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Asymptomatic bacteriuria in pregnancy: evaluation of reagent strips in comparison to microbiological culture

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Summary

Screening for asymptomatic bacteriuria during pregnancy, the major risk factor for symptomatic urinary tract infection during pregnancy have been recommended.This cross sectional study was conducted to determine prevalence of asymptomatic bacteriuria in Ibadan and evaluate the diagnostic accuracy and relative cost effectiveness of dipstick tests for nitrite and leucocyte esterase in comparison to laboratory culture. Two hundred and five patients, presenting for their first antenatal visit at the University College Hospital, Ibadan, participated in the study. Urine samples obtained from the participants were subjected to two tests; reagent dipstick test for nitrite and leucocyte esterase and routine laboratory culture, which is the gold standard for diagnosis. Main outcome measures were sensitivity, specificity, positive and negative predictive values of the reagent dipstick tests as well as likelihood ratios. The prevalence of asymptomatic bacteriuria in pregnancy with routine laboratory culture and using combined leucocyte esterase and nitrite strip tests were 10.7% and 11.7% respectively. Compared with laboratory culture, combined strip tests had sensitivity, specificity and negative predictive values of 50%, 92.9% and 93.9% respectively, indicating a statistically significant lower level of accuracy ($P < 0.05$). The corresponding likelihood ratios for positive and negative strip tests (LR+ and LR-) were 7 and 0.5 respectively. The study concludes that combined Leucocyte esterase-nitrite dipstick test is not sufficiently sensitive and specific to be used for routine screening of bacteriuria in pregnancy in place of laboratory culture, though may be more cost effective in low resource settings.

Keywords: *Asymptomatic bacteriuria, pregnancy, dipstick tests, laboratory culture*

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Résumé

Le dépistage de la bactériurie symptomatiques pendant la grossesse, le risqué majeur de l'infection symptomatique de la voie urinaire pendant la grossesse ont été recommandé. Cette étude était conduite dans le but de déterminer le taux de la bactériurie asymptomatique à Ibadan et évaluer le diagnostic juste et le cout effectif relatif des tests rapide par nitrite et estérase leucocyte en comparaison avec la culture au laboratoire. Deux cent cinq patients, se présentant pour leur première visite à la clinique anténatale au centre universitaire hospitalier, Ibadan participaient a cette étude. Les échantillons d'urine étaient obtenues de chaque participant et étaient soumisse a deux tests: le test à kit de nitrite et leucocyte estérase et la culture au laboratoire, qui est le standard pour le diagnostic. Les mesures des résultats étaient la sensitivité, spécificité, les valeurs prédictives positive et négative des tests à kit aussi bien que l'estimation des proportions. La prévalence de la bactériurie asymptomatique en grossesse avec la culture en laboratoire et combinant avec les tests kits de leucocyte estérase et nitrite étaient de 10.7% et 11.7% respectivement. L'étude comparée de la culture et les tests à kits combines avait une sensibilité, une spécificité et des valeurs prédictives négatives de 50%, 92.9% et 93.9% respectivement, indiquant une valeur statistique significativement faible ($P < 0.05$). Les proportions correspondantes des tests à kit positifs et négatifs (LR+ et LR-) étaient de 7 et 0.5 respectivement. Cette étude a conclut que le combiné test kit de leucocyte estérase- nitrite n'est pas suffisamment sensible et spécifique à être utilisé de façon routinier dans le dépistage de la bactériurie pendant la grossesse que la culture, bien que qu'elle peut être plus coûteux dans les pays sous développés.

Introduction

Asymptomatic bacteriuria refers to the situation in which there is persistent, actively multiplying bacteria within the urinary tract without symptoms [1]. The search for an ideal screening method to detect asymptomatic bacteriuria continues to propel research

globally. An ideal screening test should be inexpensive, simple and rapid, and should have high sensitivity in addition to specificity [2]. Detection schemes must also consider the prevalence of infection in the population screened and the cost associated with the modalities. This becomes more pertinent in developing countries like Nigeria where resources for health are meager, majority of the people especially women are not economically empowered to take care of their health, and over 90% of people are living below poverty line [3].

Quantitative urine culture of a midstream clean-catch specimen has been widely recognized as the optimal screening test [4]. By this method, bacteriuria is said to be significant when there are at least 100,000 bacteria colonies of a single pathogen per milliliter (ml) in freshly voided urine collected by the mid - stream clean catch technique [3]. The original criterion for diagnosing asymptomatic bacteriuria was bacteria count of greater than 10^5/ml on two consecutive clean catch samples. However, the detection of 10^5 bacteria/ml or more in a single voided midstream urine sample is accepted as adequate and more practical alternative [5,6].

