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Research Article

A study on asymptomatic bacteriuria in pregnancy: prevalence, etiology and comparison of screening methods

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ABSTRACT

Background: Asymptomatic bacteriuria is common in women with prevalence of 4-7% in pregnancy. The traditional reference test for bacteriuria is quantitative culture of urine which is relatively expensive time consuming and laborious. The aim of this study was to know the prevalence of asymptomatic bacteriuria in pregnancy, to identify pathogens and their antibiotic susceptibility patterns and to device a single or combined rapid screening method as an acceptable alternative to urine culture.
Methods: Clean catch mid-stream urine were collected from 250 pregnant women aged between 18-45 years attending antenatal clinic, for a period of one year (November 2008-2009). Screening tests such as gram staining of uncentrifuged urine, pus cell count, nitrite test and leukocyte esterase test were done. Identification of organisms and antibiotic sensitivity tests were performed as per standard methods.
Results: Out of the 250 pregnant women, 21 (8.4%) had significant bacteriuria. High percentage of asymptomatic bacteriuria was seen in 2nd trimester (42.86%) and in primigravidas (52.38%). E. coli (57.14%) was the most common organism. Among screening tests gram staining of uncentrifuged urine had a sensitivity of 85.71%. Nitrite and leukocyte esterase tests alone showed sensitivity of 71.42%. However, the combination of these two tests, either tests positive, showed sensitivity and negative predictive value of 90.47% and 99.09% respectively.
Conclusion: Asymptomatic bacteriuria in pregnancy can be identified by simple and combined rapid screening methods and urine culture along with antibiogram so that early treatment can be started thereby preventing complications.

Keywords: Bacteriuria, Pregnancy, Screening tests

INTRODUCTION

Asymptomatic bacteriuria is defined as the presence of more than 100000 colonies of a single bacterial species per milliliter of urine, cultured from midstream sample in the absence of declared symptoms.[1] Asymptomatic bacteriuria is common in women and increases in prevalence with age and/or sexual activity.[2] The prevalence of asymptomatic bacteriuria in pregnancy varies form 4-7% (range 2-11%) and is similar to that observed in non-pregnant women.[3] Although pregnancy does not predispose a woman to the acquisition of asymptomatic bacteriuria, it does predispose her to acute upper urinary tract infection or pyelonephritis[4]. Pyelonephritis develops in 20-40% of pregnant women with untreated asymptomatic bacteriuria. Approximately,

40-80% of pregnancy complications caused by acute pyelonephritis could be prevented by treating asymptomatic bacteriuria. Untreated bacteriuria can have serious implications such as premature birth, low birth weight and perinatal death in foetus and hypertension, pre-eclampsia, anaemia, amnionitis and endometritis in the mother.[5]

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.[6] 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.[7]

Thus the present study aimed to find out the prevalence of asymptomatic bacteriuria in pregnant women, the common pathogens involved with their antibiotic sensitivity pattern, to compare the sensitivity of various screening methods (Gram-staining, nitrite test, leukocyte esterase test, pus cell count) and to device a single or combined rapid screening method, which may provide an acceptable alternative to screening all asymptomatic patients with urine culture.

METHODS

This was a cross-sectional study conducted for a period of one year from November 2008 to November 2009 at a tertiary care hospital in Bangalore. 250 pregnant women aged between 18-45 years attending the obstetrics and gynaecology clinics were included in this study. From these patients mid-stream clean catch urine samples were collected and processed. The study and data collection were carried out with approval from the institutional ethical committee.

Inclusion criteria

1. Pregnant women with varying gestational periods attending the antenatal clinic without a history of dysuria, loin pain, or increased frequency of micturition.
2. Patients not treated earlier with antibiotics, parenteral or local application for at least preceding one month before her attendance to the hospital.

Exclusion criteria

1. Pregnant women <18 years and >45 years of age.
2. Patients with vaginal discharge or bleeding per vagina.
3. Pregnancy induced diabetes mellitus/hypertension.
4. Known congenital anomalies of the urinary tract.

