

Original Research Article

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Evaluation of Screening Tests to Detect Asymptomatic Bacteriuria in Antenatal Women

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ABSTRACT

The aim of the study was to compare the diagnostic performance of urine microscopy, leucocyte esterase and nitrite dipstick tests as screening tests for asymptomatic bacteriuria in pregnancy with the gold standard urine culture. 1000 Antenatal women attending OPD at Govt. Kilpauk medical college were screened for asymptomatic bacteriuria and the bacteria isolated by culture. A count of $> 10^5$ colony-forming units of a single organism per millilitre of urine was taken as significant. Dipstick results were read as positive according to the manufacturer's instructions. Asymptomatic bacteriuria was prevalent in 11.8% of the 1000 women who were evaluated in the study. Urine culture was the gold standard for the detection of asymptomatic bacteriuria. Gram's stain of uncentrifuged urine was found to be the best among the screening tests which were evaluated. There was a higher prevalence of asymptomatic bacteriuria in the Ist Trimester (38.98%). *Escherichia coli* was the major isolate constituting 54(45.76%) followed by *Staphylococcus aureus* 21(17.8%). Among the screening tests, Gram stain of uncentrifuged urine is the better one and in addition with dip stick testing of Nitrite & Leucocyte esterase will increase the diagnostic performance. Though Screening at the earliest (first trimester) is better, screening in each trimester is equally important to reduce the complications associated with asymptomatic bacteriuria.

Keywords

Asymptomatic Bacteriuria,
Urine Microscopy,
Urine Culture,
Leucocyte Esterase .

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Introduction

Urinary tract infection is mainly a disease of the females, due to the anatomical structure of the female urethra (Shruthi *et al.*, 2012; Lavanya *et al.*, 2002). There are 2 types of urinary tract infection (UTI). They are asymptomatic and symptomatic urinary tract infection (Sujatha *et al.*, 2004; Jeyaseelan *et al.*, 2013; Annie *et al.*, 2014). The definition of asymptomatic bacteriuria is the occurrence of actively multiplying bacteria, more than 10^5 bacteria per ml of urine inside the urinary tract, exclusive of the distal urethra at a time when the patient has nil symptoms of UTI

(Santogita *et al.*, 2013; Chandel *et al.*, 2012). In pregnancy, urinary tract infections commonly occur, because of the physiological and morphological changes that occur in the genitourinary tract. In pregnant women, the occurrence of asymptomatic bacteriuria was found to be 2% to 10%. The relatively increased occurrence of asymptomatic bacteriuria, the consequences faced by the antenatal women, the ability to avoid undesirable outcomes with the management justifies the testing of asymptomatic bacteriuria in pregnancy. Both

screening and treatment of all pregnant women for asymptomatic bacteriuria are therefore recommended.

The gold standard test for detection of bacteriuria is urine culture, but it takes 24 - 48 hours to obtain results (Titoria *et al.*, 2014). To overcome this problem, many alternative screening methods have been evaluated, including urine microscopy, chemical analysis using a dipstick method, Gram staining, dipslide urine culture and bioluminescence. Each method has advantages and disadvantages in terms of capital investment, running costs, automation, convenience and adaptability for use in the ward or clinic. The Griess nitrite test relies on the fact that the enteric Gram-negative bacilli and some Gram-positive cocci reduce nitrate present in urine to nitrite. The leucocyte esterase test is a colorimetric test that detects specific byproducts of leucocytes in the urine. Urine reagent dipstick testing is rapid, inexpensive and requires little technical expertise.

This study was aimed at comparing the diagnostic performance of urine microscopy and reagent strip tests with urine culture as standard for detecting asymptomatic bacteriuria in antenatal women.

The main aim of this study to compare the efficacy of screening tests with urine culture to detect asymptomatic bacteriuria in antenatal women.

Materials and Methods

This study was done from January 2014 to June 2015 at Government Kilpauk Medical College and Hospital, Chennai to compare the efficacy of screening tests to detect asymptomatic bacteriuria in pregnancy. Totally 1000 urine samples were collected from 18-40 years of age group among pregnant women and were studied during this period.

Sample Collection

Mid stream urine specimens were collected in a wide mouthed sterile container from the antenatal women who attended antenatal clinic at Government Kilpauk Medical College and Hospital were included in the study after obtaining informed consent. Specimens were transported immediately to the microbiology laboratory and processed without delay.

Sample Processing (Kheya *et al.*, 2014; Cheesbrough, 2014)

Macroscopic examination

Colour, turbidity and deposits of the urine samples were observed macroscopically and the results were recorded (Cheesbrough, 2014).

Screening Tests

One half of collected urine was taken for urine microscopy and culture and the other half was used for commercial reagent strip test for the nitrite and leucocyte esterase.