A single screening test using culture method at 12–16 weeks of pregnancy will identify 80% of women with asymptomatic bacteriuria of pregnancy. However, performing a urine culture is expensive, requires laboratory facilities and competent personnel which may not be available in low resource settings [7]. Therefore, other bacteriuria screening methods have been described and used [2,7,8]. These include the relatively cheap and affordable dipstick test for nitrites and leucocyte esterase. Meta - analysis by Deville et al, revealed dipstick test for bacteriuria using a combination of nitrite and leucocyte esterase to have a sensitivity of 68-88% [9]. The review also demonstrated that the urine dipstick test alone was useful in all population to exclude the presence of infection if the results of nitrites or leucocyte esterase tests are negative. Formal microbiological culture is then recommended only for those with positive dipstick test [4,9].

Others include routine urinalysis, Gram staining of urine, enzymatic method for detecting catalase in urine, semi quantitative dipstick culture and chromogenic limulus amoebocyte assay [7,10-13]. The use of urinary interleukin-8 (IL-8), an inflammatory cytokine, and chlorhexidine for detecting bacteriuria in pregnancy have also been reported in some isolated studies [14,15].

Bacteriuria is a common and important complication of pregnancy[16]. Pregnant women with asymptomatic bacteriuria have been reported to be at a higher risk for a number of complications for both mother and the unborn child [7,17]. Maternal complications include overt urinary tract infection in 30-40% of patients as pregnancy advances [7,17]. Whether or not symptomatic urinary tract infection ensues, the foetus is still at risk for prematurity, low birth weight and even fetal wastage [17].

The overall prevalence of bacteriuria in pregnancy varies from 4-7%, although a range of 2-11% has been reported [1,18]. The prevalence rate among pregnant Nigerian women has been variously reported to be between 4-23.9% [19–22]. Bacteriuria is typically present at the time of first pre-natal visit and only approximately 1-2% of pregnant women develop bacteriuria after a negative screening early in pregnancy [4,16]. The condition is detectable and largely treatable. Its consequences are also preventable, hence screening for asymptomatic bacteriuria is justifiable and ultimately cost-effective [23] and has therefore been recommended [24,25].

Generally, few local studies have evaluated the use of cheaper and simpler methods of screening for bacteriuria in pregnancy. Also, routine screening for asymptomatic bacteriuria is not practiced in most maternity units in this environment despite overwhelming evidence clearly demonstrating its benefits in preventing symptomatic urinary tract infection and the associated adverse pregnancy outcome [7,16,18].

This study was therefore performed to determine the prevalence of asymptomatic bacteriuria in our centre and to evaluate the diagnostic accuracy and cost effectiveness of leucocyte esterase-nitrite dipstick in detecting asymptomatic bacteriuria in comparison to routine urine culture.

Methods

The study was a cross sectional, descriptive study of the diagnostic accuracy of urinary dipstick tests for nitrite and leucocyte esterase compared to microbiological culture in detecting asymptomatic bacteriuria in pregnancy. It was conducted among healthy pregnant women presenting for the first antenatal (Booking) visit at the University College Hospital, Ibadan between 1st of April and 30th of May 2006.

All consecutive patients presenting for the first antenatal visit (booking) during the period of study were counseled about asymptomatic bacteriuria and recruited into the study after obtaining their written informed consent. Relevant information was obtained from all patients recruited for the study. This included their age, parity, educational level, last

menstrual period, occupation and blood genotype when known. Effort was made to retrieve the result of blood genotype done at the booking clinic from the records department in those who did not know their genotype and to confirm the ones obtained.

Pregnant women who had symptoms of acute urinary tract infection and those who were on, or had been on antibiotic treatment in index pregnancy prior to booking were excluded from the study. Others excluded were those known to have underlying renal disease and those who could not give informed consent.

Sample collection and processing

On presentation at each booking antenatal clinic, the patients were instructed adequately by the nursing staff on how to collect clean catch mid-stream urine. This involved initial cleaning of the perineum with running water. The first part of the urine was voided and about 10-15mls of the mid-stream urine was collected into the sterile universal bottles which had been correctly labeled and distributed to them.