Specimen collection

After thorough instructions to every patient, clean catch midstream urine of about 20 ml was collected in a sterile universal container and transported to the Microbiology laboratory within one hour. In case of delay, the sample was refrigerated at 4°C for as long as 24 hours.[8] The urine samples were observed macroscopically for its color, turbidity and deposits and the findings were recorded and then subjected to various tests as follows:

Screening procedures

Gram stain of uncentrifuged urine

A drop of well-mixed urine was smeared on slide, allowed to air-dry, fixed, stained and examined under oil-immersion. Presence of at least one organism per oil immersion field (examining 20 fields) correlates with significant bacteriuria.

Pus cell count of the uncentrifuged urine

Pyuria is the hallmark of inflammation and pus cells were counted using a Neubauer's counting chamber. The presence of > 8 PMNs/mm^3 correlates with significant bacteriuria. A 1:1 dilution of 0.1 ml of urine and WBC diluting fluid (Turck's fluid) was taken and charged on to the Neubauer's counting chambers and the pus cells were counted at all the 9 squares under the microscope using low (10x) objective and calculated using the formula.

$$\text{Pus cell count} = \frac{\text{Number of cells x depth factor x dilution factor}}{\text{Area covered}}$$

Nitrate reductase (Griess) test and leucocyte esterase test

A dipstick strip COMBUR 10 (Boehringer Mannheim & Co.) which determines 10 different parameters was used.

Most of the urinary tract pathogens produce nitrate reducing enzymes that reduce nitrate to nitrites, which react with an amine impregnated on the dipstick pad to form a diazonium compound resulting in a pink color

reaction within 60 seconds. Leucocyte esterase, an enzyme produced by inflammatory cells reacts with the chloro acetate stain impregnated in a dipstick pad resulting in an iodoxyl moiety that is oxidized by room air and produces a color change in 1-2 minutes.

The strip was dipped in the well-mixed uncentrifuged urine for no longer than a second, after withdrawing the strip, excess urine along the rim of vessel was wiped. After 1 minute the colour change in the strip was compared with the color scales provided with the kit. Pink color produced was considered as nitrate positive and a violet colour was considered as leucocyte esterase positive. Any colour change appearing only along the edges of the test patches or developing after more than 2 minutes were considered insignificant.

Urine culture

Urine samples were inoculated on CLED agar by a semi-quantitative method using a calibrated loop, which delivers 0.01 ml of urine. The culture plates were incubated aerobically at 37°C for 24-48 hours. After counting the colonies, the number of CFUs was multiplied by 100 to determine the numbers of microorganisms per milliliter in the original specimen. Count with 10^5 CFU/ ml was considered as significant.

The isolates were further identified on the basis of colonial morphology, cultural characteristics, Gram's staining and a battery of biochemical reactions.

Antibiogram

The antibiotic sensitivity test of isolates was performed on Mueller Hinton agar plates by Kirby Bauer disc diffusion method. Zone diameter was measured and interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.[10] For quality control of disc diffusion tests control strains of Escerichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were used. The antibiotic discs were obtained from Hi-media, Mumbai. The following antibiotics were employed: amoxicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), cephalexin (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), nitrofurantoin (300 µg), nalidixic acid (30 µg) and co-triamoxazole (1.25/23.75 µg).

All patients were advised to take antibiotics as per the sensitivity testing.

The results of prevalence of asymptomatic bacteriuria, organisms isolated, their antibiograms and their distribution among pregnant females are expressed as percentages. Microsoft excel was used for the interpretation of these results. For comparisons of screening tests - sensitivity, specificity, positive predictive value and negative predictive values were calculated using SPSS-7.5 version software.

RESULTS

Out of the total 250 pregnant women included in this study 21 (8.4%) patients were identified by culture to have significant bacteriuria of which 61.9% of them were between 26-30 yrs. High percentage of asymptomatic bacteriuria was seen in 2nd trimester (42.86%) and in primigravidas (52.38%) (Table 1).

Table 1: Age and gestational characteristics of pregnant women screened for asymptomatic bacteriuria.

