

Asymptomatic Bacteriuria among Pregnant Women Attending Antenatal: Evaluation of Screening Test

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ABSTRACT:

Introduction: Asymptomatic bacteriuria in pregnancy is common with a prevalence of 2 – 10% and is similar to that observed among non-pregnant women. It is however more likely to progress to symptomatic urinary tract infection during pregnancy because of the physiological changes associated with pregnancy. The value of screening for asymptomatic bacteriuria in pregnancy cannot be over-emphasize, but the kits and methodology need to be evaluated before adoption. **Methodology:** The study was a prospective, cross sectional, hospital based study. All the subjects were given plastic universal sterile transparent container with screw cap and were enlightened to collect clean catch midstream urine for urinalysis dipstick and microscopy methods using a calibrated wireloop and tested against culture method (which was considered gold standard). **RESULT:** The urinary strip for nitrite gave a sensitivity of 25.0%, a specificity of 99.1% and a negative predictive value of 90.0% and positive predictive value of 80.0%. From the foregoing the false positive rate was 1.0% while the false negative rate was 10.0%. The efficacy of microscopy method was evaluated as evidence by the presence of pus cells and positive Gram's stain (positive or negative Gram organism) and compared with gold standard culture, sensitivity (true positive) of 81.3%, a specificity of 94.5% was obtained. **Conclusion:** Microscopy method has appreciable sensitivity and specificity, biochemical methods have low sensitivities but high specificities when compared to the gold standard.

KEY WORDS: Evaluation of methods, screening, asymptomatic bacteriuria and pregnancy

I. INTRODUCTION

Urine is the amber colored fluid excreted from the kidney at the rate of about 800-1500ml every 24 hours in an adult^{1,2}. The urinary tract comprises of the following organs: Kidneys, Ureters, Bladder and Urethra.^{3,4}

Bacteria are most commonly responsible for Urinary tract infection, although Yeast and Viruses may also be involved^{2,5}.

Asymptomatic bacteriuria is one in which urine culture reveals a significant growth of pathogen that is greater than 10^5 bacteria/ ml⁹ but without symptoms of urinary tract infection. Asymptomatic bacteriuria in pregnancy is common with a prevalence of 2 – 10% and is similar to that observed among non-pregnant women⁵. It is however more likely to progress to symptomatic urinary tract infection during pregnancy because of the physiological changes associated with pregnancy^{5,6}. Asymptomatic bacteriuria in pregnancy if left untreated, about 30% of the affected mothers will develop acute pyelonephritis compared to 1.8% of non-bacteriuric controls⁵. Pyelonephritis can lead to perinatal and maternal complications, including preterm delivery, low birth weight, foetal mortality, hypertension, anaemia and renal insufficiency^{5,7,8}. Randomized controlled trials and cohort studies have shown that detection and treatment of asymptomatic bacteriuria in pregnancy can decrease the occurrence of acute pyelonephritis later in pregnancy and also decrease the occurrence of intrauterine growth restriction⁵.

The value of screening for asymptomatic bacteriuria in pregnancy may be judged by the screening criteria of Wilson and Jungner⁹. These criteria are (1) the condition sought must be an important health hazard; (2) a latent phase of the condition must be recognizable by simple and acceptable test; (3) the natural history of

the condition must be understood and the beneficial effect of the treatment upon the natural history must be established and (4) the cost of case finding and treatment should be economically balanced against the expenditure on medical care as a whole.

Screening for asymptomatic bacteriuria in pregnancy has become a standard for obstetric care and most guidelines in developed countries include screening and treatment of asymptomatic bacteriuria^{5, 6, 7}. Screening for asymptomatic bacteriuria has been shown to be cost effective when compared with treating urinary tract infection and pyelonephritis without screening^{10,11}. There are, however, inadequate data to determine the optimal frequency of subsequent urine testing during pregnancy¹². Some authors have suggested that urine should be cultured in each trimester to improve the detection of bacteriuria in those who were not bacteriuric at initial screening¹³.

In a prospective study of 3,254 pregnant women, Stenqvist¹⁴ concluded that if a single time point was chosen for screening, the 16th week was optimal time to screen, based on the greatest number of bacteria-free gestational weeks gained from treatment. But there are several point of care test kits for the evaluation of pregnant women, with varying prediction accuracy, although the commonly available kits are simple, easy to handle and easy to use, but in reality their use relies mostly on the manufacturers' instructions^{4,6,7}. The main screening techniques for detecting bacteriuria include urinalysis to detect the presence of protein, leucocyte esterase activity, nitrite (Griess) test, urine microscopy and urine culture. Others include uriscreen, urinary interleukin-8, and chlorhexidine reaction. The gold standard for screening for asymptomatic bacteriuria is growing bacteria cultures of urine samples from women in early pregnancy (12-16 weeks gestation), or at the first prenatal visit^{7, 12, 15, 16}.