Leukocyte Esterase Test and Nitrite Test (Gayathree *et al.*, 2010)

Evidence of a host response to infection is the presence of PMN, polymorphonuclear leucocytes in the urine. Because inflammatory cells produce Leukocyte esterase, a simple and rapid method that measures this enzyme has been developed. The nitrate reductase test is a screening procedure that looks for the presence of urinary nitrite, an indicator of UTI. Nitrate reducing enzymes that are produced by the most common urinary tract pathogens reduce nitrate to nitrite. Uncentrifuged urine specimens were used for the presence of nitrite and leukocyte esterase activity. The

presence of nitrite and leucocyte esterase is noted by specific colour change in the panel provided by the manufacturer. The samples were tested as per the manufacturer's instructions. The results were compared with the colour code on the reagent strip container between 30 and 60 seconds as per the instruction manual. The positive results were recorded (Douglas, 1998).

Microscopic examination

Pus cell count of the uncentrifuged urine (Titoria *et al.*, 2014)

Presence of polymorphonuclear neutrophils (PMNs) was detected by urine wet mount examination. Microscopic analysis was done with 0.05 ml of the uncentrifuged urine.

≥ 5 pus cells / high power field - positive finding.

Gram's Staining of Uncentrifuged Urine

A loopful of uncentrifuged, well mixed urine was placed on a grease free slide and it was air dried. Then, the smear was stained by Gram's stain and was observed under oil immersion. The presence of 1 bacteria / Oil immersion field in 20 fields correlated with the diagnosis of significant bacteriuria of 10^5 CFU/ml of urine¹³

Urine culture

Semi-quantitative method (Titoria *et al.*, 2014; Collee *et al.*, 1996; Sushama *et al.*, 2012)

To isolate the organism, a semi-quantitative calibrated loop technique was used. One loopful of properly mixed urine that was not centrifuged was inoculated onto the surface of Nutrient agar, 5% sheep Blood agar, MacConkey agar and Cysteine Lactose

Electrolyte Deficient agar using a calibrated loop that delivers 0.01 ml of urine sample. The culture plates were incubated under aerobic conditions at 37⁰ C for 18-24 hours. The colonies were counted using colony counter and the number of colony forming units were multiplied by 100 to find out the number of microorganisms present per millilitre of urine.

≥ 10⁵ colony forming units / ml - significant bacteriuria.

The diagnostic criteria for asymptomatic bacteriuria was considered when atleast two consecutive urine samples showed more than or equal to 10⁵ colony forming units in 1ml of urine of the single species without any UTI symptoms (Lindsay, 2003). To differentiate pathogens from commensals, standard microbiological methods were followed. The importance of the isolates were identified based on presence of pus cells in direct gram staining, pure growth and colony morphology on culture plates and biochemical reactions by using standard microbiological techniques.

Results and Discussion

Antenatal women attending Obstetrics OP at Government Kilpauk Medical College and Hospital, Chennai were included in the study for asymptomatic bacteriuria detection . The observations were recorded and analysed. The results were as follows:

Out of the 118 isolates isolated from 1000 antenatal women, *Escherichia coli* 54(45.76%) was the predominant isolate followed by *Staphylococcus aureus* 21(17.8%). *Proteus mirabilis* was the least common organism isolated 2(1.69%).

The culture positives with asymptomatic bacteriuria in pregnancy were 11.8%. The culture positives were more in the 21-30

years age group, primigravida and in the first trimester. Gram negative bacilli were the predominant bacteria 78(66.10%), isolated in women with asymptomatic bacteriuria in antenatal women. This study aims at comparison of efficacy of screening tests with the urine culture to detect asymptomatic bacteriuria in pregnancy. The study was conducted from January 2014 to June 2015, among 1000 antenatal women from 18 to 40 years of age without any symptoms of urinary tract infection. The observations were recorded, analysed and discussed as follows.

In this study, number of culture positive urine samples was 118(11.8%). The culture positives were more in the 21-30 years age group 78(66.10%), primigravida 58(49.2%) and in the first trimester 46(38.98%). Gram negative bacilli were the predominant bacteria 78(66.10%) isolated in women with asymptomatic bacteriuria. *Escherichia coli* was the major isolate constituting 54(45.76%) followed by *Staphylococcus aureus* 21(17.8%)

Pus cells were present in 67(56.7%) by wet mount method. Direct Gram stain smear positive for organisms were 93(78.81%). Leukocyte esterase test was positive in 71(60.16%) and Nitrite test was positive in 89(75.4%) by dipstick method.

Urine culture was taken as the gold standard, against which the comparison of various screening tests were done. Statistical

formulas were applied and thus sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy were calculated.

