Research Article

Evaluation of screening tests to detect asymptomatic bacteriuria in obstretic patients at Noor Hospital, Warudi, Jalna

Sufia M Siddiqui[1*], Arvind B Deshmukh[2], Uzma Afreen[3], Prasad L Bhanap[4]

{[1]Associate Professor, [2]Professor &HOD, [3]Resident, Department of Microbiology} {[4]Professor and HOD, Department of OBGY}
IIMSR, Warudi, Jalna, Maharashtra, INDIA.
Email: imran1374@rediffmail.com

Abstract

Introduction: Urinary tract infections in pregnant women are a major cause for maternal and perinatal morbidity. The semiquantative culture is considered as the gold standard for the diagnosis of UTI in patients but considering the heavy load of Antenatal care women and the time consuming nature of this test, it becomes necessary to evaluate other tests for screening so that the diagnosis can be obtained at the earliest possible time and with the maximum sensitivity and specificity. **Aims and Objectives:** Five tests ie wet mount examination, gram stain of uncentrifuged urine, catalase test, triphenyl tetrozoleum test and nitrate reduction tests were compared with the semiquantative culture tests of 206 women attending the antenatal clinic at Noor Hospital, Warudi, Jalna. **Results**: Of 206 women, 18 showed significant bacteriuria with the semiquanitative culture technique. Of all these screening tests catalase test was found to be the most useful test and wet mount was the least useful one in assessing significant bacteriuria in pregnant females.
Keywords: Asymptomatic bacteriuria, semiquantative culture, screening tests, significant bacteriuria.

[*]**Address for Correspondence:**
Dr. Sufia M Siddiqui, Associate Professor, Department of Microbiology, IIMSR, Warudi, Jalna, Maharashtra, INDIA.
Email: imran1374@rediffmail.com
Received Date: 02/10/2015 Accepted Date: 10/10/2014

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	DOI: ---

INTRODUCTION

"Asymptomatic bacteriuria," or asymptomatic urinary infection, is isolation of a specified quantitative count of bacteria in an appropriately collected urine specimen obtained from a person without symptoms or signs referable to urinary infection[1]. This if untreated can lead to overt urinary tract infection in later months, acute cystits, acute pyelonephritis in mother and prematuriry, low birth weight, perinatal mortality and intrauterine growth retardation in foetus[2,3]. The relatively high prevalence of asymptomatic bacteriuria during pregnancy, the significant consequences for women and for the pregnancy, plus the ability to avoid sequelae with treatment, justify screening pregnant women for bacteriuria.[4] The urine microbiologic culture is considered the gold standard for laboratory diagnosis of UTI. It is the most accurate method to identify and quantify bacteria in the urine with high sensibility[5]. Its drawbacks are the relatively higher costs, the longtime needed to achieve the number of bacterial colonies necessary for a sensitive result and the need for professionals and laboratories qualified for its elaboration[6]. Hence it was decided to evaluate other tests like wet mount, gram stain, catalase test, triphenyl tetrazoleum test and nitrate test to screen for asymptomatic bacteriuria and to compare them with respect to sensitivity and specificity.

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology of Indian Institute of Medical Sciences and Research, Warudi after obtaining an approval from the ethical committee of the institute. All the antenatal women were asked to collect midstream urine sample in a sterile wide mouthed container and two samples were

How to site this article: Sufia M Siddiqui, Arvind B Deshmukh, Uzma Afreen, Prasad L Bhanap. Evaluation of screening tests to detect asymptomatic bacteriuria in obstretic patients at Noor Hospital, Warudi, Jalna. *MedPulse – International Medical Journal* October 2014; 1(10): 623-626. http://www.medpulse.in (accessed 14 October 2014).

collected at two separate visits. The samples so collected were divided into two parts One part was processed for culture and the other part was subjected for screening tests.

Culture

Urine was cultured on Blood and Mackonkey agar using a calliberated loop of 4 mm in diameter. After overnight incubation at 37^0Cfor 24 hours on culture plate, if bacterial counts equal or more than 10^5per ml were seen, they were taken as positive in asymptomatic women. (as per Kass concept of significant bacteriuria)[7]. Samples with less than these colonies were repeated .Samples showing significant growth and with a predominant single isolate were included in the study. The isolates were identified by colony morphology, gram stain and biochemical reactions. The Antibiotic Sensitivity Testing (AST) was performed as per CLSI guidelines.

Screening tests: Following screening tests were done.