The procedure involves inoculation of urine specimen on cholcolate and blood agar plates and incubating at 37^o C for 24 hours, after which the colonies are counted¹⁷. A diagnosis of significant bacteriuria is made when there are 10⁵ or more colony forming units (CFU) of a single pathogen per a millilitre of urine, provided a calibrated wire loop was used to inoculate¹⁷. Further culture in Cysteine Lactose Electrolyte Deficient agar (CLED) for isolate characterization and antibiobiotic sensitivity test.

A dipslide culture method is substantially less costly, allows immediate inoculation after specimen collection and requires no special equipment for incubation as it may be left at room temperature¹⁸. A dip slide is a plastic paddle coated on both sides with agar that is attached to a plastic cap that screws onto a sterile plastic vial^{19,18}. One side of the dipslide is coated with cysteine-lactose-electrolyte-deficient which supports the growth of all bacteria known to cause urinary tract infections. The other side is coated with reddish MacConkey medium selective for Gram negative bacteria^{19,18}. However to identify the causative organism and its antibiotic sensitivity, the traditional quantitative culture is still needed^{19,18}.

Microscopic urinalysis which involves analysis of urinary sediments to detect bacteria and white blood cells has also been evaluated as a screening test for bacteriuria¹⁷. Pyuria is deemed significant when there are ten pus cells per high-power field¹⁷. In pregnant women, microscopic analysis, with either bacteriuria or pyuria indicating a positive test, was found to have a sensitivity of 83% but a specificity of only 59%¹².

The dipstick test is rapid, inexpensive, and requires little technical expertise. The dipstick leucocyte esterase test, which detects esterases released from degraded white blood cells, is an indirect test for bacteriuria. It is an alternative method for detecting pyuria when it is not possible to examine fresh urine microscopically for white blood cells or when the urine is not fresh and likely to contain mostly lyzed white blood cells²⁰. When compared to culture, it has a sensitivity of 72-97% and a specificity of 64-82%¹². It is however subject to false negatives in cases of bacteriuria without pyuria or when urine contains boric acid or excessive amounts of protein(> 500mg/100ml) or glucose (>2g/100ml)²⁰ and false positives with contamination from vaginal secretions.

The nitrate reduction test detects nitrites produced by urinary bacteria. The immediate development of pink-red colour gives a positive nitrite test²⁰. The ability to reduce urinary nitrate to nitrite is usually possessed by all Gram negative bacteria belonging to the genera *Escherichia*, *Klebsiella*, *Citrobacter*, *Proteus* and *Shigella*. This property is also possessed by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus albus*, but never found in *Streptococci*^{17, 21}. It has a variable sensitivity (35-85%), but a good specificity (92-100%)¹².

Uriscreen, a rapid enzymatic urine test for asymptomatic bacteriuria, is a reliable alternative to culture screening of pregnant women²². This test enables the detection of catalase produced by bacteria²². It has said a sensitivity of 100%, specificity of 81%, negative predictive value of 100%, and positive predictive value of 30%²². With the low cost of uriscreen compared with culture screening (\$1.00 versus \$18.00), a policy of performing urine culture only on patients with positive uriscreen will save as much as 80% of unnecessary cultures, saving as much as \$1,340,000.00 for every 100,000 specimens²². It is however not readily available in our environment. Detection of urinary interleukin-8 (IL-8), an inflammatory cytokine by chemiluminescent immunoassay has been studied²³. Using an optimal cut off point of 264 pg/ml interleukin-8 was found to have a sensitivity of 70% and specificity of 67% for predicting asymptomatic bacteriuria²³. It is however not an acceptable screening method for asymptomatic bacteriuria because it fails to detect 30% of women with this condition²³.

In university of Benin Teaching Hospital, a study was conducted to evaluate the value of chlorhexidine reaction in the detection of asymptomatic bacteriuria in pregnancy²⁴. The accuracy of 1:1000 concentration of chlorhexidine in detection of bacteriuria was compared with bacteriological method.