In our evaluation of the screening tests like Gram's stain of uncentrifuged urine, the Leukocyte esterase test and the Nitrite test, we found Gram's stain of uncentrifuged urine to have a good sensitivity (78.81%), specificity (98.98%), positive predictive value (91.18%) and negative predictive value (97.22%) with a good diagnostic accuracy value of 96.61 % than other screening tests vis-a -vis urine culture . Though the Leukocyte Esterase alone showed a good specificity (98.98%), it was less sensitive (60.17%) than Gram's stain (78.81%). The nitrite tests has comparative sensitivity (75.42%) and positive predictive value (89%) with that of Gram's stain of uncentrifuged urine, but have low specificity (88.78%) and negative predictive value (75%). Combined Leukocyte esterase and Nitrite tests show a good sensitivity and specificity that of Gram's stain of uncentrifuged urine.

Among the screening tests evaluated, we observed that Gram's stain of uncentrifuged urine was the best screening method for Asymptomatic bacteriuria, as in other studies. The Dipstick test for Leukocyte esterase and Nitrites can also serve as a rapid screening method for asymptomatic bacteriuria, as its sensitivity and specificity is nearer to that of Gram's stain and the urine culture.

Table.1 Asymptomatic bacteriuria in antenatal women (n=1000)

Asymptomatic Bacteriuria In Antenatal Women	
Total number of Antenatal women	Antenatal women with Asymptomatic bacteriuria
1000	118(11.80%)

Out of the 1000 urine samples, 118 (11.8%) were culture positive.

Table.2 Relationship of Parity and culture positives (n=118)

Gravida	Culture Positives
Primi gravida	58(49.2%)
Second gravida	41(34.7%)
Third gravida	18(15.3%)
Gravida more than three	1(0.8%)
Total	118(100%)

Out of 118 bacterial isolates, the culture positives were more in the Primigravida 58(49.2%).

Table.3 Relationship of duration of pregnancy and culture positives (n=118)

Trimester	Culture positives
I	46(38.98%)
II	40(33.89%)
III	32(27.11%)
Total	118(11.8%)

Out of the 118 bacterial isolates the culture positives were more in the first Trimester 46(38.98%)

Table.4 Screening tests for asymptomatic bacteriuria in pregnancy (n =1000)

	Pus cells		Direct gram stain smear		Nitrate reduction		Leucocyte esterase	
	Positive	Negative	Positive	Negative	Reduced	Not Reduced	Positive	Negative
Culture Positivity								
Culture Positive	67	51	93	25	89	29	71	47
Culture Negative	30	852	9	873	11	871	36	846
Sensitivity%	56.78		78.81		75.42		60.17	
Specificity%	96.6		98.98		88.78		98.98	
PPV%	69.07		91.18		89		66.36	
NPV%	94.35		97.22		75		94.74	
Diagnostic Accuracy%	91.39		96.61		81.48		91.7	

Out of the 118 culture positives , 67 had significant pus cells, 93 had organisms in Gram stain, 89 reduced nitrate and 71 were positive for leucocyte esterase by urine dipstick.

Table.5 Distribution of organisms (n=118)

Organisms	Percentage
<i>Escherichia coli</i>	54(45.76%)
<i>Staphylococcus aureus</i>	21(17.80%)
<i>Klebsiella pneumoniae</i>	19(16.10%)
<i>Staphylococcus saprophyticus</i>	10(8.45%)
<i>Enterococcus faecalis</i>	9(7.63%)
<i>Pseudomonas aeruginosa</i>	3(2.54%)
<i>Proteus mirabilis</i>	2(1.69%)

In conclusion, asymptomatic bacteriuria was prevalent in 11.8% of the 1000 women who were evaluated in the study with *Escherichia coli* as the major isolate constituting 54(45.76%) followed by *Staphylococcus aureus* 21(17.8%). Urine culture remained the gold standard for the detection of asymptomatic bacteriuria. Gram's stain of uncentrifuged urine with diagnostic accuracy value of 96.61% was observed to be the best among the screening tests which were evaluated. Addition of combined dipstick testing (Nitrite and Leucocyte esterase) with urine microscopy will improve further its diagnostic performance.

Screening at the earliest may reduce the sequelae of asymptomatic bacteriuria during pregnancy preferably in the first trimester by initiating antimicrobial treatment early in pregnancy. Screening is also important in all three trimesters to prevent the dangerous complications associated with asymptomatic bacteriuria during rest of the pregnancy. The present study showed high occurrence of asymptomatic bacteriuria in pregnant women which if not treated, might lead to various maternal and neonatal complications. Hence, screening and management of asymptomatic bacteriuria need to be incorporated as a routine antenatal care for an integrated approach to safe motherhood and newborn health.

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