ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.4.L123-128

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Medicine for Novel Therapy

Evaluation of Diagnostic Methods for Asymptomatic Bacteriuria (ASB) During Pregnancy

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Abstract: Asymptomatic bacteriuria (ASB) is the existence of bacteria in a patient's properly gathered urine who has no obvious symptoms of a urinary tract infection, which is common in females, with the prevalence rate of 2-11%, ASB during pregnancy is influenced by a range of factors including; mechanical compression, changes in the immune and renal systems, if ASB untreated, it can lead to cystitis and pyelonephritis. Our aim is to evaluate the sensitivity and specificity of the screening tests compared with the culture method for detection of ASB during pregnancy, and to identify the most common etiologic agents of ASB in pregnant women. An analytical cross- sectional study was carried out on 110 pregnant women, selected using nonprobability sampling methods their age between 18-45year- old with no signs and symptoms of urinary tract infections (UTIs). Clean-catch Midstream urine specimen from each participant was collected and submitted for routine analysis by dipstick to detect leukocyte esterase, nitrite reductase tests and microscopic examination to detect pyuria (pus cells), as well as urine culture, and biochemical test. About 14.5% of pregnant women were positive for ASB, the most prevalent bacterium Escherichia *coli* (37.5%) followed by *Klebsiella pneumoniae* (18.7%). There was no significant association between age, parity, gestation, recurrent urinary tract infections, Diabetes mellitus, hypertension and ASB. For screening tests; the nitrite test was more specific (98.9%) than leukocyte esterase and pus cells, on the other hand, pus cell detection was more sensitive (81.2%) than the other tests for detection of ASB. The prevalence of ASB was relatively high, no one used routine screening tests revealed both high sensitivity and specificity. So, urine culture remains the gold standard method for detection of ASB in pregnant women.

Keywords: ASB, Screening Test, Sensitivity And Specificity, Pregnant Women

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Received On 19 April 2022

Revised On 14 June 2022

Accepted On 23 June 2022

Published On 01 July 2022

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Reem Anwar Abdelaal, Ghalia Mohammed Ibrahim, Ali Hashim Baraka, Islam Hamed Mohammed, Nuha Hassan Ali, Rayyan Yousif Abdelrahman, Hadia Babiker Abdelbasit, Sara Abdelghani, Lienda Bashier Eltayeb , Evaluation of Diagnostic Methods for Asymptomatic Bacteriuria (ASB) During Pregnancy.(2022).Int. J. Life Sci. Pharma Res.12(4), L123-128 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.4.L123-128

1. INTRODUCTION

Urinary Tract Infections (UTIs) in pregnancy including A Significant Bacteriuria (ASB) is associated with maternal morbidity and adverse pregnancy outcomes including; preterm birth, stillbirth, low birth weight and early onset neonatal sepsis, however in pregnancy the capacity for screening and treatment of UTIs as well as ABU is limited [1]. ASB during pregnancy is influenced by a range of factors including mechanical compression and changes in the immune and renal system [2], if untreated; can leads to cystitis and pyelonephritis, which result in serious complications such as acute respiratory distress, transient renal failure, septic shock during pregnancy, and more likely to intrauterine growth restriction, preeclampsia and premature deliveries low-birth weight infants [3, 4]. Physiological and hormonal disturbances during pregnancy can cause the susceptibility of UTIs, which if not detected and treated early during the pregnancy can lead to severe complications both for the mother and the fetus. Locally, the evaluations for ASB are limited, relying solely on commonly performed urine analysis, which in most cases does not indicate UTIs [5].Screening tests for ASB include macroscopic examination to detect the color of urine sample, the appearance, and the dipstick test to detect the nitrite reductase enzyme, produce by enteric Gram negative bacteria; which reduce the nitrate present in urine to nitrite, the leukocyte esterase enzyme, it is positive when bacterial infection is present, and the second screening method is microscopic examination to detect the present of pyuria (present of pus cell in urine), red blood cells in the urine, and use the gold standard urine culture, Gram stain and biochemical tests to identify the genus and species of bacteria if present [6-8]. The screening tests alone are insensitive for diagnosis of ASB during pregnancy [4], and must use culture methods also to diagnose ASB during pregnancy [9]. Thus, the current study was aimed to evaluate the sensitivity and specificity of the screening tests compared with the culture method for detection of ASB during pregnancy, and to find out the most etiologic agents causing ASB in pregnant women.

