apt treatment to avoid complications.

Advanced maternal age and multiparity were the important risk factors found in this study which also have been mentioned by other authors [18-20]. Higher rate of infection was noted in the third trimester as compared to the first and second trimester which is in alignment with the studies by Harshika *et al* and Senthinath *et al*. Most pregnant females who had ASB were home makers. No relation could be established between ASB and area of residence and literacy levels. Common occurrence of ASB in lower socioeconomic groups has been mentioned in many studies but in our study we could not find any such relation [18, 20].

Conclusion:

Due to lack of symptoms and harmful consequences in pregnancy, asymptomatic bacteriuria needs special considerations. All the pregnant females should be screened for asymptomatic bacteriuria during pregnancy and treated as per antimicrobial sensitivity pattern of the isolate to prevent maternal and perinatal morbidity. Rapid strip test could be used as an OPD diagnostic test to screen ASB among pregnant women and reserve urine culture for those who turnout positive in screening tests.

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