In the study, 10 drops of midstream urine were placed in a test tube and 5 drops of 1:1000 dilution of chlorhexidine were added. The mixture in the test tube was shaken and observed after 1 minute and a cloudy colour indicated a positive result²⁴. The sensitivity of chlorhexidine in detecting bacteriuria was 100% but with a very low specificity of 28.5%²⁴. The study concluded that chlorhexidine reaction is of little value in detecting bacteriuria, but is however recommended as a screening tool for urinary tract infection in pregnancy because of its high sensitivity, cost effectiveness and ease of performance²⁴. At low concentrations, chlorhexidine affect membrane integrity of bacteria and at high concentrations, cause coagulation of cytoplasmic constituents and resulting in precipitation of cellular proteins and nucleic acids^{24,25}. It also adsorbs on pus cells, serum and red blood cells and forms insoluble salts with phosphates, silicates, and bicarbonates which are occasionally present in urine^{24, 25}.

Currently, paucity of data in Nigeria regarding the efficacy of point of care and evaluation of screening tools for patients with asymptomatic bacteriuria, which have militated against a nationwide adoption of a particular test kit for screening of pregnant patients. The slow pace at evaluation urinary test kits in pregnancy have reduced the detection of urinary tract infections and thus increased in pregnancy related complication, most especially among asymptomatic patients and had contributed to poor understanding of its merits and thus made preventive and control programme very difficult both to the patients and healthcare provider.

Therefore, the aim of this study was to evaluate the screening tool in pregnant women with asymptomatic bacteriuria at the University of Abuja Teaching Hospital (UATH).

II. MATERIALS AND METHODS

Study background

The study was conducted at the antenatal clinic, department of Obstetrics and Gynaecology, University of Abuja Teaching Hospital, Gwagwalada, Abuja, Federal Capital Territory (F.C.T). The Hospital is located in Gwagwalada whose geographical coordinates are 8° 56' 29" North and 7° 5' 31" East. It has an area of 1,043 km². The Federal Capital Territory had a population of 1,406,239 inhabitants in the year 2006, of which 157770 (11.22% approximately) inhabitants reside in Gwagwalada.²⁶ Projected population of Gwagwalada city in 2012 was over 1 million people. The Hospital has an average of 3,000 deliveries annually, and annual attendees at the booking antenatal clinic well over 5,000.

Study population

The study was conducted on pregnant women in the antenatal booking clinic during the study period. The population was a mixture of rural and urban dwellers. Those who consented to participate in the study were enrolled and were informed about the need for this work using the study tools (questionnaire and consent form).

Study design

The study was a prospective, cross sectional, hospital based study.

INCLUSION CRITERIA:

- i. All pregnant women with no symptom who gave consent
- ii. All women booked for antenatal care in the hospital

EXCLUSION CRITERIA

- i. Patient that discontents to be part of the study.
- ii. History of diabetes mellitus, renal disease or sickle cell anaemia
- iii. All pregnant women who are diagnosed of parasitic plasmodiasis.

STUDY SAMPLE SIZE

The sample size was calculated using the statistical formula of Fischer²⁷:

$$N = z^2 p q / d^2$$

P = prevalence of asymptomatic bacteriuria (8%)²⁵ as local prevalence.

N= the minimum sample size = 113.

Therefore, the sample size was 125

III. METHODS

This study was conducted in conjunction with the Clinical Microbiologist. The purpose of this study was explained to the subjects before their consent to participate was sought. The consent form was filled by the investigator and the subjects recruited signed the form. Interviewer-administered, structured questionnaires were used as the study tool. The questions outlined in the data forms were explained to the subjects and completed forms which contain information that included the bio-demographic data (such as subject age, gravid age, parity, educational status), provisional diagnosis and laboratory processes, such that the eventual result was noted in the data forms and communicated to the Obstetrician and the patients.

IV. SPECIMEN COLLECTION, TRANSPORTATION AND PROCESSING

All the subjects were given plastic universal sterile transparent container with screw cap and were enlightened to clean the genital area three times with lukewarm water and allowed to air dry, avoiding chemicals^{28,29}. In addition, the cleaning should be anterior to posterior in unidirectional with the labial majora and minora held apart. Clean catch midstream urine was collected and submitted to the microbiology and immunology research laboratory where macroscopy, microscopy, cultural characterization and antibiogram were performed.

