

Original Research Article

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Evaluation of different screening tests for detection of Urinary Tract Infections in pregnant women attending the antenatal clinic

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ABSTRACT

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Detection of asymptomatic bacteriuria (ASB) and its treatment in pregnant women is important. It will be helpful in early detection of Urinary tract infection (UTI) and prevention of untoward future complications in the mother and baby due to UTI. Aim- To know most prevalent bacterial pathogen causing UTI and evaluate different screening tests in comparison with culture method in the diagnosis of UTI. Material and method-Total 300 midstream urine specimens were processed. On one part of sample semiquantitative culture was done and on another part various screening test (wet mount, Gram's stain, catalase test, triphenyl tetrazolium chloride test and modified Griess nitrate test) were performed and compared with culture. Result-Out of 300 urine samples, 138(46%) samples were culture positive. *E. coli* was most common bacteria pathogen isolated. Among screening tests, modified Griess nitrate test and TTC test were more specific. While catalase test and Gram stain were both sensitive and specific. wet mount examination found to be the least useful test. Conclusion- A combination of screening tests should be used for diagnosis of UTI as there is no single test with 100% sensitivity and specificity in resource limited area still culture remain gold standard for diagnosis of UTI.

Introduction

Infection of the urinary tract (UTI) is a common problem in pregnancy due to the morphological and physiological changes that takes place in the genitourinary tract. In addition, other factors like low socio economic status, multiparity, illiteracy, medical disorders like diabetes mellitus & sickle trait are also associated with increased incidence of UTI in pregnancy. It may be of two types, symptomatic or asymptomatic.¹ Symptomatic bacteriuria poses no problems because of its easy

diagnosis and treatment due to its overt symptoms but asymptomatic bacteriuria (ASB) is difficult to diagnose. Pregnant women with ASB are more likely to develop acute pyelonephritis in later pregnancy, postpartum urinary tract infection, hypertensive disease of pregnancy, anaemia, chronic renal failure, prematurity, low birth weight babies and prenatal death if untreated. Therefore to prevent untoward complications in the mother and baby that may arise due to ASB, it has been suggested

to do routine screening for all pregnant women attending the Antenatal clinic even in the absence of symptoms.^{2,3}

Many prompt diagnostic methods are available including screening tests like wet mount microscopy, Gram stain, catalase test, Nitrate test, Triphenyl tetrazolium chloride test, but gold standard method for diagnosis of UTI is quantitative urine culture.⁴ Urine culture is an expensive, time consuming procedure and needs a well-equipped microbiology laboratory with experienced technicians.⁵ While On the other side, Screening tests of urine sample are designed to allow early detection of infection in the emergency department and an earlier initiation of the treatment.⁶ They are inexpensive, less time-consuming, easy to perform, no expertise required to perform and are useful in small laboratories having no culture facility.

Due to clinical significance of early diagnosis of UTI, different screening tests are used widely, but their performance characteristic are still questionable.⁷ The aims of the present study were 1.To study the prevalence of bacterial pathogens causing urinary tract infection among pregnant women 2.To evaluate the accuracy of different screening test for diagnosis of UTI, keeping semi quantitative culture as the gold standard.

Materials and Methods

The study was carried out in the Department of microbiology, Dr. V.M. government medical college Solapur, Maharashtra in women coming for their antenatal check-up in obstetric OPD. They were asked to collect clean catch midstream urine sample in a sterile wide mouth container after proper explanation. Total 300 urine sample were collected and each of them was divided

into 2 parts – one for culture and the other for screening tests.

