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Original Article

Comparison of the Accuracy of Uriscreen Test with Urine Culture in the detection of Asymptomatic Bacteriuria during Pregnancy, at a Medical Center.

Running Title: Uriscreen test can compare favorably with urine culture in detecting asymptomatic Bacteriuria in pregnancy

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Abstract

Context: Routine screening of all pregnant women during antenatal clinic visits is being advocated so as to detect Asymptomatic Bacteriuria (ASB) and avert associated complications. Urine culture technique is the gold standard for diagnosis of ASB but it is unaffordable in developing countries. There is paucity of data on the use of Uriscreen test as an alternative screening tool to urine culture in detecting ASB in obstetric practice. **Aim:** To compare the accuracy of Uriscreen with urine culture method for the detection of asymptomatic bacteriuria in pregnancy. ***Study Design:*** Comparative Cross-sectional study. **Methods and Materials:** Four hundred and twenty-five (425) consecutives booked, consenting women without symptoms of urinary tract infection during ANC were used. Clean catch midstream urine specimen was collected into two properly labeled containers to detect bacteriuria using Uriscreen and culture methods. The prevalence, accuracy and costs were calculated and compared between the two methods. Data management was with SSPS with significant p-value set at <0.05 **Results:** The prevalence of ASB was 43.3% and 35.1% using Uriscreen and urine culture respectively (P <0.001). The accuracy, sensitivity and specificity of Uriscreen compared to urine culture were 92.42%, 100% and 88.78% respectively. The cost of Uriscreen was 33.4% less than that of urine culture (P < 0.05). **Conclusion:** Uriscreen is a useful alternative screening tool to the urine culture method because it is cheap, has good sensitivity and specificity in detecting bacteriuria in asymptomatic pregnant women.

Keywords: Asymptomatic Bacteriuria, Uriscreen, Urine Culture

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Introduction

Asymptomatic bacteriuria is the presence of significant bacteriuria (≥100,000cfu/ml) in a patient without urinary symptoms[1, 2]. The physiological, anatomical, immunological and biochemical changes in pregnancy make the pregnant woman with asymptomatic bacteriuria at risk of progression to pyelonephritis- a condition linked with preterm labour, intrauterine growth restriction, low birth weight, and other complications[1,2,3,4]. Therefore, there have been several global calls for the inclusion of routine screening for bacteriuria in pregnant women as a component of antenatal care services[5]. The United States Preventive Services Task Force (USPSTF) and indeed many developed countries recommend screening for asymptomatic bacteriuria with urine cultures for pregnant women at 12 to 16 weeks gestation or at their first antenatal visit, if later[6,7]

Urine culture method is the gold standard for screening but it is expensive, time consuming, requires electricity and trained personnel to perform the test[8].

URISCREEN is a simple, rapid cost-effective enzyme test primarily intended for screening of asymptomatic population for significant bacteriuria[9]. This study was intended to compare the performance of Uriscreen test against urine culture for the detection of bacteriuria among asymptomatic pregnant women at FMC, Katsina.

Subjects And Methods

This was a hospital based, comparative cross-sectional study conducted at the Obstetrics and Gynaecology department of the Federal Medical Center, Katsina (North-west, Nigeria), between 4th September, 2019 and 17th January, 2020. Four hundred and twenty-five (425) consecutive consenting pregnant women seen at the antenatal clinic and without symptoms of urinary tract infection formed the study population for the study. All pregnant women with symptoms of urinary tract infection such as frequency, dysuria or loin pains, and all those currently on antibiotics or have had antibiotic treatment in the preceding two weeks and women at higher risk for urinary tract infections like hemoglobinopathy, diabetes mellitus, and previous history of urinary tract infection were excluded from the study.

The recruited women had to undergo pre-test counseling and a structured interviewer-administered questionnaire was administered to them.

An informed consent was obtained from each eligible participant. The protocol for the collection of clean catch mid-stream urine specimen was well explained to the women and they were assisted (when needed) by a nurse in order to reduce the chances of contamination. Each participant was given a sterile, dry, wide mouthed container with an instruction to clean the vulva with clean water. Cleaning with water removes leucorrhoea and other vaginal secretions that might give false positive result. She then used her index and ring fingers of any preferred hand to part her labia and then start voiding and use the container to collect about 20millilitres of urine halfway, having already voided out the initial one that might have genital bacterial contaminants.