A portion of each urine sample in the sterile universal bottles (about 5mls) was poured into another clean container. The remaining urine samples in the sterile universal bottles were transported to the laboratory for processing within one hour and where immediate processing was not possible, the samples were promptly refrigerated at 4°C to avoid multiplication of bacteria at room temperature. They were then subjected to routine microscopy, culture and sensitivity according to standard practice.

The other half of each urine sample in the clean container was tested immediately with a reagent test strip (Multistix 10SG Bayer Diagnostics, Mfg Ltd Bridgend, UK) for nitrites and leucocyte esterase by the first author according to the manufacturer's instructions. Each of these reagent strips had panels that could detect ten different parameters in the urine such as protein, blood, ketone bodies, nitrite and leucocyte esterase in urine. The test results were compared visually with the colour charts on the reagent strip bottle and read at the time specified in the instruction. Tests showing positive and negative results were noted and recorded accurately. The scientists and the microbiologist involved in the laboratory microscopy and culture of the urine samples were blinded to the results of the reagent strip in order to avoid bias in reporting culture results. The results of microbiological culture and dipstick tests for leucocyte esterase and nitrite were reviewed and compared.

The main outcome measures were prevalence of asymptomatic bacteriuria as revealed by routine culture and reagent strip test, sensitivity, specificity, positive and negative predictive values of the reagent strips.

Data obtained from the test results, microbiological culture as well as the participants' demographic parameters were entered into a proforma and fed into the computer. Statistical analysis was performed with chi-square test, Fisher's exact test and McNermars test where applicable using SPSS version 11. Level of significance was set at 5%. The accuracy of the dipstick tests for nitrite and leucocyte esterase was further evaluated by estimating the sensitivity, specificity, positive and negative predictive values as well as the likelihood ratios of the tests.

Ethical consideration

Ethical approval was obtained from the joint Institutional Review Board (IRB) of University of Ibadan / University College Hospital Ibadan, Nigeria, before the commencement of the study.

Results

The mean age of patients involved in this study was 30.6 ± 4.3 years with a range of 19-43 years. The parity of the patients ranged from 0 to 7 with para 0 being the modal parity. The mean gestational age at booking during this study was 20.9 ± 7.0 weeks with a range of 6 – 40 weeks. Table 1 shows the socio-demographic and obstetric characteristics of the patients screened for asymptomatic bacteriuria in pregnancy and the associated prevalence rates (using standard laboratory culture method).

Out of 245 women who presented for booking for antenatal care at the University College Hospital Ibadan during the study period, two hundred and five (205) participated in the study. With routine laboratory microbiological culture, significant bacteriuria was found in 22 patients giving a prevalence of 10.7%. However, using results of nitrite and leucocyte esterase strip tests separately and in combination, 11, 17 and 24 patients were positive for bacteriuria giving a prevalence of 5.4%, 8.3% and 11.7% respectively (Table 2).

Evaluation of the nitrite strip test in comparison to routine culture revealed that out of 11 patients diagnosed to be positive for asymptomatic bacteriuria by nitrite dipstick test, 8 were confirmed positive by culture constituting true positive result (TP) while the remaining 3 were found to be negative for bacteriuria by culture, and thereby constituting false positive result (FP) as shown in Table 3. Similarly, of the 194 patients identified to be negative for asymptomatic bacteriuria by nitrite strip test, 180 were confirmed negative by culture while the

Table1: Socio-demographic and obstetric characteristics of patients screened for asymptomatic bacteriuria

Characteristics	Bacteriuric(22)	Non-Bacteriuric (183)	Total (205)	Prevalence of AB (%)
Age in years				
16 -25	2	19	21	9.5
26 -35	18	139	157	11.5
36 - 45	2	25	27	7.4
Mean age + SD	30.73±4.41	30.61±4.27	30.6±4.30	
Educational level				
≤ Primary	1	10	11	9.1
Secondary	6	35	41	14.6
≥ Tertiary	15	138	153	9.8
Religion				
Christianity	20	138	158	12.7
Islam & Others	2	45	47	4.3
Genotype				
AA	12	101	113	10.6
AS	9	46	55	16.4
SS / SC	0	5	5	0.0
AC	1	5	6	16.7
Unknown	0	26	26	0.0
Parity				
Para 0	9	79	88	10.2
Para 1 - 2	12	79	91	13.2
Para 3 - 4	1	21	22	4.5
≥ Para 5	0	4	4	0.0
Gestational age (GA) in trimester				
1st trimester	2	29	31	6.5
2nd trimester	14	111	125	11.2
3rd trimester	6	43	49	12.2
Mean GA (weeks + SD)	21.91±6.80	20.70±7.04	20.9±7.0	