2. MATERIALS & METHODS

2.1 Design of Study

An analytical cross- sectional study was carried out at ANC in Police Hospital has frequency of the pregnant women in the range of 400-500 pregnant women/month. The second area was ANC Omdurman Military Hospital (0.5 Kilometer westward the White Nile bridge) the frequency of ANC is high; in the range of 600-800 pregnant women/month. The third area was Tal medical clinic located in Arcawiet area in Khartoum which has a frequency of 200-300 pregnant women/month. Any pregnant women between the age group from 18- to 45-year-old and who agreed to participate in this study were included. Any pregnant women who have had signs of UTIs or vaginal bleeding, all pregnant women Utilizing antibiotics less than two weeks' prior to the request to participate in this study, were excluded from the study. Ethical clearance was obtained from the Ethical Committee of Al-Far College of Science and Technology Program of Medical Laboratory Sciences ethical committee, NO MC NU: 153. verbal consent was obtained from participants after adequate provision of information regarding the study requirements.

2.2. Sample Size and Sample Technique

One hundred and ten pregnant women were enrolled in the study, sample size calculated using non probability sampling methods, a clean-catch midstream urine sample was collected from each participant, the selection of these participants was convenient because of the limited time to finish this study.

2.3 Laboratory Methods

2.3.1 Urine Culture

Under aseptic condition a sterile disposal standard calibrated delivering 0.001 ml was used to culture urine samples in CLED media by cross- streak method (Horizontal line and zigzag line) all the inculcate plates were incubated in the aerobic incubator at 37° overnight incubations (18-24 hours).

2.3.2 *Macroscopic and Microscopic Examination*

To perform a routine examination (screening) test, all the samples were examined first macroscopically by observing the color and pH using urine strip, and by urine dipstick strips bomber ten mainly use to detect leukocyte esterase enzyme and nitrate reductase, and the remains part of sample examined microscopically by take about 2 mL of urine sample and centrifuged at 1500 rpm for 5 minutes to detect pus cells (any pus cells >10 per high power field was significant for pyuria). A drop of sediment was placed on a glass slide and examined under a light microscope at high power 40× to detect pyuria (pus cells), all sample processed as soon as possible without delay.

2.3.3 Significant Growth Determination

All the plates were examined to determine for the significance growth for bacteriuria (growth of 100 or more colonies were considered significant (use reference. All plates were examined to determine if they are significant growth (105 CFU/ml) to notes colonial morphology in CLED and comment on the organism's fermentation of sugar (yellow colony) or non-ferment (green colony), colony size we found that large colony for Gram negative bacteria however small colony are Gram positive.

2.3.4 Gram Stain and Biochemical Test

Smears from the growth were prepared to be stained by Gram stain as follows: heat fixed smear was flooded by crystal violet stain for 1 mines, gentle washing of smear by distilled water, then add Logl's iodine for 30- 60 seconds, washed with cleaned water, covered with safranin stain for 2 minutes, then washed and let to air dry and examined microscopically using oil immersion lens (100X). The Gram's reaction, morphological appearance and arrangement of bacteria were reported with caution. Catalase, and coagulase tests used for differentiation and conformation of gram positive staphylococci from streptococci. Kligler Iron Agar (KIA), Urea hydrolysis test, Citrate utilization test, Indole production test were used for differentiation of gram negative bacteria [10].