The type and quality of specimens submitted to the laboratory usually determine the success of isolating the bacteria. Each specimen received should therefore be examined for quality, in terms of amount, sterility and presence or absence of debris.^{28,30}

The urine specimen was macroscopically examined for turbidity, presence of blood and divided into two equal parts. Urine obtained from the first part (uncentrifuge urine) was used for urinalysis, microscopy analysis and Gram's stain procedure while urine from the second container were aseptically centrifuged at 3000 rpm for 5 minutes, with the supernatant discarded and the residue used in inoculating blood agar and Cysteine Lactose Electrolyte Deficient (CLED) and the remaining residue was microscopically examined, presence of pus cells was noted (microscopy analysis of centrifuge urine). Calibrated wire loop with internal diameter of 5mm that delivers 0.002ml was used to inoculate the samples on those media. The cultures were incubated at 37°C for 18-24 hours with adequate moisture. Positive urinary nitrite and leucocyte esterase on urinalysis and presence of pus cells were considered features suggestive of urinary tract infection but the presence of at least one Gram organism per oil-immersion field in uncentrifuged urine or colony count of greater than 10⁵/ul of urine from overnight growth on blood agar plate was considered significant bacteriuria. After overnight incubation on the CLED, the growth characteristics were noted and pure growth was Gram stained^{28,29,31}.

Data Analysis

Data were analysed using SPSS 16.0 software. The chi square-test and Fischer exact test was used to perform and establish any statistical difference. Probability values of <0.05 was considered as statistically significant. Sensitivity, specificity, positive and negative predictive value of various techniques were determined following the methods of Galen and Gambino³³.

V. ETHICAL CONSIDERATIONS

The study was approved by the Ethical Committee of University of Abuja Teaching Hospital, Gwagwalada, Abuja, F.C.T.

VI. RESULTS

A total of 125 consecutive consenting women who met the inclusion criteria were screened for asymptomatic bacteriuria. Their ages ranged between 19 and 41 years with a mean of 28.9 years \pm 4.2. The median and modal ages were 28 and 30 years respectively. Most of the women were married (98.4%) while 1.6% was single. Out of 125 urine samples examined, 16 women had positive cultures, giving a prevalence rate of 12.8% for asymptomatic bacteriuria. The highest frequency ratio (relative ratio) was among age group <20 years (0.33) and the lowest were among age group 25-29 years (0.06). The association was not statistically significant (p=0.245, Table 1).

The bacterial pathogens isolated from 16 women with asymptomatic bacteriuria. *E. coli* was the commonest bacteria isolated (56.3%), followed by beta haemolytic *Streptococcus* (18.3%), *Klebsiella pneumoniae* (12.5%) and *Staphylococcus aureus* (12.5%).

The biochemical methods were also used for screening the women using the urinary nitrite and leucocyte esterase and their sensitivities compared using culture as the gold standard. The urinary strip for nitrite truly detected bacteriuria in only 4 of the 16 cases of significant bacteriuria with 108 true negative cases. There were 12 false negative cases and 1 false positive case. This gave a sensitivity of 25.0%, a specificity of 99.1% and a negative predictive value of 90.0% and positive predictive value of 80.0%. From the foregoing the false positive rate was 1.0% while the false negative rate was 10.0%.

The efficacy of microscopy method was evaluated as evidence by the presence of pus cells and positive Gram's stain (positive or negative Gram organism) and compared with gold standard culture. It detected true bacteriuria in 13 of the 16 cases of significant bacteriuria and false positive case of 6, using this technique the true negative was 103 cases. This gave sensitivity (true positive) of 81.3%, a specificity of 94.5%. Positive predictive value of 68.4% was obtained whereas the negative predictive value from the cases was 97.2%. False positive rate was 5.5% while the false negative rate was 2.8% using the microscopy methods.

The leucocyte esterase was able to truly detect bacteriuria in 7 cases and truly excluded bacteriuria in 107 cases. There were 2 false positive and 9 false negative cases. This gave a sensitivity of 43.8%, specificity of 98.2% and negative predictive value of 92.2% positive predictive value of 77.8%. False positive rate was 1.8% while the false negative rate was 7.8% with this technique.

Table 1: Age distribution and significant bacteriuria among asymptomatic pregnant women.

Maternal age(Years)	Total	Percent	Culture methods	Percent
< 20	3	2.4	1	6.3
20 - 24	13	10.4	1	6.3
25 - 29	50	40.0	3	18.8
30 - 34	45	36.0	8	50.0
>35	14	11.2	3	18.8
Total	125	100.0	16	100.0

p= 0.245, $\chi^2= 5.090$, df= 4

Table 2: Gestational age distribution and significant bacteriuria among asymptomatic pregnant women.