Urine was cultured on Blood and MacConkey agar using a calibrated loop of 4 mm in diameter. After overnight incubation at 37 °C for 18 to 24 hours on, if bacterial counts $\geq 10^5$ per ml were seen, they were taken as positive in asymptomatic women. (as per Kass concept of significant bacteriuria).⁸ The isolates were identified by colony morphology, gram stain and biochemical reactions.⁹ The antibiotic susceptibility testing (AST) was done by Kirby-Bauer disc diffusion method on Muller Hinton agar for following antibiotics - Norfloxacin (10µg), Nitrofurantoin (300 µg), Cephalexin (30 µg), Amoxicillin (25 µg), Gentamicin (10µg) and ciprofloxacin (5µg) (Hi-media Mumbai).¹⁰ Following screening tests were done.

1. Direct wet mount⁸: Pus cells/high power field (hpf) were counted. About 20 fields were searched. Finding >1 pus cell/ 7 hpf indicates significant pyuria. Apart from pus cells, RBC, any casts, bacteria, yeast cells were also noted.

2. Gram Staining⁸: The presence of ≥ 1 bacteria/Oil immersion field in 20 fields correlated with the diagnosis of significant bacteriuria of $\geq 10^5$ CFU/ml of urine.

3. Catalase test¹¹: Four drops of 10% hydrogen peroxide were added in 2 ml of urine in the test tube. The mixture was shaken gently for 5 seconds. Formation of effervescence to form a complete ring or layer on the surface of the liquid within 1 to 2 minutes was taken as positive test. Positive control- *Staphylococcus aureus* and Negative control - *Enterococci* species.

4. Triphenyl tetrazolium chloride (TTC) test¹²: 2 ml of urine was taken in a sterile test

tube and 0.5 ml of working triphenyl tetrazolium chloride reagent was added. This mixture was incubated at 37⁰C for 4 hours. Formation of red precipitate indicated a positive test.

5. Modified Griess Nitrite Test ¹³: 8 ml. of urine was taken in a test tube and centrifuged this for 15 minutes. The supernatant was decanted. To the precipitate, 0.5 ml. of a 10% solution of potassium nitrate was added. This was incubated for one and half hour at room temperature. Then, 1 ml of the Griess reagent (0.5ml of solution A: Sulphanilic acid + 0.5ml of solution B: α -naphthylamine) was added to it. The development of a pink or a red colour in a matter of seconds was considered to be a positive test. Asepsis was strictly observed. Positive control – *E.coli* and Negative control – *Enterococci* species.

Results and Discussion

Out of 300 samples taken from pregnant females 138 (46%) showed significant bacteriuria by the semiquantative culture method. Most common organisms isolated was – *E.coli* (69.56%) followed by *Klebsiella* species (10.86%), *Proteus* species (5.07%), *Pseudomonas aeruginosa* (5.07 %), *Staphylococcus aureus* (4.34%), *Enterococci* species (2.89%) and *Acinetobacter* species (2.17%). For all the organisms isolated (Gram positive and Gram negative), nitrofurantoin was the most sensitive drug (94.20%), followed by cephalexin (89.85%), amoxicillin (88.40%), norfloxacin (86.95%), gentamicin (78.98%) and ciprofloxacin (71.01%). Evaluation of various screening tests in relation to the culture is as given below. (Table II and Table III).

The global prevalence of bacteriuria in

pregnancy varies from 4% to 23.9% in various studies.¹⁴ The prevalence of ASB in the present study was 46% which correlates with the studies done by Rani et al (51.2%).¹⁵ This high prevalence was attributed to their low socioeconomic status, lack of personal and environmental hygiene. The diagnosis of UTI is currently based on concept of quantitative bacteriuria, but for mass detection of UTI especially in risk groups like diabetics, antenatal cases and children in health surveys, reliable screening method is essential. The purpose of urine screening is to eliminate those specimens that do not contain significant number of bacteria.