Direct wet mount

0.05 ml of well mixed un-centrifuged urine sample was placed on a clean microscopic slide and a coverslip of dimension 22 x 22 mm was placed on the drop and was scanned in microscope with 10X objective lense, any sample showing pus cells was later on seen under microscope with 40x objective lens. Pus cells/high power field (hpf) was 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.[7]

Gram Staining

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 $\geq 10^5$ CFU/ml of urine[8]

Catalase test

1.5 to 2 ml of urine was placed in a test tube. Four drops of 10% hydrogen peroxide were added to the test tube, and the mixture was shaken gently for 5 seconds. A positive finding was defined as the formation of effervescence sufficient to form a complete ring or layer on the surface of the liquid within 1 to 2 minutes of the addition of the hydrogen peroxide. The test result was considered negative in the absence of effervescence or when the ring of effervescence was incomplete after 2 minutes.[9]

Positive control- *Staphylococcus aureus*

Negative control - *Enterococci spp.*

Triphenyl tetrazolium chloride test (TTC)

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 37ºC for four hours. Formation of red precipitate indicated a positive test.[10]

Modified Griess Nitrite Test

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 nitratewas added. This was incubated for one and half hour at room temperature. Then, 1 ml of the Griess reagent.ie 0.5ml of solution - A: Sulphanilic acid and0.5ml of solution B: αnaphthylamine were added to it. The development of a pink or a red color in a matter of seconds was considered to be a positive test. Asepsis was strictly observed.[11,12]

Positive control – *E.coli*

Negative control – *Enterococci spp*.

RESULTS

Out of 206 pregnant females 18 (8.7%) showed significant bacteriuria by the semiquantiative culture method. Assessment of various screening tests in relation to the culture is as follows.

Table 1: Statistical analysis of various screening tests with respect to culture

Tests	True positive (culture is positive screening test positive)	False positive (culture is negative screening test positive)	False negative (culture is positive sreeening tests negative)	True negative culture negative screening test negative)
Wet mount	11	25	7	163
Grams stain	14	12	4	176
Catalase test	17	8	1	180
TTC test	10	4	8	184
Modified Griess Nitrite test	13	13	5	175

Table 2: Sensitivity, specificity and predictive value of various screening tests

Tests	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Wet mount	61.1	86.70	30.56	95.88
Grams stain	77.77	93.61	53.85	97.78
Catalase test	94.44	95.75	68	99.44
TTC Test	55.55	97.87	71.43	95.83
Modified Griess Nitrite test	72.22	93.09	50.00	97.22

As shown above, catalase test showed both high sensitivity and specificity but the positive predictive value was less than TTC test (table2)

DISCUSSION

The screening tests were evaluated in relation to the semiquantitative culure method. The sensitivity and specificity of wet mount (pyuria) was 61.1%and 86.7%respectively.There were 25 samples in which pus cell were found in urine but there was no significant growth on culture media indicating sterile pyuria pointing towards the noncultural organisms like Chlamydia as the causative agent. Thereby wet mount was less sensitive than that shown by Usha Rani *et al*[12] and more than that shown by Lavanya *et al*[13] Also the positive predictive value was very less i.e. 30.56% making this test less useful in the diagnosis of urinary tract infection. In gram stain, we had observed no. of bacteria per OIF in an uncentrifuged sample and the sensitivity and specificity was also higher(77.78% and 93.62%respectively).The sensitivity and specificity of gram stain as shown by Ali was 92.5% and 98.8% respectively.[14] and Bachman *et al* was 91.7% and 89.2% respectively.[15] Our sensitivity of gram stain corresponded with that shown by Yap Hui kim *et al*l i.e. 80% but specificity was more than that shown by them (83%)[16] Thus it was observed that there is a fair approximation between the presence of bacteria in direct smear and bacteriological counts as said by Kass in 1957.[17] Catalase test was found to be 94.44% sensistive and 95.75% specific .which was more than that shown by Usha Rani *et al*[12] and Lavanya *et al*[13]. There were 8 samples which were catalase positive but did not show any growth on culture media which might be due to haematuria (as seen in wet mount) in the patients. The positive predictive value was 68% making it a useful test in the diagnosis of asymptomatic bacteriuria. The TTC test showed a high positive predictive value of 71.43% and specificity of 97.87% but low specificity of 55.55%. Lavanya *et al*[13] and Usha Rani *et al*[12] have also shown a high specificity of TTC test as compared to its sensitivity. This test because of low sensitivity fails to qualify as a good screening indicator of urinary tract infection. Nitrate reduction test: The sensitivity of this test was 72.22% which was more than that shown by Stephen Berger *et al* (41.5%)[18] by Brigul Kacmaz *et al* (60%)[19] and Ali *et al* (66.66%)[14] but less than that shown by Usha Rani *et al*(87.5%) (creeen 2).Specificity of modified Greiss nitrite test was found to be very high93.9% which corresponded to that shown by Berger *et al*(92.3%)[18] but was shown to be less than that shown by other authors.[12,19,14] Next to catalase test, this test can also be used as a good screening test for detection of significant bacteriuria in urine.

