

Screening Tests for Asymptomatic Bacteriuria in Pregnant Women

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Asymptomatic bacteriuria (ASB) in pregnant women leads to complications like pyelonephritis, preeclampsia, prematurity, low birth weight, and intrauterine death. The present study was undertaken to evaluate screening methods with quantitative culture. Midstream clean catch urine collected from 750 asymptomatic antenatal women were subjected to wet mount, Gram stain, catalase test and triphenyl tetrazolium chloride test (TTC). Cultures were put-up by standard loop method and pour-plate method. Isolates with significant growth were identified by standard methods and antibiotic susceptibility test was done by Kirby- Bauer disc diffusion method. ASB was detected in 7.87% of antenatal women. Among the rapid screening methods, catalase test showed the highest sensitivity (100%), followed by Gram stain (98.30%), TTC test (95.45%) and wet mount (27.12%). Wet mount and Gram stain showed highest specificity (100%), followed by TTC test (98.24%) and catalase test (94.73%). Semi- quantitative culture by standard loop method showed a sensitivity of 96.61% and specificity of 100%. *Escherichia coli* was the predominant organism isolated (66.66%) and majority of isolates were found sensitive to nitrofurantoin (70%). Gram stain can be a reliable rapid screening method and standard loop method, an adequate substitute for pour plate method.

Key words: Asymptomatic bacteriuria, Gram stain, Standard loop method, *Escherichia coli*, Nitrofurantoin

Infection of the urinary tract, so called "The Problem Tract", is one of the commonest of all infections, next to those of the upper respiratory tract¹ and is predominantly a disease of females.² In women, the short length of the urethra and sexual intercourse facilitate the ascent of bacteria into the bladder.³ During pregnancy specific

physiologic and anatomic changes do occur and, the net effect of these changes results in infection of the urinary tract to develop.⁴ Asymptomatic bacteriuria (ASB) is defined as significant bacterial count $\geq 10^5$ organisms or colony forming units present per milliliter in the urine of a person without symptoms⁵ and is present in approximately 5 to 10% of the pregnant women,⁶ and if untreated, it leads to the development of symptomatic cystitis and pyelonephritis in up to 50 percent of patients.⁷ Infection may be complicated by low birth weight, prematurity, preeclampsia, maternal anemia,

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amnionitis, and intrauterine death.⁸ Early treatment of bacteriuria not only could avert the occurrence of acute or chronic pyelonephritis, but it could also diminish the risk of prematurity and perinatal mortality.¹ All these factors justify screening for ASB during pregnancy. But, the optimal method of screening has not been established.⁹ Many studies evaluated screening methods such as nitrite test, leukocyte esterase test, microscopic urine analysis, triphenyl tetrazolium chloride test and catalase test with inconsistent results.^{8,10,11} Considering the above factors, the present study was undertaken to evaluate the rapid screening methods and urine cultures in detecting ASB in antenatal women.

MATERIALS AND METHODS

The present prospective study was conducted in the Department of Microbiology, Government Medical College, Mysore, from June 2004 to August 2005. Seven hundred and fifty antenatal women, in the first, second and third trimester, without any symptoms of urinary tract infection, attending Obstetric Outpatient Department, Cheluvamba Hospital, Government Medical College, Mysore, were randomly selected as the study group. The patients were instructed to collect midstream clean catch urine into a labeled wide mouthed sterile container.^{12,13,14} The sample was processed immediately. After noting macroscopic appearance, uncentrifuged urine sample was subjected to wet mount examination, Gram stain, catalase test and triphenyl tetrazolium chloride (TTC) test (procured from Grand chemicals, Mysore).¹²⁻¹⁵ Culture was put-up by standard loop method on 5% sheep Blood agar

and MacConkey agar (Himedia Mumbai, India) and by pour-plate method on nutrient agar.¹²⁻¹⁵ Colony count of $\geq 10^5$ CFU/ml was taken as significant bacteriuria and repeat urine culture was done at > 24 hours interval.²¹ If the same organism was isolated in repeat culture with significant bacteriuria, diagnosis of asymptomatic bacteriuria was made.^{3,16,17} The antimicrobial susceptibility test was done by Kirby-Bauer disc diffusion method standardized as per NCCLS (CLSI) guidelines.¹⁸ (Study had been approved by Ethical committee of the Institution).