Table 2: Prevalence rates of asymptomatic bacteriuria of pregnancy by different screening methods

Methods	Number with significant bacteriuria (N = 205)	Prevalence (%)
Culture	22	10.7
Nitrite test alone	11	5.4
LE test alone	17	8.3
Combined nitrite and LE tests	24	11.7

remaining 14 were found to be positive by culture constituting true negative (TN) and false negative (FN) results respectively (Table 3). Statistically, the difference in the figures obtained with nitrite strip test alone compared with culture was significant ($p < 0.05$). Further evaluation of accuracy of nitrite strip test revealed sensitivity of 36.4% and a high specificity of 98.4%. Positive and negative predictive values were 72.7% and 92.8% respectively. Likelihood ratios for a positive and negative nitrite strip test were 22.8 and 0.6 respectively.

Table 3: Comparison of results of strip tests and culture in patients screened for asymptomatic bacteriuria

	Culture results			
Strip tests	Positive	Negative	Total	Significance*
Nitrite test				
Positive	8 (TP)	3 (FP)	11	P = 0.000(S)
Negative	14 (FN)	180 (TN)	194	Odds ratio = 0.02995%
Total	22	183	205	CI (0.007-0.122)
LE test				
Positive	7 (TP)	10 (FP)	17	P = 0.000 (S)
Negative	15 (FN)	173 (TN)	188	Odds ratio = 0.12495%
Total	22	183	205	CI (0.041-0.372
Combined nitrite and LE				
Positive	11 (TP)	13 (FP)	24	P = 0.000 (S)
Negative	11 (FN)	170 (TN)	181	Odds ratio = 0.07695%
Total	22	183	205	CI (0.03-0.21)

**Fisher's exact test and McNemers test*
S = Significant LE = Leucocyte Este

Calculation of indices of accuracy of strip tests (Values for Nitrite)

Sensitivity = $\frac{TP}{TP+FN} = \frac{8}{8+14} = 36.4\%$

Specificity = $\frac{TN}{TN+FP} = \frac{180}{180+3} = 98.4\%$

Positive Predictive Value(**PPV**)= $\frac{TP}{TP+FP} = \frac{8}{8+3} = 72.75\%$

Negative Predictive Value (**NPV**) = $\frac{TN}{TN+FN} = \frac{180}{180+14} = 92.7\%$

Likelihood ratio for positive test (**LR+**) = $\frac{\text{Sensitivity}}{\text{1- Specificity}} = \frac{36.4}{1.6} = 22.8$

Likelihood ratio for negative test (**LR-**) = $\frac{\text{1- Sensitivity}}{\text{Specificity}} = \frac{63.6}{98.4} = 0.6$

Key

Positive result for combined nitrite and leucocyte esterase test = Positive strip test result for at least either nitrite or leucocyte esterase.

Negative Result = Negative strip test results for both nitrite and leucocyte esterase.

Table 4: Summary of accuracy indices of strip tests for asymptomatic bacteriuria in pregnancy

Indices	Strip tests		
	Nitrite	Leucocyte esterase	Combined nitrite & LE
Sensitivity (%)	36.4	31.8	50
Specificity (%)	98.4	94.5	92.9
PPV (%)	72.7	41.2	45.8
NPV (%)	92.9	92.0	93.9
LR+	22.6	5.6	7
LR-	0.6	0.7	0.5

Table 3 also shows performance of leucocyte esterase in detecting asymptomatic bacteriuria compared to culture. The corresponding true positive, false positive, true and false negative values are also shown. Compared with culture method, there was statistically significant difference in the performance of leucocyte esterase in detecting asymptomatic bacteriuria ($p < 0.05$).

When combined together as a single result, strip tests for nitrite and leucocyte esterase compared to culture method, still revealed a significant difference statistically ($p< 0.05$) as shown in table 3. In this wise, a result was regarded as positive for bacteriuria if either nitrite or leucocyte esterase segment of the strip test was positive. Also, a negative result in this wise was when both tests were negative.

Indices revealing relative levels of accuracy of the reagent strip tests in comparison to microbiological culture are presented in table 4. While highest level of sensitivity was obtained when the strip tests were combined, nitrite strip test alone was associated with highest specificity, positive predictive value and likelihood ratio for positive test. All the 3 modalities for strip test however had sensitivities of 50% or less.