2.4 Data Collection Tools

All the participants were informed and made aware about the procedure of the study and verbal consent was taken. demographic information and medical history was collected

through self-designed questionnaire. A- socio- demographic (age, educational level, economic status) medical information data (parity, gestation, age, history of UTI, D.M and hypertension) were collected through the written constructed questionnaire.

2.5 STATISTICAL ANALYSIS

The statistical analysis of the data was performed using the statistical analysis system (SPSS). Version 25 was used to detect specificity and sensitivity of Screening Tests compared with the culture method and to study the variable effect asymptomatic bacteriuria (educational level, age, number of previous pregnancies, past history of UTI and duration of pregnancy) and to found.

3. RESULTS

A total of 110 pregnant women were examined for ASB. Table (1) describes the demographic and clinical status of the participants and their urine culture results. Out of 110 pregnant women Sixteen were positive for ASB with a prevalence of (14.5%). ASB commonly found in age of (26-30) the number was 9 (53%) third trimester 10(70%), university education 10(57%) multigravida 11(75%) medium economic status 15 (93%) and recurrent UTIs 13(8%) we found no significant effect of D.M and hypertension on ASB. But statically we didn't find any significant association between age (P. value= 0.6) trimester (P. value 0.3) and multi-gravida (P. value 0.4 - OR =0.8%) previous UTI (P. value 0.9 – OR 0.9%). University education (P. value 0.7) and ASB in pregnant women. Screening tests dip- stick; leukocyte esterase test (LE), nitrate reductase test (NR) and microscopic pus cells were compared with positive urine culture (N = 16) (14.5%) for the pregnant women (table 2) it was found NR was positive (1/16) (6.25) and negative in 15(15/16) (93.7%) LE was positive in 7(7/16) (43.7%) and negative 9(9/16) (56.2%) and pus cell were positive in 13 (81.2%) and negative in 3 (2.7). The sensitivity, specificity and efficiency of NT, LE, and pus cells were calculated (table 3) was found NT more specific (98.9) than LE and Pus cells and Pus cells are more sensitive (81.2) than NT and leukocyte esterase test. And has high positive and negative predictive value compared with others Prevalence of ASB: Out of 110 participants; sixteen (14.5%) had asymptomatic bacteriuria, the most prevalent organism isolated was *E. coli* at 6(37.5%) followed by the *klebsiella pneumoniae* at 3 (18.7%) after then was come (mix growth *staphylococcus aureus* and *Enterococcus faecalis* at 2(12.5%) after then was come *staphylococcus saprophyticus* 1(6.25%). Figure 1 display frequency of isolated bacteria from positive urine culture

Table (1): Shows the demographic and clinical status of pregnant women compared with urine culture results:

Variables		Frequency	Percent %	Urine culture		P. value	Odds ratio
				Positive	negative		
Age	< 20	8	7.3	0	8	0.6	
	20-25	26	23	5	22		
	26-30	58	53	8	50		
	36-40	15	13.6	3	12		
	41-45	2	1.8	0	2		
Education	Not Literate	5	4.5	0	5	0.7	
	Primary	10	9	2	8		
	High	32	29	4	28		
	university	63	57	10	53		
Economic Status	Low	0	0	0	0	0.9	
	Medium	103	93	15	88		
	High	7	6.4	1	6		
Parity	Primer	27	24	5	22	0.4	1.3%
	Multi	83	75	11	72		0.8%
Gestation	First trimester	14	12.7	4	10	0.3	
	Second trimester	19	17	2	17		
	Third trimester	77	70	10	66		
Recurrent UTIs	No	89	81	13	77		0.9%
	Yes	21	19	3	18		1%
D.M	Yes	2	1.8	0	2	0.5	
	No	107	97	16	92		
Hypertension	Yes	3	2.7	1	2	0.3	3%

D.*M: Diabetes Mellitus, Chi-square was used to calculate p value, P. value less than 0.05 considered significant*

