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Original Research Article

EVALUATION OF THE ROLE OF SCREENING TESTS AND CULTURE IN THE DETECTION OF ASYMPTOMATIC BACTERIURIA IN PREGNANCY

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ABSTRACT

Background: Relatively high prevalence of asymptomatic bacteriuria during pregnancy, significant consequences for women and for pregnancy, and ability to avoid sequelae with treatment, clearly justify need of screening pregnant women for bacteriuria. But this area remains unexplored. **Objective:** To study prevalence of asymptomatic bacteriuria in antenatal women, to identify common pathogens involved, with their antibiotic sensitivity pattern.
Material and Methods: Hospital-based, cross-sectional, clinico-microbiological study conducted among 106 pregnant women aged 18-45 years. 100 non-pregnant women in same age group, with no history of symptoms suggestive of Urinary Tract Infection, 50 of whom were married, and 50 unmarried, were Control subjects. Socio demographic data, previous history of UTI, and history of Renal calculi were collected from all subjects, by using a pre-structured questionnaire.
Results: Prevalence of asymptomatic bacteriuria was 10.5% in antenatal women compared to only 2.3% in non-pregnant women in 20-25 years. It was 22.1% vs. 3.6% in 26-35 years. It was more in third trimester than first and second trimester; more in primi than multi. With gram stain and motility test with TTC positivity was 17.9% while it was 19.8% with leukocyte esterase test and only 8.5% with griess nitrite test. Staphylococcus aureus was 100% sensitive to ampicillin/sulbactam, piperacillin/tazobactam, ticarcillin/clavulanic acid. Enterococci were sensitive to all the antibiotics used for sensitivity. Gram positive bacilli were 100% sensitive to ampicillin/sulbactam, piperacillin/tazobactam, ticarcillin/clavulanic acid.
Conclusion: A combination of three Screening tests - Leukocyte esterase test which demonstrated a sensitivity of 100%, a negative predictive value of 100%, a specificity of 97.70%, will provide an acceptable alternative to screening all asymptomatic pregnant women with urine culture.
Keywords: screening test, bacteriuria, pregnancy.

INTRODUCTION

Urinary tract infections (UTI), caused by the presence and growth of microorganisms in the urinary tract, are perhaps the single commonest bacterial infections of mankind.[1] Predominantly a disease of females, the prevalence of bacteriuria increases gradually with time, from 1-3% in girls between 5 to 17 years of age, to as high as 10% to 20% in older women.[2] The shortness of the urethra, its proximity to the anus, and urethral trauma during intercourse, cause women in the sexually active age group, particularly, pregnant women, to be more prone to Urinary tract infection.[3] Urinary tract infections (UTIs) in pregnant women continue to pose a clinical problem and a great challenge for physicians. Although the incidence of bacteriuria in this population is only slightly higher than in non-pregnant women, its consequences for both the mother and the unborn child are more severe. There is a much higher risk (up to 40%) of progression to pyelonephritis, and possibly increased risk of pre-eclampsia, premature birth and low neonatal birth weight,[4] that is related to profound structural and

functional urinary tract changes, typical for pregnancy. Common symptoms include urgency, and frequency of micturition, associated with discomfort or pain. Upper UTI is characterized by loin pain, tenderness, and high fever with rigors, while lower UTI usually presents with dysuria, frequency, and fever with chills. However, the anatomical and hormonal changes in pregnancy can sometimes cause UTI without any symptoms. Asymptomatic Bacteriuria is the presence of actively multiplying bacteria in an appropriately collected urine specimen, obtained from a person without symptoms or signs of Urinary tract infection. It is commonly defined as the presence of more than 100,000 organisms/ml in two consecutive urine samples in the absence of declared symptoms. Untreated asymptomatic bacteriuria is a risk factor for acute cystitis (40%) and pyelonephritis (25-30%) in pregnancy. These cases account for 70% of all cases of symptomatic UTI in unscreened pregnant women.[5] Untreated bacteriuria can have serious implications such as intra uterine growth retardation, premature birth, low birth weight and perinatal death in foetus and hypertension, pre-eclampsia, anaemia, amnionitis and endometritis in the mother. Early detection of this condition is therefore extremely important. The traditional reference test for bacteriuria is the quantitative culture of urine, which is relatively expensive, time consuming, laborious and needs the infrastructure of a laboratory and of qualified trained staff. This is not available in many areas of the world, especially in regions where preterm delivery, low birth weight and other complications of asymptomatic bacteriuria are associated with higher morbidity and mortality. Hence the best screening test which can be used remains to be determined. The relatively high prevalence of asymptomatic bacteriuria during pregnancy, the significant consequences for women and for the pregnancy, and the ability to avoid sequelae with treatment, clearly justify the need of screening pregnant women for bacteriuria. But this area has not yet been adequately explored.[6]