Discussion

The prevalence of asymptomatic bacteriuria (detected by microbiological culture) among pregnant women attending the first antenatal (Booking) clinic at University College Hospital Ibadan, was 10.7%. However, the prevalence rates were much lower when nitrite and leucocyte esterase were used separately to detect bacteriuria among the study population (5.4% and 8.3% respectively). When combined as a single test result, the strip tests produced a slightly higher prevalence rate (11.7%). The prevalence rates of bacteriuria in pregnancy obtained by both microbiological culture and reagent strip tests in this study fall within the reported rates of 2-11% in most studies and that of 4-23.9% reported among Nigerian pregnant women [1,18-22].

The use of nitrite or leucocyte esterase strip tests separately produced low sensitivity in detecting asymptomatic bacteriuria in pregnancy. However, the high specificity and corresponding negative predictive value may suggest usefulness in excluding bacteriuria when the tests are negative. Also, combination test, using results of both nitrite and leucocyte esterase produced a higher level of sensitivity (50%) with high specificity and negative predictive value. Other studies and reviews elsewhere have reported similar findings when dipstick tests were compared with urine culture [11,26]. Garigalo-Molina in his overview of diagnostic approaches to asymptomatic bacteriuria reported an improvement in accuracy when combined strip tests are employed [11]. However, the level of sensitivity obtained with combined strip test in this study (50%), is lower than the range of 68% – 88% reported in a meta – analysis by Deville et al [9].

Since statistical analysis in this study has also revealed significant difference in the diagnostic accuracy of strip tests and microbiological culture (p- value < 0.05), routine laboratory culture remains the gold standard for screening for asymptomatic bacteriuria in pregnancy as had been widely acclaimed. However, when cost of screening is an

important consideration especially in low-resource settings, combined strips tests for nitrite and leucocyte esterase may be useful since it demonstrated fair level of sensitivity and high specificity. A negative strip tests for nitrite and leucocyte esterase may be useful in ruling out urinary tract infection generally as reported in meta-analysis by Deville et al [9]. This is further strengthened by a fairly high likelihood ratio for positive test and low likelihood ratio for negative result. Likelihood ratio (LR) is the ratio of the probability of having a particular test result among those that have the condition to the probability among those who do not have the condition, in this case, asymptomatic bacteriuria as diagnosed by culture result. The LR is used to assess the value of a diagnostic test. It has advantages over sensitivity and specificity because they are less likely to change with prevalence of the disorder and can be used to calculate post test probability of a target disorder. A high likelihood ratio (e.g LR > 10) is useful for ruling in a diagnosis while a very low LR (e.g LR < 0.1) virtually rules out the diagnosis. A LR around 1 however produces no useful information to either rule in or rule out the diagnosis [27].

In a study of cost-effectiveness and cost-benefit analysis, Rouse et al found that screening for, and treatment of asymptomatic bacteriuria to prevent pyelonephritis is cost – beneficial whether based on the leucocyte esterase-nitrite dipstick or on urine culture when compared with a policy of no screening at all [28]. However, the culture strategy was not beneficial when compared with the dipstick strategy. Similarly, Tincello and Richmond reported that reagent strip testing of antenatal urine specimen is effective and accurate when used to screen for bacteriuria in an effort to reduce the cost of urine analysis and culture [27]. Etherington and James reported in a study of 898 samples that routine use of reagents strips would result in a saving of over £4000 a year in a busy inner city hospital [29]. In our environment, the cost of performing laboratory urine culture and sensitivity varies between US $330 and $660 per 100 samples while dipstick test of equal number of samples will cost US $65 - $130. This amounts to a saving of US $265 - $530 for every 100 samples screened by dipstick test. The cost for dipstick test can further be reduced when it is done at the outpatient clinic (antenatal clinic) where laboratory personnel are not required and cost of the test can directly reflect the cost of the strips (US $30 per 100 strips). Therefore, there is convincing evidence that in low resource settings, dipstick test for asymptomatic bacteriuria is a more cost-effective and cost-beneficial approach to screening for asymptomatic bacteriuria.

Major strength of this study lies in the methodology; the microbiologist and other staff involved in urine culture were unaware of the initial result of dipstix test, thus eliminating bias in the microbiological culture.

In conclusion, this study has revealed that the leucocyte esterase-nitrite dipstick tests in combination are only fairly accurate and not optimal (compared to laboratory culture), though may be more cost effective in detecting asymptomatic bacteriuria.

Larger multi-centre studies are recommended for generalization of the findings of this study. Appropriate cost-benefit and cost-effectiveness analysis study on various screening modalities for asymptomatic bacteriuria is also recommended.

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