The urine sample was taken to the laboratory where it was divided into two and placed in properly labeled containers- one for the culture and the other for the Uriscreen test. The Uriscreen test was carried out based on the manufacturer's instruction manual by the laboratory scientist with the assistance of the researcher. The URISCREEN^{TM} test kit (Catalog no.101-010) from Savyon Diagnostics Ltd, Israel was used. A Positive Uriscreen test is noted with the appearance of foam seen on the surface of the liquid. The quantity of the resulting foam indicates the presence and relative level of catalase originating from bacterial and/or somatic cells in the urine. Lack of foam indicated negative test results.

The samples for urine culture were immediately processed by routine quantitative culture within one hour by medical microbiologist and /or laboratory scientist. A semi-quantitative calibrated loop technique was used for the primary isolation of the organism using sterile calibrated wire loop, delivering 0.002ml (1/500ml) of urine. A loopful of urine was inoculated on dried plates of cysteine-lactose electrolyte-deficient (CLED) agar. The plates were incubated aerobically at 37^0C for at least 24hour, and the number of colony-forming units (CFU) was multiplied by the number of colonies on agar by 500. A urine sample that grew 10^5 CFU/ml or more of pure isolates was deemed

significant (positive culture), but a count of less than 10^5 CFU/ml was regarded as insignificant bacteriuria or due to contamination (negative culture). Isolates were identified to species level using standard methods and antibiotic sensitivities were by disc infusion technique.

The results were tabulated. Sensitivity, specificity, false negative, false positive rates, positive and negative predictive value as well as accuracy of Uriscreen were calculated using the urine culture as the gold standard.

The study was undertaken after due approval from the Ethics and Research Committee of Federal Medical Center, Katsina.

Results

Out of 425 pregnant women recruited for this study, 184 (43.3%) tested positive for bacteriuria with Uriscreen, while remaining 241 (56.7%) had negative screening results for bacteriuria with Uriscreen test. Similarly, of 425 pregnant women, urine culture detected 149(35.1%) women with bacteriuria (positive culture), while the remaining 276 (64.9%) subjects had negative culture results for bacteriuria with the urine culture method, as shown in Figure 1. There was a significant difference in the prevalence of bacteriuria detected by Uriscreen and urine culture (43.3% versus 35.1%, $\chi 2$= 197.476, p< 0.001).

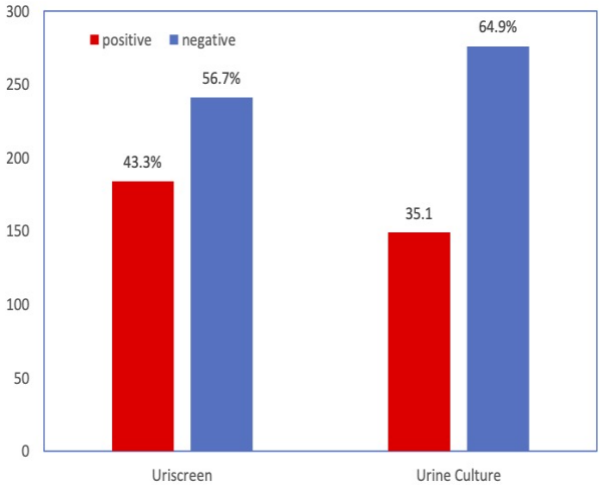

Figure 1: Prevalence of asymptomatic bacteriuria with Uriscreen compared with urine culture.

Table 1: sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Uriscreen test compared with urine culture

	DISORDER	NO DISORDER
POSITIVE TEST RESULT	TP (True positive) 35.1%	FP (False positive) 8.2%
NEGATIVE TEST RESULT	FN (False negative) 0%	TN (True Negative) 64.9%

Sensitivity = TP/TP+FN = (35.1/35.1+0) = 100%
Specificity = TN/TN+FP = (64.9/64.9+8.2) = 88.78%
Negative predictive value = TN/FN+TN= (64.9/0+64.9) = 100%
Positive predictive value = TP/FP+TP = (35.1/8.2+35.1) =81.06%
Accuracy of Uriscreen = TP + TN / TP+FP+TN+FN = 92.42%

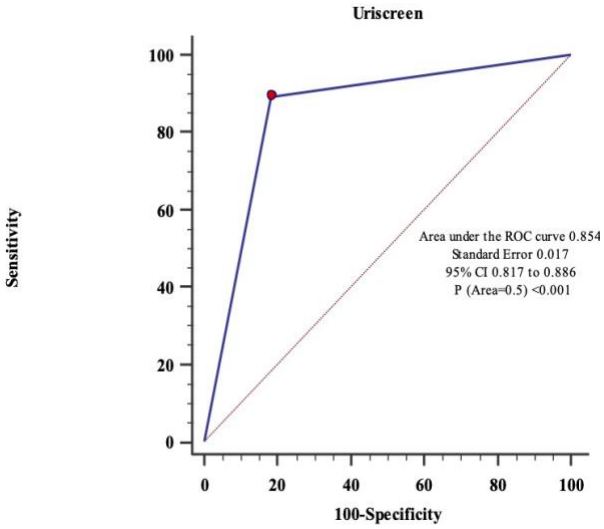

Figure 2: The area under the receiver operating characteristic (ROC) curve (Uriscreen).

