Asymptomatic bacteriuria in women attending an antenatal clinic at a tertiary care centre

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Objective. To compare the diagnostic performance of urine microscopy, leucocyte esterase and nitrite dipstick tests and various combinations of these as screening tests for asymptomatic bacteriuria in pregnancy.
Methods. Pregnant women (*N*=800) attending an antenatal clinic were recruited at their first visit. Urine microscopy, culture and dipstick testing were performed on a random clean-catch midstream urine sample. 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.
Results. A total of 800 eligible women were screened. The prevalence of asymptomatic bacteriuria as diagnosed by urine culture was 5.0% (*n*=40). *Escherichia coli* was the most prevalent uropathogen isolated by culture (60.0%). Neither urine microscopy nor the leucocyte test was found to be sufficiently sensitive to be used as a single screening test for asymptomatic bacteriuria in pregnant patients. The nitrite test alone had a sensitivity of 82.5% and a specificity of 99.9%. Combined dipstick testing had an improved sensitivity of 87.3% and a specificity of 96.2%. Addition of urine microscopy to combined dipstick testing increased the sensitivity to 95.0%, and the specificity became 92.4%.
Conclusion. Combined dipstick testing is a useful screening test for asymptomatic bacteriuria in pregnancy. Addition of urine microscopy to combined dipstick testing further improves its diagnostic performance.

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Asymptomatic bacteriuria is defined as the presence of ≥105 colony-forming units (cfu) of a single type of bacteria per millilitre of urine detected by culture of a midstream urine specimen in asymptomatic patients.[1] Depending on the patient population, 2 - 10% of pregnant women have asymptomatic bacteriuria at their initial prenatal visit.[2] Without antimicrobial treatment, as many as 30% of these women will develop symptomatic urinary tract infection during pregnancy.[2,3] The sequelae of urinary tract infection in pregnancy include pyelonephritis, premature labour and preterm rupture of the membranes. Treating bacteriuria in pregnancy decreases the rate of subsequent pyelonephritis by 80 - 90%.[3,4] Both screening and treatment of all pregnant women for asymptomatic bacteriuria are therefore recommended. Unfortunately universal screening is not practised worldwide, especially in developing countries where the costs of standard plate cultures are prohibitive for limited healthcare budgets and there is a lack of adequate laboratory facilities and trained microbiologists.

The gold standard for detection of bacteriuria is urine culture, but this test is costly and it takes 24 - 48 hours to obtain results. To overcome these problems, 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. Another method is the use of urine dipsticks that can detect protein, blood, nitrite and leucocyte esterase, all of which are associated with urinary tract infection. Urine reagent dipstick testing is rapid, inexpensive and requires little technical expertise. The objectives of this study were: (*i*) to evaluate the diagnostic performance of urine microscopy and reagent strip tests in detecting significant bacteriuria on subsequent urine culture, used as standard; and (*ii*) to see whether combinations of tests could minimise the need for diagnostic urine cultures.

Methods

This prospective study was conducted from December 2008 to February 2010 in the antenatal outpatient clinic, Department of Obstetrics and Gynaecology, Lok Nayak Hospital and Maulana Azad Medical College, a tertiary care teaching hospital in north India. Based on the prevalence of asymptomatic bacteriuria in pregnancy in the Indian population, which is around 4 - 7%,[5-7] the sample size was calculated as 750, from an alpha risk of 5%, with a power of 90.0%. All the women attending the antenatal clinic were included in the study. Clinical information regarding each patient's medical and obstetric history was recorded on a predesigned proforma. Patients were excluded if they had symptoms of urinary tract infection, had taken antibiotics during the previous week, or had any signs of labour.

Urine microscopy, culture and dipstick tests were performed on a random sample of urine. We used randomly voided specimens because of concerns regarding delays in the processing of specimens from patients who presented at the antenatal clinic in the afternoon. Patients were instructed how to collect a clean-catch midstream urine sample in a sterile container. One aliquot was tested by the resident obstetrician in the antenatal clinic using reagent dipsticks (Lab Strip U11 Plus) to detect the presence of leucocyte esterase and nitrite, according to the manufacturer's instructions. The reagent dipsticks were dipped in urine and the result was interpreted by comparison with the colour chart provided. For leucocyte esterase, the result was read 2 minutes and for nitrite 1 minute after dipping. The nitrite portion of the test was read as positive if the reagent square turned pink, and the leucocyte esterase portion as positive if the reagent square matched a colour coded + or ++. A second aliquot was sent to the microbiology laboratory within 4 hours of sample collection for microscopy and urine culture. The microbiologist was blinded to the results of dipstick test. Microscopic analysis was performed on 0.05 ml of the uncentrifuged urine sample. A count of ≥5 pus cells per high-power field or the presence of any bacteria was considered a positive finding.[8,9] A sample from the remainder of the urine specimen was processed for culture and plated on blood agar and MacConkey agar using a 0.01 ml calibrated loop. The plates were incubated aerobically and read at 12, 24 and 72 hours. A count of >105 cfu/ml of a single organism was taken as significant. Specimens with less than 105 cfu/ml or contaminated specimens, i.e. with growth of two or more organisms on culture, were considered not to indicate significant bacteriuria. Antibiotic sensitivities were determined by the streak method. Patients with a positive culture were treated with the appropriate antibiotics.