Characteristics	Number of cases (n=250)	Number of Significant bacteriuria (%) (n=21)
Age in years		
18-25	48	4 (19.05)
26-30	154	13 (61.90)
31-35	32	3 (14.29)
36-40	12	1 (4.76)
41-45	4	0 (0)
Parity		
Primigravida	121	11 (52.38)
Gravida 2	93	6 (28.57)
Gravida 3	29	4 (19.05)
Gravida 4+	7	0 (0)
Gestational age		
1st Trimester	102	5 (23.81)
2nd Trimester	110	9 (42.86)
3rd Trimester	38	7 (33.33)

Gram negative organisms were predominant (80.95%) causative agents than gram positive organisms (19.05%). E. coli (57.14%) was the most common organism isolated (Figure 1, Figure 2, Table 2).

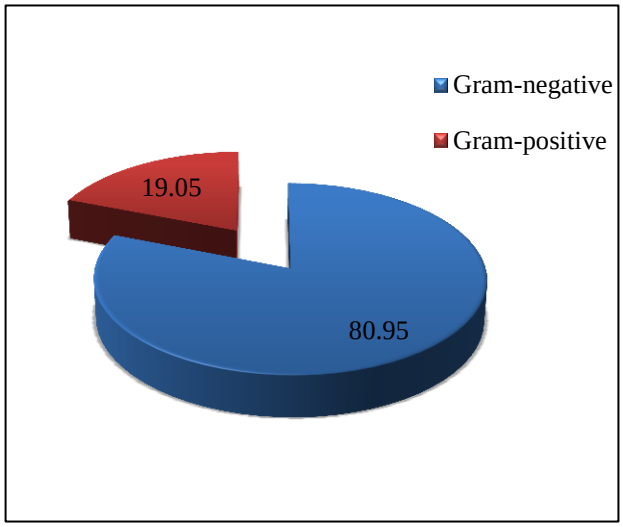

Figure 1: Percentage of gram positive & gram negative organisms causing asymptomatic bacteriuria.

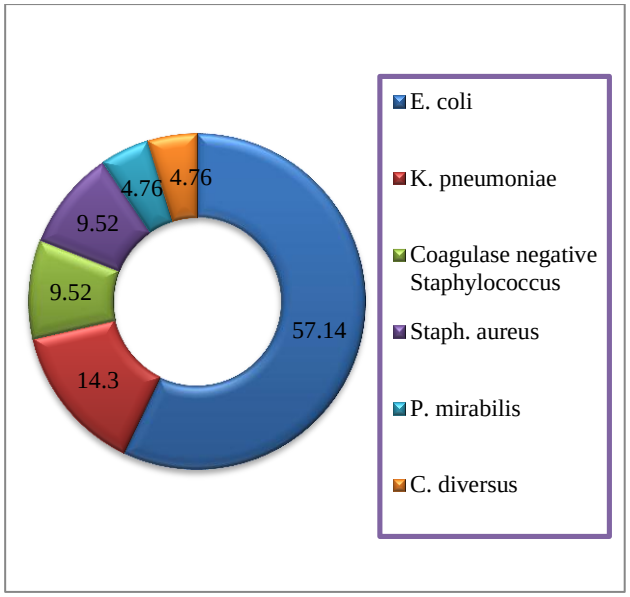

Figure 2: Relative percentage of organisms causing significant bacteriuria (n=21).

Table 2: Organisms causing asymptomatic bacteriuria expressed in percentage.

Organism	No. of organism	Percentage % (n=21)
E. coli	12	57.14
K. pneumoniae	3	14.30
Coagulase negative Staphylococcus	2	9.52
Staph. aureus	2	9.52
P. mirabilis	1	4.76
C. diversus	1	4.76
Total	21	100

The antibiograms revealed that 95.23% of isolates were sensitive to ciprofloxacin, followed by cefotaxime (80.95%), ceftriaxone (76.19%) and gentamicin (66.67%). Among the antibiotics ciprofloxacin, cefotaxime and ceftriaxone were most effective (100% sensitive against S. aureus, P. mirabilis and C. diversus) while amoxicillin and cephalexin were the least effective with only 23.80% and 33.33% sensitivity rates (Table 3).

Table 3: Pattern of antibiotic sensitivity of bacterial isolates.