Table (2): Shows screening tests compared with urine culture

Screening tests		Frequency	Percent	Urine culture		P. value	Odds ratio
				Positive	Negative		
Nitrate test	Positive	1.8	2	1	1	0.1	6%
	Negative	98	108	15	94		0.9%
Leukocyte test	Positive	16	18	7	9	0.001	3.6%
	Negative	83	92	9	86		0.6%
Pus cell	Less than 10	93	103	13	91	0.02	0.8%
	More than 10	6.4	7	3	4		5%

Table (3): Sensitivity and specificity predictive value of screening test

Screening Tests	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Efficiency
Nitrate Test	6.25	98.9	50	68.1	85.4
Leukocyte Test	43.7	90.2	43.7	90.2	83.3
Pus Cells	81.2	65.7	76.4	96.2	93.6

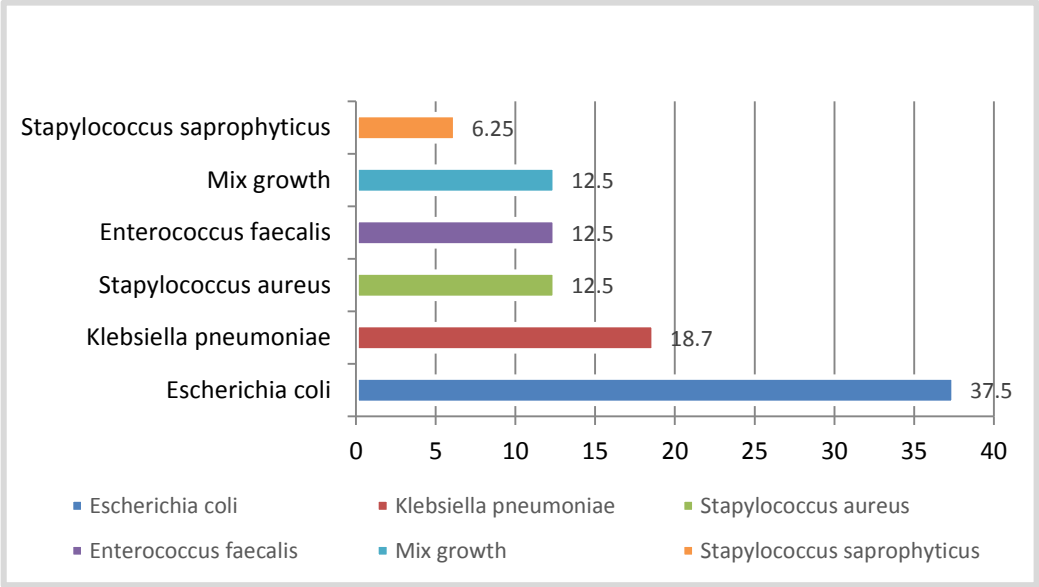

Fig (1): Frequency of isolated bacteria from positive urine culture

4. DISCUSSIONS

Pregnant women are more likely to have a urinary tract infection because of their functional, anatomical physical and hormonal alteration, moreover; urinary tract infection is usually a symptomatic [11]. As early intervention is critical for them, it is mandatory to assess and determine the most effective method for diagnosis of ABU. Thus current study was aimed to evaluate the sensitivity and specificity of the screening tests compared with the culture method for detection of ASB during pregnancy, and to find out the most etiologic agents causing ASB in pregnant women attending different antenatal units in Khartoum state Sudan Current study revealed that the prevalence of ASB was 14.5%, this finding is consistent with Hamdan, H. Z et al in Sudan 2011, who noted that ASB 14.6% [12]. Relatively our result is more than the result noted by Tugrul, S et al 2005 [13] in Turkey in which (8.1%) of pregnant women were diagnosed with ASB, and by Uncu *et al* (2002) [14] who concluded that pregnant women were evaluated and prevalence of ASB was 9.3%. In our study there was no association between age, parity, education, economic status, D.M, hypertension, previous UTIs, length of gestation, this a greed with studies that found the age, parity and gestational age were not significant influence on ASB [15, 16]. In recent study done by Desmond *et al* in Ghana (2018) [17] found the ASB was not associated with educational level, but this disagreed with another study done by Solomon *et al* [18] in Ethiopia (2018) found there was association with low educational status, may be due to our sample size was small we didn't find any association between variables and ASB and educational level.In our study, the most predominant isolated bacteria were *Escherichia coli* (37.5%) . These agreed with different studies that reported the prevalence was (42.4%) [12,] and 46.4% [19]. However, a much higher frequency, 60% was revealed in a study done by A Titoria *et al* in India (2014) [20], followed by *klebsiella* (18.7%), the same result found (22.5%). and in other study done by Khan *et al.* (2015) it was found, (17.03%) [21], followed by S *taphylococcus aureus and Enterococcus faecalis (12.5%),* in other studies found *Staphylococcus aureus* was the predominantly isolated Gram- positive pathogen from Ethiopia [22, 23], India [24], Sudan [12]. With regard the sensitivity and specificity used tests, present study noted that sensitivity and