For the purpose of the study, the urine culture was taken as the gold standard test. A positive result for an individual test that was also culture positive was taken as 'true positive'. Sensitivity for each test was calculated as true positive/all culture positive, and specificity as true negative/all culture negative. The positive predictive value was calculated as true positive/all positive by that individual test, and the negative predictive value as true negative/all negative by that individual test. Sensitivity and specificity of the two tests were combined in parallel to improve the diagnostic performance of the combined test. Combination in parallel means that the combined test result was interpreted as positive if any one test was positive, and interpreted as negative only when both the tests were negative. The combined sensitivity in parallel was calculated as: (sensitivity A + sensitivity B) – (sensitivity A × sensitivity B). The combined specificity in parallel was calculated as: specificity A × specificity B.

Results

A total of 800 eligible pregnant women were screened. Their sociodemographic characteristics are shown in Table 1. The mean period of gestation at which patients were screened was 18.45 weeks (±standard deviation (SD) 5.2). The prevalence of asymptomatic bacteriuria as diagnosed by urine culture was 5.0% (*n*=40). *Escherichia coli* was the most prevalent uropathogen isolated by culture (60.0%, *n*=24). Other organisms isolated were *Klebsiella pneumoniae* (22.5%, *n*=9), *Staphylococcus aureus* and *Pseudomonas aeruginosa* (5%, *n*=2 each), and *Proteus mirabilis*, *Enterococcus faecalis, Klebsiella oxytoca* and *Acinetobacter baumannii* (2.5%, *n*=1 each). Microscopy detected 24 cases of asymptomatic bacteriuria (60.0%) out of a total of 40 cases that showed growth on culture plates. The leucocyte esterase dipstick test was positive in 11 culture-positive cases, whereas the nitrite dipstick test was positive in 33 culture-positive cases (Table 2).

The sensitivity, specificity, and positive and negative predictive values of the individual tests are shown in Table 3.

Sensitivity and specificity were calculated for the leucocyte esterase test combined with the nitrite dipstick test. When they were combined in parallel, i.e. either test positive, the sensitivity improved to 95%, as shown in Table 4.

Table 5 compares pregnancy complications in women with asymptomatic bacteriuria with those in women who had sterile urine cultures: 2 women with asymptomatic bacteriuria had symptomatic urinary tract infection as opposed to only 1 women in the control group, the difference being statistically significant (*p*=0.007). Preterm birth was defined as delivery before 37 weeks' gestation and low birth weight as a birth weight less than the 10th percentile for gestational age. The prevalence of low-birth-weight babies was significantly higher in the asymptomatic bacteriuria group than in the control group (*p*=0.002).

Two women developed symptomatic urinary tract infection later in their pregnancies. Both had renal stones that might have caused recurrent urinary tract infection. Although the numbers were small, the increased occurrence of symptomatic urinary tract infection in women with asymptomatic bacteriuria was significant (*p*=0.007).

Discussion

The prevalence of asymptomatic bacteriuria diagnosed on urine culture in the present study is 5.0%, which correlates well with that

Table 1. Sociodemographic characteristics of women screened for asymptomatic bacteriuria (*N*=800)

Characteristics	
Age (years), mean (±SD) (range)	26.5 (±3.4) (18 - 35)
Education, *n* (%)	
No formal schooling	125 (15.6)
Primary	324 (40.5)
Secondary	231 (28.9)
Tertiary	120 (15.0)
Parity, *n* (%)	
Primigravidas	325 (40.6)
Multigravidas	475 (59.4)
Gestational age at the time of inclusion (weeks), *n* (%)	
<12	143 (17.8)
12 - 28	527 (65.9)
>28	130 (16.2)

SD = standard deviation.