RESULTS

Of 750 cases studied, 59 cases (7.87%) had significant bacteriuria, 61 cases (8.13%) had insignificant bacteriuria and no growth was observed in 630 cases (84%). Out of four rapid screening methods, catalase test had highest sensitivity (100%) followed by Gram stain (98.30%), triphenyl tetrazolium chloride (TTC) test (95.45%) and urine wet mount for pus cells had least sensitivity (27.12%). Urine wet mount and Gram stain had highest specificity (100% each) followed by TTC test (98.24%) and catalase test (94.73%). As compared with the reference method – pour-plate method, standard loop method had sensitivity of 96.61% and specificity of 100%. The commonest organism isolated was *Escherichia coli* 40 (66.66%), followed by *Klebsiella pneumoniae* 8 (13.33%), *Staphylococcus saprophyticus* 5 (8.33%) and *Staphylococcus aureus* 2 (3.33%). *Enterobacter aerogenes*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Acinetobacter* spp. were isolated in 1 (1.67%) case each. 70% of isolates were sensitive to

Table 1. Sensitivity and specificity of screening methods as compared to pour-plate method

Screening method	True positive	False positive	True negative	False negative	Sensitivity (%)	Specificity (%)
Urine wet mount (for pus cell)	16	0	692	43	27.12	100
Gram stain	58	0	691	1	98.30	100
Catalase test	22	12	216	0	100	94.73
*TTC test	21	4	224	1	95.45	98.24
Standard loop method	57	0	691	2	96.61	100

*TTC - Triphenyl tetrazolium chloride

nitrofurantoin, 31.66% to amoxicillin-clavulanic acid and 30% to cephalexin. 90% and 86.66% of the isolates were resistant to cotrimoxazole and amoxicillin respectively.

DISCUSSION

Asymptomatic bacteriuria (ASB) during pregnancy is a common cause of serious maternal and perinatal morbidity; with appropriate screening and treatment, this morbidity can be avoided.⁷ In the present study, among the rapid screening methods, urine wet mount for pus cells showed a sensitivity of 27.12% and specificity of 100% (Table 1). This finding correlated with study of Lavanya SV *et al.* (2002).¹⁵ The lower sensitivity observed with urine wet mount may be due to insufficient inflammatory response in ASB and hence no symptoms of UTI.¹⁹ It may also be due to the fact that leukocyte number depends on urine flow, hydration status, physicochemical composition of the urine (alkaline urine causes disintegration of white cells) and previous antibiotic therapy.^{2,8,20} Because of its lower sensitivity, urine wet mount for pus cells cannot be taken as an acceptable screening method for ASB in antenatal cases.²¹ Gram stain of the uncentrifuged urine smear showed a sensitivity of 100% and specificity of 94.73%. Gram stain results correlated well with significant bacteriuria. Gram stain provides information about stain characteristics and organism morphology. It is also a least expensive and rapid method. The limitations of Gram stain is that methodically reviewing the smears is too labour intensive and may have technical variations.¹³ In the present study, catalase test showed a sensitivity of 100% and specificity of 94.73%. This finding correlated with study of Hagay Zion *et al.* (1996).¹ The high rate of false positive results (12) and low specificity observed in the present study may be due to the fact that catalase test detects catalase activity that is present not only in bacteria, but also in somatic cells (RBC, inflammatory exudates or renal cells).^{10,13} In the present study, triphenyl tetrazolium chloride (TTC) test showed a positivity rate of 95.45%. But many studies reported variable results.²²⁻²³ The false positive (4) and false negative (1) results observed in the present study may be due to the fact that various species of bacteria vary widely in the level of dehydrogenase activity, which is detected in

the TTC test.²¹ In the present study, standard loop method results correlated well with the results of pour-plate method in detection of significant bacteriuria (sensitivity 96.61% and specificity 100%). This method can be considered as an alternate substitute for quantitative urine cultures by pour-plate method.^{11,22,23} *Escherichia coli* possess the virulence factors required to colonize and infect the urinary tract hence commonly isolated in community acquired infections.³ Next commonly isolated organisms, *Klebsiella* spp. and *Staphylococcus saprophyticus* produce urease which is an important virulence factor which helps in development of UTI.¹⁴ In the antibiogram, there was a good correlation as regards to the general trends about the susceptibility pattern of different organisms, i.e., majority of the isolates in each study being sensitive to nitrofurantoin, amoxicillin-clavulanic acid and cephalexin.^{15 16}

CONCLUSION

To conclude, given the potential sequelae of undiagnosed asymptomatic bacteriuria (ASB) in the obstetric population, we suggest that all pregnant women should be screened for ASB. The ideal screening test should correctly identify the negative samples, i.e., one with high sensitivity and reasonably good specificity. Other factors include accuracy, ease of test performance, reproducibility and turnaround time. Although urine culture is expensive and labour intensive, it is the screening method of choice and culture has to be done to know antibiogram of isolates. Gram stain can be used as a reliable screening method where facilities for culture are not available. Since standard loop method results correlated well with the results of pour-plate method in detection of significant bacteriuria, this method can be considered as an adequate substitute for quantitative culture.

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