In present study, sensitivity and specificity of wet mount (pyuria) was 63.04% and 86.42% respectively. Similar results were noted by Siddiqui et al.¹⁶ There were 38 samples in which pus cell were found in urine but there was no significant growth on culture media indicating either sterile pyuria pointing towards the noncultural organisms like Chlamydia as the causative agent or disintegration of the pus cells due to the presence of *Proteus*, *Klebsiella* and *Pseudomonas* in alkaline urine.¹⁵ Also the positive predictive value was less i.e. 69.60% making this test less useful in the diagnosis of urinary tract infection. According to Kass ¹⁷, there is a fair approximation between the presence of bacteria in direct smear and bacteriological counts. In present study we also observed similar correlation, out of 138 samples positive on culture, 108 had shown presence of ≥ 1 bacteria/Oil immersion field in 20 fields on Gram stain suggestive of significant bacteriuria. Sensitivity and specificity of Gram stain was 78.26% and 88.89% respectively which was comparable to results noted by Siddique et al¹⁶ and Yap Hui kim et al¹⁸.

Table.1 Frequency of Organisms Isolated from Urine Sample on Culture (N=138)

Organism isolated	Number (%)
<i>E.coli</i>	96 (69.56)
<i>Klebsiella species</i>	15 (10.86)
<i>Proteus species</i>	7 (5.07)
<i>Pseudomonas aeruginosa</i>	7 (5.07)
<i>Staphylococcus aureus</i>	6 (4.34)
<i>Enterococci species</i>	4 (2.89)
<i>Acinetobacter species</i>	3 (2.17)

Table.2 Statistical Analysis of Various Screening Tests with Respect to Culture. (n=138)

Tests	True positive (culture is positive screening test positive)	False positive (culture is negative screening test positive)	False negative (culture is positive screening tests negative)	True negative culture negative screening test negative)
Wet mount	87	38	51	124
Gram stain	108	18	30	144
Catalase test	131	15	7	147
TTC test	119	7	19	155
Modified Griess Nitrite test	125	9	13	153

Table 3 Sensitivity, Specificity and Predictive Value of Various Screening Tests

Tests	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Wet mount	63.04	86.42	69.60	70.85
Gram stain	78.26	88.89	85.71	82.76
Catalase test	94.93	90.74	89.73	95.45
TTC test	86.23	95.67	94.44	89.08
Modified Griess Nitrite test	86.95	94.44	93.02	89.47

In this study, Catalase test was found to be 94.94% sensitive and 90.74% specific. Similar results was noted by Siddique et al.¹⁶ There were 15 samples which were catalase positive but did not show any growth on culture media which might be due to haematuria in the patients. By Triphenyl Tetrazolium chloride(TTC) test we obtained 86.23% sensitivity and 95.67%

specificity. Other worker like Agrawal et al (81.3%)¹⁹ and Wagle et al (86.9%)²⁰ also noted similar sensitivity of test but low specificity compared to our study. According to Hinton et al¹², the frequency of positive TTC test was directly related to number of bacteria per ml of samples and not by other non-specific factors like glucose, ketones, urobilinogen, cast,

crystals, number of pus cells, RBCs in sample. But main drawback of test is that it was not well standardized because different bacteria respire at different rates. Specificity (94.44%) of Modified Griess nitrate test was more than Sensitivity (86.95%) in present study so test is more useful in rolling out UTI in patients. Similar finding were noted by Rani et al¹⁵ and Siddique et al¹⁶, while in contrast result was noted by Najeeb et al.⁴ Total 13 false negative samples were noted as nitrate test does not detect organisms unable to reduce nitrate to nitrite, such as Enterococci, Staphylococci species, Acinetobacter etc.²¹

There is variation in result from different studies while comparing performance of different screening tests, it may be because of different sample population in different studies like high risk population, Gender, Children or because of variation in procedure while performing tests.

Based on this study, a combination of screening tests should be used for diagnosis of UTI as there is no single test with 100% sensitivity and specificity. The selection of screening tests will be depend on the facilities available and patient load at the given laboratory. Screening tests are suitable for screening large population at high risk in resource limited area depending on that empirical treatment can be given to patient. But laboratories where adequate culture facilities are available, it remains the gold standard method for detection of urinary tract infection.

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