Table 2. Screening test results in culture-positive cases

	Culture positive	Culture negative	Total
Urine microscopy, *n*			
Positive	24	30	54
Negative	16	730	746
Total	40	760	800
Leucocyte esterase by dipstick, *n*			
Positive	11	28	39
Negative	29	732	761
Total	40	760	800
Nitrite test by dipstick, *n*			
Positive	33	1	34
Negative	7	759	766
Total	40	760	800

Table 3. Diagnostic performance of individual tests

	Urine microscopy	Leucocyte esterase test	Nitrite test
Sensitivity, %	60.0	27.5	82.5
Specificity, %	96.1	96.3	99.9
Positive predictive value, %	44.4	28.2	97.1
Negative predictive value, %	97.9	96.2	99.1

Table 4. Sensitivity and specificity for combinations of screening tests when applied in parallel

	Sensitivity, %	Specificity, %
Combined dipstick test (LE + nitrite)	87.3	96.2
Urine microscopy plus combined dipstick test	95.0	92.4

LE = leucocyte esterase.

Table 5. Pregnancy complications

	Women with ASB (*N*=40)	Women without ASB (*N*=760)	*p*-value
Symptomatic UTI, *n*	2	1	0.007
Gestational hypertension, *n*	1	35	1.000
Pre-eclampsia, *n*	1	28	1.000
Anaemia, *n*	10	300	0.07
Preterm labour, *n*	2	42	1.000
PPROM, *n*	1	22	1.000
Low birth weight, *n*	12	98	0.002

ASB = asymptomatic bacteriuria; UTI = urinary tract infection; PPROM = preterm premature rupture of the membranes.

described in the Indian literature.[5-7] In our study, 65.9% of women were diagnosed with asymptomatic bacteriuria between 12 and 28 weeks. According to the literature, the 16th week of pregnancy is the optimal time for a single screen for bacteriuria because it is the time when rates of bacteriuria are highest.[10] The most common uropathogens found in our study were *E. coli* (60.0%) and *K. pneumoniae* (22.5%). The fact that pregnancy-related urinary stasis allows *E. coli* to grow faster may explain its high prevalence in all studies.

Antibiotic sensitivity patterns differ from population to population and also from hospital to hospital. We found found that gentamicin, amikacin and nitrofurantoin were effective against most urinary isolates. Although gentamicin and amikacin are effective in treating asymptomatic bacteriuria in pregnant women, they are known to be nephrotoxic[11] and should therefore only be used when absolutely necessary. Most of the organisms were resistant to amoxicillin and cephalosporins. The upsurge in antibiotic resistance may be due to antibiotic abuse and self-medication.

A variety of urine screening tests have been developed over the past several years in an effort to avoid screening all pregnant patients with costly urine cultures. It has consistently been shown that most screening methods used in the laboratory have a low sensitivity and are therefore unreliable for the diagnosis of urinary tract infection. The sensitivity and specificity of urine microscopy in our study were 60.0% and 96.1%, respectively. In a study by Bachman *et al.*[8] the sensitivity and specificity were 83% and 59%, respectively, and in a study by McNair *et al.*[9] they were 80.6% and 71.5%, respectively. We found the sensitivity of the leucocyte esterase dipstick test used alone to be low (27.5%), which is in agreement with the available literature.[2,12,13] Used alone, this test is therefore a poor screening test for asymptomatic bacteriuria. In our study the nitrite dipstick had a sensitivity of 82.5% and a specificity of 99.9%, making it a good screening test for asymptomatic bacteriuria. Previous studies have reported the sensitivity of the nitrite test to be 67.5%,[2] 60%[14] and 75%,[15] while the specificity was 99.7%, 99.2% and 99%, respectively. Combined dipstick tests (LE + nitrite) when used in parallel (either test positive) in our study increased the sensitivity to 87.3%, with a specificity of 96.2%. In two previous studies the sensitivity of the combined

dipstick test was 92%[16] and 70%[17] and the specificity 95% and 83.4%, respectively. Dipsticks can be stored at room temperature and do not require trained personnel and laboratory facilities. Furthermore, results are available immediately so presumptive treatment can be started. The combined dipstick test is therefore a useful screening tool for asymptomatic bacteriuria in pregnancy in settings where resources are limited.

Using a combination of urine microscopy and combined dipstick tests in parallel (either test positive), the sensitivity further improved to 95.0% and the specificity became 92.4%. With the availability of a microbiologist and laboratory facilities, the combined dipstick tests with urine microscopy can be used to screen for asymptomatic bacteriuria in pregnant women, especially in a low-resource setting. In the study by McNair *et al.*,[9] the sensitivity and specificity of urine microscopy plus reagent strip tests when combined in parallel were lower than in our study at 83.3% and 64.6%, respectively.

We found that women with asymptomatic bacteriuria had a significantly higher rate of low-birth-weight deliveries (p=0.025) than women with sterile cultures. A meta-analysis by Romero *et al.*[18] also showed that asymptomatic bacteriuria was associated with an increased risk of low birth weight, the relative risk being 1.54 (95% confidence interval 1.35 - 1.75). Previous studies have also shown asymptomatic bacteriuria to be associated with preterm delivery,[18,19] but we did not find a significant difference in the rate of preterm birth in our patients with asymptomatic bacteriuria.