% Sensitive								
Antibiotics	**E. coli**	**K. pneumoniae**	**CONS**	**S. aureus**	**P. mirabilis**	**C. diversus**	**Total sensitive isolates**	**Total % sensitive (n=21)**
Ciprofloxacin	91.67	100	100	100	100	100	20	95.23
Cefotaxime	83.33	66.67	50	100	100	100	17	80.95
Ceftriaxone	75	66.67	50	100	100	100	16	76.19
Gentamicin	66.67	66.67	50	50	100	100	14	66.67
Amoxicillin-clavulanic acid	50	33.33	50	100	0	100	11	52.38
Nitrofurantoin	66.67	66.67	0	0	0	0	10	47.61
Co-trimoxazole	58.33	33.33	50	50	0	0	10	47.61
Nalidixic acid	50	66.67	0	0	100	0	09	42.85
Cephalexin	41.67	0	50	50	0	0	07	33.33
Amoxicillin	25	33.33	0	50	0	0	05	23.80

Table 4: Distribution of statistical values for various screening tests.

Test	True positive	True negative	False positive	False negative	Total number of cases
Gram's stain	18	226	03	03	250
Pus cell count	09	218	11	12	250
Nitrite test	15	228	01	06	250
Leukocyte esterase test	15	211	18	06	250
Combined nitrite and leukocyte esterase test	11	229	0	10	250
Either nitrite test or leukocyte esterase test	19	220	09	02	250

Table 5: Statistical analysis for various screening tests.

Test	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %
Gram's stain	85.71	98.68	85.71	98.68
Pus cell count	42.85	95.19	45	94.78
Nitrite test	71.42	99.56	93.75	97.43
Leukocyte esterase test	71.42	92.13	45.45	97.9
Combined nitrite and leukocyte esterase test	52.38	100	100	95.71
Either nitrite test or leukocyte esterase test	90.47	96.069	67.86	99.09

Among the screening tests gram staining of uncentrifuged urine had a sensitivity of 85.71% and negative predictive value of 98.68%. Nitrite and leukocyte esterase tests alone showed sensitivity of 71.42%. However, the combination of these two tests, either tests positive, showed sensitivity or negative predictive value of 90.47% and 99.09% respectively (Table 4, Table 5).

DISCUSSION

Asymptomatic bacteriuria of pregnancy needs special attention, due to lack of symptoms & its adverse consequences in pregnancy.[11] A cost evaluation study reported that screening for pyelonephritis is appropriate when the prevalence of ASB is greater than 2%.[12]

The present cross-sectional study was conducted to know the prevalence of asymptomatic bacteriuria and to evaluate the diagnostic efficacy of various screening methods.

This study showed the overall prevalence of asymptomatic bacteriuria of 8.4% (21/250) which was almost similar to a study done in Iran[13] (8.9%) and Raichur[14] (9%). But studies done in Nepal,[15] Lucknow[16] and Srilanka[17] showed higher and lower prevalence rates of 26%, 16.9% and 3.6% respectively.

In our study, the prevalence was seen to be higher in women belonging to 26-30 years of age group (61.90%), in primigravidas (52.38%) and during second trimester (42.86%).

These findings were similar to those reported in other studies done in Belgaum[11] and Raichur[14] where 57% and 52% of cases belonged to 26-35 years of age, 59% and 56% were primigravidas and 54.54% and 44.40% of cases were in second trimester respectively. However a study done in Ghana[18] showed higher prevalence of asymptomatic bacteriuria (36.80%) in 30-34 years age group, while a study done in Hassan[19] showed 61.77% of cases in third trimester of pregnancy.

Our study did not encounter polybacterial isolation. It showed that gram negative organisms were predominant isolates (80.95%) than Gram positive (19.05%) with E. coli (57.14%) being the most common followed by K. pneumoniae (14.3%). Most of the other studies[11,14,15,17] have reported E. coli as the most common pathogen but with higher isolation rates than our study (72.72%, 77.77%, 70.8%, 67%), while studies done in Iran[13] and Hassan[19] showed almost similar isolation rate of E. coli (58.96% and 51.61%) as our study. But one study done in Nigeria[20] 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 95.23% of isolates were sensitive to ciprofloxacin, followed by cefotaxime (80.95%), ceftriaxone (76.19%) and gentamicin (66.67%). We found that the sensitivities to amoxicillin-clavulanic acid (52.38%), nitrofurantoin and cotrimoxazole (47.61%), nalidixic acid (42.85%), cephalexin (33.33%), and amoxicillin (23.80%) which are used as drugs of choice in treating asymptomatic bacteriuria were comparatively lower posing problems in treating these patients.