The present study was undertaken, to study the prevalence of asymptomatic bacteriuria in antenatal women, to identify the common pathogens involved, with their antibiotic sensitivity pattern, to compare the sensitivity of various screening methods (Microscopy of wet Mount, Gram-staining, Greiss Nitrite test, Leukocyte esterase test, Triphenyl tetrazolium chloride test, Motility test) with culture, and to find a single or combined rapid screening method, which may provide an acceptable alternative to culture, with guidance for empirical treatment, in situations where culture facilities are unavailable or unaffordable. In other situations where Urine culture and Antibiotic Sensitivity can be done, the alternative provided in the present study can help provide an early report, and thereby, facilitate early treatment.

MATERIALS AND METHODS

The present study was a hospital-based, cross-sectional, clinico-microbiological study conducted in the Dept. of Microbiology, MNR Medical college, Sangareddy, from February 2016 to June 2017. 106 pregnant women aged 18-45 years from among those attending the Obstetrics and Gynaecology Out Patient Department, and those admitted in the Antenatal ward, MNR Hospital, were selected for the study. 100 non-pregnant women in the same age group, with no history of symptoms suggestive of Urinary Tract Infection, 50 of whom were married, and 50 unmarried, were selected to be Control subjects. The study was conducted after approval by the institutional Research & Ethics Committee. Informed consent was obtained for sample collection and for enrolment in the study, from participants of both groups – pregnant and non-pregnant women, selected for the study. Socio demographic data such as age, occupation, annual income, parity, previous history of UTI, and history of Renal calculi if any, were collected from all Antenatal as well as Control subjects, by using a pre-structured questionnaire.

Inclusion Criteria

- All Antenatal women registered as Outpatients or Inpatients in the Department of Obstetrics & Gynaecology, MNR Hospital, irrespective of their period of gestation, and
- Antenatal women NOT treated with Antibiotics for at least 4 weeks prior to their visit to the hospital, were included in the study.
- Non pregnant women in the same age group, with no history of symptoms suggestive of Urinary tract infection were included as Control subjects.

Exclusion Criteria

- Antenatal women with symptoms suggestive of UTI,
- Those with known congenital anomalies of the urinary tract,
- Those with vaginal discharge or bleeding per vagina, and
- Those with Diabetes and Hypertension were excluded from the study.

Specimen Collection & Transportation

Each participant was given two sterile leak-proof containers for the collection of a single urine sample. Samples from hospitalized patients were collected before the administration of antibiotics. Clear instructions were given to each participant 41 in the study, regarding how to collect a clean catch mid-stream urine specimen in order to reduce chances of contamination. Each container was labelled with a unique number, date and time of collection. The specimens were transported to the Microbiology laboratory soon after collection, and processed within one hour. A second specimen was collected from all pregnant and non-pregnant subjects who were Culture positive, and processed again, for confirmation.

Macroscopic Examination

All urine samples were first observed macroscopically for color, turbidity and deposits if any. The findings were recorded, and the samples were then subjected to various tests

Screening Tests

Each specimen of uncentrifuged urine from all 106 Antenatal women, and from the 100 non-pregnant women who served as Controls in the present study, was subjected to a) Microscopy - Wet Film b) Microscopy - Gram stain Examination, c) Dip Stick Tests - Griess Nitrite test, Leukocyte esterase test, pH estimation d) Motility test with Triphenyl tetrazolium chloride, and e) Dip Slide Culture – on CLED, MacConkey's and Bile Esculin media.