1. Lorentzon S, Hovelius B. The diagnosis of bacteriuria during pregnancy. Scand J Prim Health Care 1990;8(2):81-83. [http://dx.doi.org/10.3109/02813439008994935]
2. Etherington J, James DK. Reagent strip testing of antenatal urine specimens for infection. BJOG 1993;100(9):806-808. [http://dx.doi.org/10.1111/j.1471-0528.1993.tb14303.x]
3. Macejko AM, Schaeffer AJ. Asymptomatic bacteriuria and symptomatic urinary tract infections during pregnancy. Urol Clin North Am 2007;34(1):35-42. [http://dx.doi.org/10.1016/j.ucl.2006.10.010]
4. Mittal P, Wing DA. Urinary tract infections in pregnancy. Clin Perinatol 2005;32(3):749-764. [http://dx.doi.org/10.1016/j.clp.2005.05.006]
5. Gayathree L, Shetty S, Deshpande SR, Venkatesha DT. Screening for asymptomatic bacteriuria in pregnancy: An evaluation of various screening tests at the Hassan district hospital. J Clin Diagn Res 2010;4:2702-2706.
6. Jayalakshmi J, Jayaram VS. Evaluation of various screening tests to detect asymptomatic bacteriuria in pregnant women. Indian J Pathol Microbiol 2008;51(3):379-381. [http://dx.doi.org/10.4103/0377-4929.42516]
7. Bandyopadhyay S, Thakur JS, Ray P, Kumar R. High prevalence of bacteriuria in pregnancy and its screening methods in North India. J Indian Med Assoc 2005;103(5):259-266.
8. Bachman JW, Heise RH, Naessen JM, Timmerman MG. A study of various tests to detect asymptomatic urinary tract infections in an obstetric population. JAMA 1993;270(16):1971-1974. [http://dx.doi.org/10.1001/jama.270.16.1971]
9. McNair RD, MacDonald SR, Dooley SL, Peterson LR. Evaluation of the centrifuged and Gram-stained smear, urinalysis, and reagent strip testing to detect asymptomatic bacteriuria in obstetric patients. Am J Obstet Gynecol 2000;182(5):1076-1079. [http://dx.doi.org/10.1067/mob.2000.105440]
10. Davison J, Dunlop W. Urinary tract in pregnancy. In: Chamberlain G, ed. Turnbull's Obstetrics. 3rd ed. Edinburgh: Churchill Livingstone, 2002:383-400.
11. Shanson DC. Infection of the urinary tract. In: Microbiology in Clinical Practice. 2nd ed. London: Butterworth, 1989:430-450.
12. Millar L, Debuque L, Leialoha C, Grandinetti A, Killeen J. Rapid enzymatic urine screening test to detect bacteriuria in pregnancy. Obstet Gynecol 2000;95(4):601-604. [http://dx.doi.org/10.1016/S0029-7844(99)00597-9]
13. Hagay Z, Levy R, Miskin A, Milman D, Sharabi H, Insler V. Uriscreen, a rapid enzymatic urine screening test: Useful predictor of significant bacteriuria in pregnancy. Obstet Gynecol 1996;87(3):410-413. [http://dx.doi.org/10.1016/0029-7844(95)00451-3]
14. Kacmaz B, Cakir O, Aksoy A, Biri A. Evaluation of rapid urine screening tests to detect asymptomatic bacteriuria in pregnancy. Jpn J Infect Dis 2006;59(4):261-263.
15. Kodikara H, Seneviratne H, Kaluarachchi A, Corea E. Diagnostic accuracy of nitrite dipstick testing for the detection of bacteriuria of pregnancy. Public Health 2009;123(5):393-394. [http://dx.doi.org/10.1016/j.puhe.2009.01.007]
16. Robertson AW, Duff P. The nitrite and leukocyte esterase tests for the evaluation of asmptomatic bacteriuria in obstetric patients. Obstet Gynecol 1988;71(6):878-881.
17. McNeeley SG,Baselski VS, Ryan GM. An evaluation of two rapid bacteriuria screening procedures. Obstet Gynecol 1987;69(4):550-553.
18. Romero R, Oyarzun E, Mazor M, Sirtori M, Hobbins JC, Bracken M. Meta-analysis of the relationship between asymptomatic bacteriuria and preterm delivery/low birth weight. Obstet Gynecol 1989;73(4):576-582.
19. Fatima N, Yasmin S, Ishrat S. Prevalence and complications of symptomatic bacteriuria during pregnancy. Professional Medical Journal 2006;13(1):108-112.