Sensitivity, Specificity, Negative and Positive Predictive values of Uriscreen against Urine Culture using receiver operating characteristic (ROC)
The receiver operating characteristic plotted for Uriscreen showed the best area under the curve (diagnostic accuracy) of 0.854 (95% CI, 0.817 to 0.866), with a sensitivity of 89.26% and specificity of 81.52%.

The Cost Implications of Uriscreen with Urine Culture in Detecting

The unit cost of Uriscreen per pregnant woman was #799.35($2.19) while the total cost for the recruited subjects was #339,723.75 ($931.51). Similarly, the unit cost per pregnant woman for the urine culture

Table 2: Various Cut-Off Points for The ROC of Uriscreen Against Urine Culture

Criterion	Sensitivity	95% CI	Specificity	95% CI	+PV	95% CI	-PV	95%CI
≥0	100.00	97.60-100.00	0.00	0.00-1.30	35.10	30.50-39.80	0.00	
>0	89.26	83.10-93.70	81.52	76.40-85.90	72.30	65.20-78.60	93.4	89.40-96.20
>1	0.00	0.00-2.40	100.0	98.70-100.00	0.00		64.9	60.20-69.50

CI-Confidence Interval; +PV- Positive Predictive Value; -PV- Negative Predictive Value

Table 3: Comparison of Cost of Uriscreen with Urine Culture

Variable	Uriscreen	Urine culture	Paired t-test	P-value	Differential cost
Unit cost # ($)	799.35 (2.19)	1,200.85 (3.29)	-		401.50 (1.10)
Total cost # ($)	339,723.75 (930.75)	510,361.25 (1,398.25)	-		170,637.50 (467.50)
Mean ±SD # ($)	800.00 (15.36)	1,200.00 (21.72)	310.001	<0.001	-

1$= #365.00

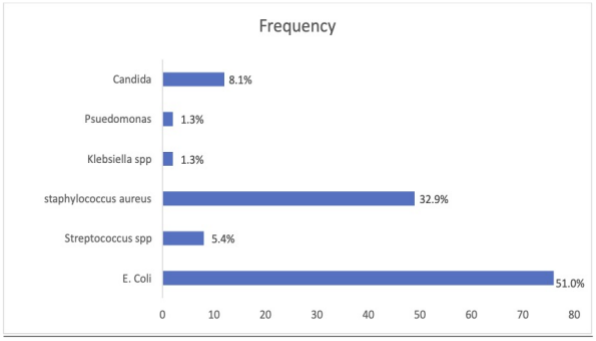

Figure 3: The pathogens isolated among the pregnant women (Urine Culture)

Table 4: Antimicrobial sensitivity patterns of the bacteria isolates

Antimicrobial	*E. coli* Sensitivity (%)	*Staphylococcus aureus* Sensitivity (%)	*Streptococcus spp* Sensitivity (%)	*KlebsiellaSpp* Sensitivity (%)	*Pseudomonas Sensitivity* (%)
Nitrofurantoin	89.5	67.3	37.5	50.0	50.0
Augmentin	93.4	57.1	100.0	50.0	00
Ceftriaxone	94.7	55.1	62.5%	50.0	50.0
Cefuroxime	57.9	8.2	25.0	50.0	0.0
Erythromycin	38.8	36.7	12.5	50.0	0.0
Gentamycin	92.1	53.1	75.0	50.0	50.0

was #1,200.85($3.29) with total cost of #510,361.25 ($1,398.25). The price of Uriscreen test was less than that of Urine culture by 33.4%. The mean (±SD) amount of money spent on Uriscreen per pregnant woman was #800:00 (± 21.72) while the mean amount spent on Urine culture per pregnant woman was #1,200:00 (± 15.36). a paired t-test was done to compare the two costs. The result showed that the unit cost of Uriscreen was significantly lower than the unit cost of Urine culture (t=310.001, p<0.001) as shown in Table 7.