RESULTS

Table 1: Prevalence of Asymptomatic Bacteriuria in ANCs & non-ANCs

Factors		ANCs			Non-ANCs		
		No. tested	No. cult. +ve	% cult. +ve	No. tested	No. cult. +ve	% cult. +ve
Age (years)	20-25	38	4	10.5	44	1	2.3
	26-35	68	15	22.1	56	2	3.6
Monthly income (INR)	Up to 10,000	108	19	17.6	94	3	3.2
	> 10,000	0	0	0	6	0	0
1st & 2nd trimester		73	10	13.7	--	--	--
3rd trimester		32	9	28.1	--	--	--
Primi		37	10	27.1	--	--	--
Multi		69	9	13.1	--	--	--

The prevalence of asymptomatic bacteriuria was 10.5% in antenatal women compared to only 2.3% in non-pregnant women in the age group of 20-25 years. It was 22.1% vs. 3.6% in 26-35 years of age group. It was 17.6% vs. 3.2% in low-income group and null in higher income group in both groups. It was more in third trimester compared to first and second trimester. It was more in primi compared to multi.

Table 2: Various tests of urine samples in antenatal women

Name of test	Positive	Negative
Gram stain	19 (17.9%)	87 (82.1%)
Leukocyte esterase	21 (19.8%)	85 (80.2%)
Griess Nitrite test	9 (8.5%)	97 (91.5%)
Motility test with TTC	19 (17.9%)	87 (82.1%)

With gram stain and motility test with TTC the positivity was 17.9% while it was 19.8% with leukocyte esterase test and only 8.5% with griess nitrite test.

Table 3: Antibiotic sensitivity pattern

Name of drug	Staphylococcus aureus sensitivity (%)	Enterococci sensitivity (%)	Gram negative bacilli sensitivity (%)
Amoxycillin	20	100	27.3
Amoxiclav	80	100	54.5
Ampicillin	20	100	27.3
Penicillin	20	100	Not done
Ampicillin/sulbactam	100	100	100
Piperacillin/tazobactam	100	100	100
Ticarcillin/clavulanic acid	100	100	100
Nitrofurantoin	80	100	81.8
Azithromycin	40	100	63.6
Erythromycin	20	100	45.5
Vancomycin	60	100	72.7

Staphylococcus aureus was 100% sensitive to ampicillin/sulbactam, piperacillin/tazobactam, ticarcillin/clavulanic acid. Enterococci were sensitive to all the antibiotics used for sensitivity. Gram positive bacilli were 100% sensitive to ampicillin/sulbactam, piperacillin/tazobactam, ticarcillin/clavulanic acid.

DISCUSSION

The prevalence of Significant Asymptomatic Bacteriuria in pregnancy studied in relation to age, showed that the 26 - 30 yrs age group showed the highest incidence of 22.72%, followed by the < 20 yrs age group who showed an incidence of 10.53%, the 21-25 yrs age group, who showed an incidence of 08.00%, while the 31-35 yrs age group showed an incidence of 0%. The Control group of 100 Non pregnant women also showed the prevalence of Asymptomatic Bacteriuria in the same age groups. But while in Antenatal women, it was 22.72%, in the 26-30 yrs age group, the prevalence was only 4.88% in the non-ANCs, thereby showing that pregnant women are more prone to Asymptomatic Bacteriuria.

These findings were similar to those reported in other studies done by Schnarr J et al,[7] (53.1%) who also found a higher prevalence in the 26-30 yrs. age group. When studied in relation to the socio-economic status, both the Study group & the Control group showed a higher prevalence of Asymptomatic Bacteriuria in the lower socio-economic group, who had an income of less than Rs. 5000/ per month. These findings are probably indicating that this group of individuals have a poor sense of hygiene, especially during pregnancy, making them prone to Urinary Tract Infection. Studied Trimester wise, our study showed a higher prevalence of Asymptomatic Bacteriuria in the 3rd trimester (28.13%), followed by those in the second trimester who showed an incidence of 14.49%, while those in the first trimester showed an incidence of 0%. These observations are different from Schnarr J et al,[7] who found the highest incidence of Asymptomatic Bacteriuria in their studies to be in the 1st trimester.