specificity of nitrite test is 6.25% and 98.9% respectively, the same result documented by Tazebew *et al* in Ethiopia (2014) found the sensitivity and specificity of urinary for nitrite was 25% and 99.1% respectively [25], while the sensitivity and specificity of leukocyte esterase 43.7% and 90.2% respectively. Moreover, same result obtained in study done by Habiba *et al* in Abuja (2015) the sensitivity and specificity of leukocyte esterase was 50% and 89.1% respectively [26], and the sensitivity and specificity of pus cells 81.2% and 65.7% respectively, a conflict was revealed with other study who noted that the microscopy method gave the sensitivity 81.3% and specificity 94.5% [25],the nitrite and leukocyte esterase test showed high specificity and low sensitivity, but pus cells showed high sensitivity and low specificity. There is no association between routine screening tests with ASB. Another recent study recommended that urine culture should be performed at least during pregnancy to detect ASB [27]. Screening for ASB must become an essential part of antenatal care, so urine culture should be conducted as a part of routine tests for pregnant women.

5. LIMITATIONS OF THE STUDY

In our study we were supposed to increase the sample size to be more than 110 but because the period we worked in was the period of spread COVID 19. Direct Gram stain was supposed to work but studies have proven that it does not give a reliable result. The susceptibility test was not performed for the limited funds for this study.

6. CONCLUSIONS

Thus from our study , the prevalence of ASB was 14.5% the most common pathogen is *E.coli* (37.5%) other organism *klebsiella* 3(18.7%) *Staphylococcus aureus* 2 (12.5%) *Enterococcus faecalis* 2(12.5%) mix growth 2(12.5%) and *Staphylococcus saprophyticus* 1(6.25%), there is no significant association statistically between age trimester gravid previous UTI, education and ASB in pregnant women "No of screening tests NR LE and pus cells used in this study showed both high sensitivity and specificity, depended only on urine culture to detect ASB in pregnant women". Thus the urine culture remains the gold standard method for detection of ASB in pregnant women.

7. ACKNOWLEDGEMENT

This publication was supported by the Deanship of scientific research at Prince Sattam Bin Abdul Aziz University. Authors appreciated the Faculty of Medical Laboratory Sciences, Faculty of Medical Laboratory Science. Department of medical microbiology, Alfajr College for Sciences &Technology. Khartoum, Sudan

8. AUTHOR CONTRIBUTION STATEMENT

Reem Anwar Abdelaal, Ghalia Mohammed Ibrahim. Ali Hashim Baraka (Construction of questionnaire, and Scientific writing of manuscript), Islam Hamed Mohammed, Nuha Hassan Ali, Rayyan Yousif Abdelrahman (Laboratory works).Sara Abdelghani (Statistical analysis of data).Hadia Babiker Abdelbasit proofreading and review manuscript. Lienda Bashier Eltayeb (proofreading and scientific writing)

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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