Urine Culture Bacteriologic Profile

The most prevalent pathogens from the urine culture was *E. coli* 51.0% (76/149), this was followed by *Staphylococcus aureus*, 32.9% (49/149), *streptococcus spp*. 5.4% (8/149), as are shown in Figure 3.

Antimicrobial Sensitivity Patterns of The Bacteria Isolates

Among the pregnant women, all the bacteria isolates were sensitive to Nitrofurantoin, Ceftriaxone, and Gentamycin. In contrast, Pseudomonas was resistant against Augmentin, Cefuroxime and Erythromycin. Further details are shown in Table 4.

Discussion

The prevalence of asymptomatic bacteriuria in the study is 35.1%. This is above the generally accepted prevalence range of 2%-10% of all pregnancies[2,11]. It is more than 4.3% from Zaria[10], 9% reported from Kano[12] ,45.9% from Abuja[13], and 15.7%, and 21.2% from Addis Ababa and North Ethiopia[14,21]respectively. On the other hand, it is comparable to 40% from Ilorin[1] and 45.3% from Benin[15]. However, it was lower than values quoted from Abakaliki (78.8%) [16]and Aba (77.4%) [17]. These discrepancies between and within countries may be due to differences in the study participants' population, socioeconomic, educational levels, cultural and religious behaviors related to personal hygiene and cultural sexual practices, and the method of screening for bacteriuria.

Uriscreen test method detected 184 women out of 425 women with bacteriuria (as against 149 women using urine culture), leaving a higher prevalence of asymptomatic bacteriuria at 43.3%. There was a significant difference in the prevalence of bacteriuria detected by Uriscreen and urine culture (43.3% versus 35.1% respectively, $\chi 2$= 197.476, p< 0.001). Uriscreen is a rapid enzymatic screening test capable of detecting the enzyme-catalase produced by bacteria and other somatic cells in the urinary system. A Catalase positive

result suggests not only the presence of bacteriuria but renal cells or somatic cells[9]. The Uriscreen test was capable of detecting 100% of all those women whose urine tested positive with urine culture. In addition, it was also able to detect enzyme activities from both bacterial and somatic cells even when urine culture was negative. Thus, the sensitivity of Uriscreen in detecting bacteriuria in pregnancy was 100%, false positivity of 8.2%), lower specificity (88.78%) and low positive predictive value of 81.06%. This sensitivity for Uriscreen can be attributed to its reliability to detect the enzyme catalase, produced by bacteria. Uriscreen (based on the study) has 100% ability to predict that an individual without ASB will have a negative result from urine culture (Negative predictive value) and 81.06% chance of reporting a positive result in an individual with ASB (Positive predictive value).

The receiver operating characteristic plotted for Uriscreen showed the best area under the curve (diagnostic accuracy) of 0.854 (95% CI, 0.817 to 0.866), as shown in Figure 2. This result shows Uriscreen test as a good screening tool in detecting bacteriuria in asymptomatic pregnant women, with good sensitivity, specificity, negative predictive value (the probability that an individual with a negative result does not have significant bacteriuria) and positive predictive value (the probability that an individual with a positive result has significant bacteriuria). These findings are comparable to the study done in Israel which showed that Uriscreen had very high sensitivity of 100%, lower specificity (81%), high negative predictive value (100%) and a low positive predictive value (30%). The study in Israel concluded that the Uriscreen test is a reliable alternative to culture screening of all pregnant women and suggested that a policy of performing a urine culture during pregnancy only on patients with a positive Uriscreen test will save as much as 80% of unnecessary cultures[18]. The United States Preventive Services Task Force Reaffirmation Recommendation Statement evidence update on screening for asymptomatic bacteriuria highlighted Uriscreen as having good potential because of its 100% sensitivity and negative predictive value in a study of 313 pregnant women in Isreal[6]. In a multicenter study to evaluate the ability of Uriscreen to detect bacteriuria and pyouria compared with Chemostrip LN dipstick with semi-quantitative plate culture method as the reference test for bacteriuria and the gram stain was used as reference test for pyouria, Uriscreen had a better sensitivity than the urine dipstick (Chemostrip LN) for leukocyte esterase and nitrite[19].