Parity wise, our study showed that the prevalence of Asymptomatic Bacteriuria was higher in Primi gravidae (27.03%), compared to Multigravida (13.04%). The midstream urine samples from all 106 pregnant women, and from the 100 Non pregnant women chosen to serve as Controls, were subjected to Screening tests & Regular Culture. Since Regular Bacterial Culture is the Gold Standard for the detection of Asymptomatic or Symptomatic Bacteriuria, the Diagnostic efficacy of each Screening test was assessed by comparing it with Culture results. Wet Mount - Microscopic Examination: In the Wet Mount Microscopic Examination of 106 freshly passed uncentrifuged midstream urine specimens, Pus cells were seen in a total of 11 (10.38%) samples, out which 8 (7.54 %) showed plenty of pus cells, 03 (2.83 %) showed occasional pus cells, while the remaining 95 (89.62%) samples showed no pus cells.

Pyuria is the presence of an increased number of polymorphonuclear leukocytes in the urine (generally >10 WBC/hpf) and is evidence for genitourinary tract inflammation, whether due to Asymptomatic Bacteriuria. catheter use, sexually transmitted diseases, renal tuberculosis, or interstitial nephritis. The absence of pyuria is a strong indicator that UTI is not present and is hence, useful in ruling out a UTI. Since pyuria is thus indicative of infection, it is important to assess the efficacy of Wet Film Examination in comparison with Culture. In the present study, we found that while culture positivity was 17.92%, Wet Film Microscopy positivity was only 10.37%,

Microscopic Examination - Gram's Stain: Examination of the Jensen's modification of Gram Stain of uncentrifuged urine samples from 106 Antenatal women screened for Asymptomatic Bacteriuria, showed bacteria in a total of 19 (17.2%) samples, out of which, Gram Positive Cocci were seen in in 7 (38.88%), and Gram-Negative Bacilli were seen in 12 (61.11%) samples. No bacteria were seen in the remaining 87 (82.07%) samples. Our findings are in agreement with all the other studies mentioned in the table above, showing a predominance of Gram-Negative bacilli over Gram Positive cocci. One of the specimens with Gram Negative bacilli also showed Gram Positive budding yeast cells.

All 19 culture isolates (17.92%) were visualised in the Direct Gram's Stain (17.92%). There was one false +ve, and one false -ve, possibly because of low numbers of bacteria. On the whole, Gram's Stain, in our study, has proved to be a good indicator of the presence of bacteria, with a diagnostic efficacy close to that of culture. The drawback however, is that the bacteria cannot be specified, especially in the case of Gram-Negative Bacilli.

Leucocyte Esterase test: The Leukocyte Esterase test done for all 106 midstream urine samples by the Dip Stick method showed that a total of 21 urine samples tested positive for the enzyme, while the remaining 85 samples tested negative. Out of these, 9 (8.49%) samples showed 125 + pus cells; 8 (7.5%) samples showed 70 + pus cells; 4 (3.77%) samples showed 15 + pus cells, and 86 (81.1%) samples showed no pus cells. A test positive for the enzyme Leucocyte esterase indicated the presence of pus cells in urine, and is thus, a fairly good indicator of Bacteriuria.

All 19 samples which were culture positive were also positive by the Leucocyte esterase test. Two samples which were positive for the enzyme, but did not show any growth in culture, were probably cases of Sterile Pyuria. Almost equal to the results of Culture, the detection of this enzyme proved to be very valuable in predicting Asymptomatic Bacteriuria.

Griess Nitrite test: Griess Nitrite test is a nitrite detection test as it measures nitrite in a sample, based on chemical diazotization reaction, which results in change of urine color to purple.[8] The test done for the detection of Nitrate reducing bacteria in the 106 midstream urine samples tested was positive for 9 (8.49%) samples, and negative for the remaining 97 (91.5%). The samples that tested negative included some samples that showed NO bacterial growth in culture, some non-nitrate reducing bacteria like Staphylococci, as well as some Nitrate reducing bacteria that could not be detected. Since it is required that urine stays in the bladder for up to 4 hours to allow accumulation of detectable nitrite levels, these false negative samples were possibly collected before 4 hours.[9]

pH Estimation: From among 106 midstream urine samples subjected to the Dipstick test, 4 urine samples showed a pH of 7.1 to 7.5, 13 urine samples showed a pH of 6.6 to 7.0, 2 urine samples showed a pH of 6.1 to 6.5, and 87 urine samples showed a pH of 5.6 to 6.0. Although Bacteriuria is known to be associated with an Alkaline pH, the present study showed that some urine samples that were Culture positive had a neutral, or in a few cases, a slightly acidic pH as well. Hence, it was concluded that the identification of pH is not of much value.[10]