A study done by Oli et al.,[21] showed similar antibiotic sensitivities with regard to ceftriaxone (75.38%), gentamicin (60%), amoxicillin-clavulanic acid (55.38%) and cephalexin (29.23%), but was in contrast to our study with respect to, amoxicillin (44.62%), nitrofurantoin (35.38%), cotrimoxazole (33.84%) and nalidixic acid (32.31%).

E. coli, the predominant isolate in this study showed sensitivity pattern of 91.67% to ciprofloxacin, 83.33% to cefotaxime, 75% to ceftriaxone, 66.67% to gentamicin and nitrofurantoin, 58.33% to cotrimoxazole, 50% to amoxicillin-clavulanic acid and nalidixic acid, 41.67% to cephalexin and 25% to amoxicillin.

Similar sensitivity rate of E. coli to ciprofloxacin (96.9%) was seen in a study done in Kashmir [22] and to cefotaxime (88.62%) in a study done in Iran.[13] However contrasting results were reported in the same study[13] with respect to gentamicin (5.07%), nitrofurantoin (29.12%) and nalidixic acid (18.99%).

A study done by Oli et al.[21] showed similar sensitivity pattern of E. coli with respect to gentamicin (66.67%), amoxicillin-clavulanic acid (57.14%) and co-trimoxazole (42.86%) but reported higher sensitivity to ceftriaxone (85.71%) than our study.

The few contrasting results observed in different studies may be due to differences in the choice of drugs used for empirical treatment.

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, nitrite test and leukocyte esterase tests were evaluated.

It was demonstrated that, gram stain of uncentrifuged urine was the most useful single test with sensitivity of 85.7% and Negative Predictive Value (NPV) of 98.68%. Studies done by Gayathree et al.[19] and Jayalakshmi et al.[23] showed similar sensitivity (90.32%, 85.1%) and 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 42.85% 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.

In the present study, nitrite test demonstrated a high specificity of (99.56%) and Positive Predictive Value (PPV) (93.75%) but lesser sensitivity (71.42%) and negative predictive value (97.43%) compared to Gram's stain. This was because 6 positive cases were missed as false negatives that included all 4 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.[24] 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 (71.42%) and negative predictive value (97.9%) comparable with nitrite test. But because of 11 false positive, which included 6 patients with sterile pyuria identified, the specificity was lower (92.13%) than the other test. Other studies[19,23,24] showed mush lesser sensitivity of leukocyte esterase test (61.29%, 61.7% and 20.8%) than our study.

In our study, neither nitrite test nor the leukocyte esterase test was acceptable by itself as a screening test (sensitivities of both being 71.42%). Combination of these two test values was analyzed, 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 100% of the cases. The negative predictive value of a normal test (i.e. negative nitrite and leukocyte esterase) was 95.81%.

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.

CONCLUSION

Our study was conducted to find out the prevalence of asymptomatic bacteriuria in pregnant women, to identify the common pathogens and their susceptibility pattern and to compare the sensitivity and specificity of various screening methods.

250 pregnant women were screened for the presence of asymptomatic bacteriuria. Our results showed a prevalence of 8.4% and 61.9% of them were between 26-30 years. High percentage of asymptomatic bacteriuria was seen in 2nd trimester (42.86%) and in primigravidas (52.38%). E. coli was the predominant organism (57.14%) and antibiograms revealed that ciprofloxacin, cefotaxime and ceftriaxone were the most effective antibiotics. Gram staining of uncentrifuged urine was found to be most useful test. The sensitivity of nitrite test and leukocyte esterase test alone was 71.42% but the combination of these two colorimetric tests, either test positive, used as outdoor diagnostic procedure with sensitivity and negative predictive value of 90.47% and 99.09% respectively may provide an acceptable alternative to screening all asymptomatic pregnant women with urine culture.

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