The strength of this study lies in the large sample size, proper method of collection of urine as well as the fact that urine samples were analyzed within one hour of collection

The commonly used screening test for bacteriuria in pregnancy in this center is the use of simple urinalysis and bacteriuria is suspected if nitrite is detected. In a study of 330 asymptomatic pregnant women, the sensitivity and specificity of nitrite test was 35.7% and 98.0% respectively[20]. The low sensitivity of nitrite test may be due to the fact that urine samples collected in the hospital are not usually the first voided urine for that day. So, nitrite concentration in urine at the time of screening is usually low. The implication being that the urine had not stayed in the urinary bladder for at least 4hours, which is the minimum, required for obtaining an optimal number of urinary nitrites, and hence it is diluted[20]. In addition, a nitrite test does not detect organisms unable to reduce nitrates such as enterococci and staphylococcus[12, 21]. In this study, a great percentage of the organisms isolated were staphylococcus (32.9%). So, accuracy of nitrite test in detecting bacteriuria in this centre will be very low. This brings to light Uriscreen, which is also a rapid test with better sensitivity and negative predictive value.

The unit cost of Uriscreen per pregnant woman was $2.19 while the total cost for the recruited subjects was $931.51. Similarly, the unit cost per pregnant woman for the urine culture was $3.29 with total cost of $1,397.26. The price Uriscreen test was 33.4% less compared with urine culture as shown (Table 3).

Table 3 showed that the cost of using Uriscreen to detect bacteriuria in asymptomatic pregnant women was significantly lower than that of urine culture ($P < 0.05$).

This finding is comparable to that reported earlier in Israel in 1996. He concluded that 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 urine specimen[18]. Beside the direct cost, the result of Uriscreen test was within 3minutes while that of urine culture was within 48hours. This implies that the patient will

have to revisit the hospital for her result. This is not favorable in a developing economy like ours where transportation cost may be a serious issue and health facilities with well-equipped laboratory are usually located far from the rural dwellings. Also, the socio-cultural lifestyle tends to limit women's too frequent visits to the hospital. One also, will have to consider the amount of time wasted in returning to the laboratory for the culture result, the cost of getting a trained microbiologist and a well-equipped laboratory and maintaining constant electricity if urine culture is to be the ideal screening tool in this environment. All these are not available in low-income countries. On the contrary, Uriscreen is a rapid on-site screening test, relatively cheaper, simple to use kits and does not require electricity and a microbiologist.

It however has its own setback or limitations. Uriscreen cannot identify the causative organism of urinary tract infection which urine culture does. So, antibiotic sensitivity test is not possible with Uriscreen. Therefore, many have suggested that Uriscreen be used for screening and those with positive result for bacteriuria should be sent for urine culture and antibiotic sensitivity. Another limitation of Uriscreen use in our setting is its availability. Uriscreen is not readily available in our markets. The test kits have to be imported from overseas. Also, few studies have been done on the accuracy of Uriscreen. Even with these studies, their reports have been widely variable from different studies. Uriscreen is not specific. It has low positive predictive value and high false positive rate. This is because it detects catalase activity from bacteria and somatic cells which may not necessarily be as a result of infection of the urinary system.Also, Uriscreen cannot detect catalase-negative organisms, such as certain species of Streptococcus which occur in approximately 2% of all specimens screened, and 5-10% of those demonstrating positive results. So, in urine infected by these organisms, Uriscreen will give a false negative result.

The most common organism isolated this study were organisms from ano-rectal flora which has been implicated in several reports as being responsible for bacteriuria[4, 22, 23, 24, 25, 26]. The commonest organism isolated in this study is Escherichia Coli (51%). This conforms to previous studies which showed that Escherichia Coli is the commonest cause of bacteriuria in pregnancy [24, 25, 27, 28]. Studies at Ilorin and Ibadan reported Staphylococcus spp [1, 12, 23] as the commonest isolate. Other organisms isolated in this study include Staphylococcus aureus (32.9%), Streptococcus species (5.4%), Pseudomonas species (1.3%), Klebsiella species (1.3%) and candida albicans (8.1%).

Most organisms were sensitive to ceftriazone, gentamycin, nitrofurantoin and augmentin. These antibiotics were also found to be highly effective in studies in Ilorin[1] and in Zaria[10]. The study did not report on the quinolones because they are not usually prescribed in pregnancy due to their possible toxic effects on the foetus.

Conclusion

Screening for asymptomatic bacteriuria should form part of obstetric care considering its high prevalence (35.1%). The advantages of the Uriscreen method for screening over the blood culture method are its lower cost, shorter turnaround time leading to earlier diagnosis and prompt treatment, opportunity to see and treat at same visit. However, its disadvantage is the absence of the ability to isolate the exact organism for specific antibiotic treatment.

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