Motility test with TTC: Of the 106 urine samples subjected to this two-in-one test, 7 (6.06%) showed Growth with motility, 12 (11.32%) showed Growth with NO motility, & 87 (82.07%) showed NO Growth at all. Culture results however, show the growth of motile bacteria in 10 (9.43%) urine samples, nonmotile bacteria in 9 (8.49%) urine samples, and no bacterial growth in 87 (82.07%) samples. This test has thus missed detection of motile bacteria in 3 (2.83%) urine samples, probably because of low numbers of bacteria.

The test is usually more than a tenth cheaper than culture.[11] It is simple to perform and results are available immediately. It does not need special testing field and even an improvised environment will do.[12] The Dip Slide Cultures of all 106 midstream urine samples showed the growth of all 19 bacteria (100%) that grew in regular Culture (100%). The same media were used in both cases.

Growth in Culture: Of the 106 pregnant women included in this study, 26 (24.53%) were culture positive. 19 (17.92%) had Significant Bacteriuria, 7 (6.60%) had Nonsignificant Bacteriuria, and 80 (80.71%) showed NO Bacterial Growth. Our study thus shows an overall prevalence rate of 17.92% Significant Asymptomatic Bacteriuria in pregnancy.

Of the 100 non-pregnant women included in the study, 3 (3%) had Significant bacteriuria while the other (93%) women showed NO Bacterial Growth. The overall prevalence of Asymptomatic Bacteriuria of 17.92% (19/106) which was almost similar to a study done in Lucknow (16.9%).[13]

Organisms grown in Culture: Of the 19 (17.92%) isolates from midstream urine samples of 106 Antenatal women on CLED, Blood agar, and MacConkey's agar, Esch. coli was isolated in 8 (42.10%) samples, Klebsiella pneumoniae was isolated in 2(10.52%) samples, Proteus mirabilis. in one (5.26%) sample, Citrobacter in 1(5.26%) sample, Staphylococcus aureus was isolated in 1(5.26%) sample, Staphylococcus saprophyticus was isolated in 4 (21.05) samples, and Enterococcus faecalis was isolated in 2 (10.52%) urine samples These findings were similar to those of study from Nepal,[14] Iran,[15] Raichur 16 and 17 showed higher and lower prevalence rates of 26%, 8.9%, 9% and 3.6% respectively. Sri Lanka

None of the samples in our study showed polymicrobial growth. Gram negative isolates were more in number than Gram positive isolates, with E. coli (42.11%) being the most common followed by S. Saprophyticus (21.05%). Most of the other studies 14-16 have reported E. coli as the most common pathogen but with higher isolation rates than our study (72.72%, 77.77%, 70.8%, 67%). But one study done in Nigeria 18 showed S. aureus as the most common pathogen (72%) and E. Coli being the least common (2%).

The results of drug sensitivity in this study revealed that 100% of isolates of GNBs were sensitive to doripenem, etrapenem, imipenem and meropenem. 81.81% were sensitive to nitrofurantoin followed by 72.72% of isolates which were sensitive to ciprofloxacin, piperacillin/tazobactam, imipenem, cotrimoxazole and norfloxacin followed by other drugs. It was found in the present study that the sensitivities to amoxicillin-clavulanic acid (54.54%), nalidixic acid (36.36%), ampicillin (27.27%) and cefuroxime (36.36%) which are used as drugs of choice in treating Asymptomatic Bacteriuria were comparatively lower, posing problems in treating these patients. E. coli, the predominant isolate in this study showed a sensitivity pattern of (100%) to doripenem, etrapenem, imipenem and meropenem. (81.81%) to, amikacin and nitrofurantoin (72.72%) to ciprofloxacin, ceftriaxone, levofloxacin, co trimoxazole, piperacillin/tazobactam and norfloxacin (54.54%) to ampicillin, cephotaxime, cefazolin, ampicillin/sulbactam ticarcillin/clavulanic acid, cephoxitin, nalidixic acid and (42.85%) to cefuroxime, gentamycin.