Maternal age (Years)	Total	Percent	Culture methods	Percent
1 ST trimester	21	16.8	1	6.3
2 nd trimester	65	52.0	13	81.3
3 rd trimester	39	31.2	2	12.5
Total	125	100.0	16	100.0

p= 0.374, $\chi^2= 5.090$, df= 2

Table 3: Efficacy of diagnostic methods among asymptomatic pregnant women.

Methods	bacteria Screened	bacteria Screened	Total positive	negative
Culture (gold standard)	16		109	125
Urinary nitrite	4		121	125
Urinary leucocyte esterase	7		118	125
Microscopy	13		112	125

Table 4: Sensitivity, specificity, positive predictive value of the methods of screening for bacteriuria among asymptomatic pregnant patients.

Methods	Prevalence	Sensitivity	Specificity	Predictive value	
(Percent)	(%)	(%)	Positive (%)	Negative (%)	
Urinary nitrite	4 (3.2)	25.0	99.1	80.0	90.0
L. esterase	7 (5.6)	43.8	98.2	77.8	92.2
Microscopy	13 (10.4)	81.3	94.5	68.4	97.2

L. esterase = Urinary leucocytes

VII. DISCUSSION

The introduction of laboratory screening technique in specialized area of medicine have transform and improve the art of diagnosis in the tropic and indeed in the developed countries. In the latter point of care services are frequently used and are at near universal coverage. Early recognition of UTIs via screening will definitely avoid grave consequence of the disease. But interestingly, in the developing countries, resources are scarce and poor funding of the health sector couple with inadequate sophisticated machines; the use of simple, cheap and readily available kits and technology should be encourage, but the efficacy of such kits needs to be tested. Therefore this present study evaluated the accuracy test of three common methods of screening urinary tract infections among pregnant women.

Bacteria detection by microscopy showed sensitivity as high as 81.3%. This outcome was similar to studies around the world^{12,20,22,24}, high predictive true positive rate and a few false negative (3 cases), therefore it can rule out UTIs and this technique would rarely misdiagnosed the presence of bacteria in urine. However this technique does not tell us if the presence of bacteria was as a result of poor specimen collection (contamination). Presence of pus cells would not indicate the Gram's cell wall characteristic and that some non-Gram's bacteria can cause UTIs with significant bacteriuria. This method offers a good specificity (94.5%) with few false positive. Therefore combine high sensitivity and specificity ensures that the microscopy method would be accurate for screening test as well as for diagnosis and compares favourably with culture methods.

The detection technique by using urinary nitrite (urinary strips) showed specificity as high as 99.1% in subjects investigated and a low sensitivity owing to large number of false negative. This outcome was contrary to other studies^{12,20,23,24}. Urinary nitrite method had showed a lower sensitivity, hence not fit for screening tool. Urinary nitrite technique is available in most clinical and service, research laboratories and side laboratories, simple to use, cheap to acquire, not invasive and can be use at home but this method is less sensitive. Despite the Microscopy method has problems of interobserver variability, the nitrite method does not give a clue to the nature of the bacteria and possible antibiotic preliminary choice, it only suggestive of the presence of bacteria. The leucocyte esterase negative predictive value was high with 92.2% with low sensitivity and high specificity which was similar to the accuracy measurement of urinary nitrite. Both methods are in the urinary strips. Although, it had better sensitivity rate compared to that of urinary nitrite. This was contrary to reported studies^{4,20,24}. The difference may be due to methodology of specimen collection. In this study urine was collected at random whereas in the other study urine was collected as first-morning specimens and on consecutive two days⁴.

The efficacy of the methods in this study have showed that there is no one method that is ideal, microscopy remains the only method in this study closest to culture but needs expertise, while the simple urinalysis strips have low sensitivity, therefore urine tested with urinalysis strips should be submitted to the laboratorian for culture. This is very important in the sense that pregnant women can as well regularly check their urine from the comfort of their home and report to the clinic if there are problem and allow for proper urinary evaluation. Therefore, regular, rapid and non-invasive investigation using clear and unambiguous point of care test should be encouraged.

VIII. CONCLUSION

Urine culture is the gold standard for diagnosis of asymptomatic bacteriuria. Microscopy method have appreciable sensitivity and specificity, biochemical methods have low sensitivities but high specificities. The sensitivity of leucocyte esterase was however better than that of nitrite.

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