CONCLUSIONS

As per our findings, catalase test was found to be the best screening test followed by Modified Greiss Nitrite test and Gram stain. The test adapted by any laboratory will depend on the facilities available there and patient load at the given laboratory. If culture facilities are not available at the given lab then a prelimnary investigation of gram stain can provide a good indicator of infection in that patient. Also if facitlities are available there is no substitute to semiquantitative culture method which stands as the gold standard in the diagnosis of urinary tract infection.

REFERENCES

1. Nicole LE, Badley S, Colgan R *et al*. Infectious disease society of American guidelines for diagnosis and treatment of Aymptomatic Bacteriuria in adults. *Clin Infect Dis* 2005; 40: 643-54.
2. Kass EH. Pregnancy, pyelonephritis and prematurity. *Clin Obstet Gynecol*. 1970; 13:239–54.
3. Harris RE, Thomas VL, Shelokov A. Asymptomatic bacteriuria in pregnancy: antibody-coated bacteria, renal function, and intrauterine growth retardation. *Am J Obstet Gynecol*. 1976; 126:20–5.
4. JOHN E. DELZEL and MICHAEL L. LEFEVRE, Urinary Tract Infections During Pregnancy *Am Fam Physician.* 2000 Feb 1; 61(3):713-720.
5. MacLean, A.B. (2001) Urinary tract infection in pregnancy. Int J Antimicrob Agents, 17, 273-276; discussion 276-277.
6. Rosen, D.A., Hooton, T.M., Stamm, W.E., Humphrey, P.A. and Hultgren, S.J. (2007) Detection of intracellular bacterial communities in human urinary tract infection. PLoS Med, 4, e329.
7. Collee JG, Duguid JP, Fraser AG, Marmion BP, Simmons A. Laboratory strategy in the diagnosis of infective syndromes. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney, practical medical microbiology. 14th ed. Edinburgh: Churchill Livingstone; 1996. p. 84-90.
8. Pezzlo MT: Laboratory diagnoses of urinary tract infections: Current concepts and controversies, Infect Dis Clin Pract 2; 469, 1993.
9. Palmer LS, Richards I, Kaplan W. Clinical evaluation of a rapid diagnostic screen (Uriscreen) for bacteriuria in children. J Urol 1997; 157:654-657.
10. Simmons and Williams. A simple test for significant bacteriuria. Lancet 1962; 1: 1377.
11. Srihari. A*, Beeregowda Y.C, Vishnu Vardhan Reddy. T. A comparitive study of griess nitrate test and urinary culture in detection of asymptomatic bacteriuria in children.
12. Usha Rani. V, Sudhindra K. S, Nirmala Grace. B, Pradeep.G, Aanchal Kapoor, Shilpa. L Evaluation of various screening tests for detection of Urinary tract infections. Journal of Biomedical and Pharmaceutical Research 3 (2) 2014, 15-20

13. Lavanya, S.V., and Jogalakshmi, D. Asymptomatic bacteriuria in antenatal women. Indian J. Med. Microbiol. 2002 (2):105-106.
14. May Mohamed Ali .Evaluation of antimicrobial susceptibility andrapid urine screening tests in asymptomatic urinary tract infection in pregnant women in Karbala .K. J. Pharm . Sci 2011; No.2:22-34
15. Bachman JW, Heise RH, Naessens JM and Timmerman MG. A study of various tests to detect asymptomatic urinary tract infections in an obstetric population. JAMA 1993; 270(16):1971-1974.
16. Yap Hui Kim, Belinda Murugasu Urinary tract infection in children Paediatrics Shared care programme. National university of health Bulletin 6 september 1998 ;1-9
17. Kass, E. H. 1956. Asymptomatic infections of the urinary tract. Trans. Assoc. Am. Physicians 69:56-63.
18. Stephen A. Berger, Bianca Bogokowsky, and Colin block Rapid Screening of Urine for Bacteria and Cells by Using a Catalase Reagent Journal of Clinical Microbiology, May 1990, Vol 28, No5: 1066-1067
19. Birgul Kacncymaz, Ozenc Cakir, Altan Asoy, Adyan Biri. Evaluation of Rapid Urine Screening tests to detect Asymptomatic Bacteriuria in pregnancy Jpn.J.Infect.Dis2006; 59,261-263.

Source of Support: None Declared
Conflict of Interest: None Declared