Similar sensitivity rate of E. coli to ciprofloxacin (96.9%) was seen in a study done in Kashmir 19 and to cefotaxime (88.62%) in a study done in Iran.[15] However contrasting results were reported in the same study 15 with respect to gentamicin (5.07%), nitrofurantoin (29.12%) and nalidixic acid (18.99%). The few contrasting results observed in different studies may be due to differences in the choice of drugs used for empirical treatment.

Assessment of the Diagnostic efficacy of different Screening tests: An ideal screening test should be simple, rapid and accurate and must identify all positive cases. Thus, a sensitive test with a high negative predictive value is desirable. In the present study, four screening tests: Gram's stain of uncentrifuged urine, pus cell count, Dip sticks (nitrite test and leukocyte esterase tests) and Dip slide cultures were evaluated. It was demonstrated that, Gram stain of uncentrifuged urine was the most useful single test with Negative Predictive Value (NPV) of 96.6%. Studies done by Gayathree et al 20 and Jayalakshmi et al,[21] showed similar NPV (98.28%, 98.8%) as our study. Though the pus cell count of unspun urine is a very accurate method, it is very cumbersome and gave a low sensitivity of 52.63% in our study. The low sensitivity of pyuria observed may be due to loss of cells in the handling of the sample and the transfer on the slide.

Griess nitrite test demonstrated a high specificity of (100%) and Positive Predictive Value (PPV) (100%) but less sensitivity (81.82%) and negative predictive value (97.93%) compared to Gram's stain. This was because six positive cases were missed as false negatives that included all four infections caused by gram-positive cocci, indicating that even though the organism is present it may not produce nitrate reductase. A study done by Mokube et al,[22] showed similar specificity (98.7%) of nitrite test but much less sensitivity (8%), PPV (67%) and NPV (77.8%) compared to our study. Leukocyte esterase test gave sensitivity (100%) and negative predictive value (100%) comparable with nitrite test. But because of 2 false positives, which included patients with sterile

pyuria were identified, the specificity was lower (98.8%) than the other test. Other studies 20-22 showed much less sensitivity of leukocyte esterase test (61.29%, 61.7% and 20.8%) than our study. In our study, both nitrite test and the leukocyte esterase test were acceptable by themselves, as screening tests. A combination of these two test values was analysed, as single dipstick with both the parameters can be used as an office diagnostic procedure. When both the nitrite and leukocyte esterase tests were positive, positive cases of asymptomatic bacteriuria were correctly identified in most of the cases. The negative predictive value of a normal test (i.e. negative nitrite and leukocyte esterase) was 100%. Using combination of these two tests, all patients with infections caused by gram positive bacteria missed by nitrite test alone would have been correctly diagnosed.

A combination of three Screening tests - Leukocyte esterase test which demonstrated a sensitivity of 100%, a negative predictive value of 100%, a specificity of 97.70%, (although with a positive predictive value of 90.47%,) along with Gram's stain with a sensitivity of 95%, a negative predictive value of 98.83%, a specificity of 98.83%, and a positive predictive value of 95% , and Griess NO2 test which has a sensitivity of 81.82%,a negative predictive value of 97.93%, a specificity of 100%, and a positive predictive value of 100% will provide an acceptable alternative to screening all asymptomatic pregnant women with urine culture.

CONCLUSION

A combination of three Screening tests - Leukocyte esterase test which demonstrated a sensitivity of 100%, a negative predictive value of 100%, a specificity of 97.70%, (although with a positive predictive value of 90.47%,) along with Gram's stain with a sensitivity of 95%, a negative predictive value of 98.83%, a specificity of 98.83%, and a positive predictive value of 95% , and Griess NO2 test which has a sensitivity of 81.82%,a negative predictive value of 97.93%, a specificity of 100%, and a positive predictive value of 100% will provide an acceptable alternative to screening all asymptomatic pregnant women with urine culture.

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