Diagnostic Performance of Reagent Strip Testing for Antenatal Screening of Asymptomatic Bacteriuria Using Single- and Double-Urine Culture Criteria

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Objective: *To determine the prevalence of asymptomatic bacteriuria (ABU) in pregnant women and diagnostic performance of reagent strip test for screening of ABU using single and double urine culture criteria.*

Material and Method: *Pregnant women attending their first antenatal care at Rajavithi Hospital Bangkok, between August 22 and November 4, 2011 were enrolled to collect clean-catch midstream urine for reagent strip test and culture on blood and MacConkey agar plates. The second urine culture was collected only from participants who had the first positive urine culture.*

Results: *Sixty-one of the 754 cases (8.1%) got the positive first urine culture. Twenty of the sixty-one cases were still positive urine culture in the second culture. Therefore, prevalence of ABU was 8.1% and 2.7% using single and double urine culture as gold standard. Escherichia was the most common pathogen in both single and double urine culture (27.9% and 40%, respectively). The urine dipstick nitrite, leukocyte, and both test by using double urine culture had a sensitivity of 35.0%, 50.0%, and 20.0%, specificity of 86.5%, 52.2%, and 93.2%, positive predictive value (PPV) of 6.6%, 2.8%, and 7.4%, and negative predictive value of 98.0%, 97.5%, and 97.7%, respectively. All diagnostic performances of double-urine culture were comparable with single-urine culture except marked worse PPV.*

Conclusion: *Prevalence of ABU in pregnant women was decreased from 8.1 to 2.7% using single and double culture as gold standard. Reagent strip testing is not sensitive for screening of ABU; either single or double urine culture were used as gold standard.*

Keywords: *Asymptomatic bacteriuria, Pregnancy, Reagent strip, Single urine culture, Double urine culture*

Asymptomatic bacteriuria (ABU) is one of the most important problem during pregnancy because it can lead to acute pyelonephritis in 25% of untreated cases[1]. Reagent strip testing has been reported as a quick, simple, inexpensive method for routine screening of ABU[2,3] in the western population. However, many Thai studies reported opposite results especially sensitivity of the test[4-6].

All previous mentioned studies used only single urine culture and defined ABU when 10^5 colony-forming units (CFU) or more per ml were found. Usually two consecutive mid-stream, clean catch urine specimens or one catheterized specimen urine culture of the same bacteria 10^5 CFU or more per ml without symptoms of urinary tract infection is traditionally used as the gold standard for diagnosis of ABU[7,8].

Prevalence of ABU in pregnancy using single urine culture varied from 5.4 to 21.1%[2,4-6,9,10]. Prevalence of ABU in two studies using double urine culture varied from 1.9 to 4.7%[7,11]. The present study was conducted to determine the prevalence of ABU in pregnant women and diagnostic performance of reagent strip test for screening of ABU in pregnant women using single and double urine culture criteria.

Material and Method

Seven hundred fifty four pregnant women who had their first antenatal visit in the antenatal care clinic (ANC) at Rajavithi Hospital, Bangkok between August 22 and November 4, 2011 were enrolled. Exclusion criteria were received antibiotics during the past seven days, medical or obstetric complication, symptomatic urinary infection (UTI) or history of UTI, and bleeding per vagina.

After approval from the Hospital's Ethics Committee, eligible cases were asked to join the present

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study after counseling and signed the informed consent. They were explained about the proper technique to collect clean midstream urine for culture into the sterile containers. Their perineums were cleaned with soap, rinsed out with clean tap water, and dried with clean paper after their hand washing.

Then clean-catch midstream urine was collected into two sterile containers and sent to laboratory for urine culture within one hour after voiding. Blood and MacConkey agar was used as media for culture and incubated at 35 to 37°C for 24 to 48 hours and read at 12, 24, and 72 hours. The participants were appointed to be informed the results one week after collection. Those with presence of 10^5 CFU or more of a single type of bacteria per milliliter of urine, were asked to collect a second urine culture.

Contamination was interpreted when the urine culture revealed more than one type of organism. Second urine culture was not performed in those who had contamination. Presence of 10^5 CFU or more per ml of the same type of bacteria (compared with the first urine culture) indicated bacteriuria[7,8]. ABU was diagnosed when there were 10^5 CFU or more per ml of urine of the same single pathogen in two consecutive mid-stream from a pregnant woman without symptoms of urinary tract infection[7]. Antibiotic sensitivities were determined by the tube dilution method. Those who were diagnosed as bacteriuria, were treated with a single course of appropriate antibiotics, according to susceptibility tests.

Urine in the other container was tested for nitrite, leukocyte esterase, sugar, and protein by reagent strip in dipstick (Multistick® 10 SG, Bayer Bangkok Ltd., Thailand). Results were interpreted after one minute. The nitrite and leukocyte esterase portion of the test were interpreted as positive if the color on the reagent areas were positive for each portion. Tests that showed zero or traces were considered as negative. Reagent strip testing was performed with the first urine culture only. The result of nitrite and leukocyte esterase was analyzed when one of the individual test was positive, or both tests were positive.

Data were collected and statistically evaluated using SPSS version 17.0 software program. Diagnostic test of urine dipstick was performed using single and double positive urine culture as a gold standard.

Definition

1. Single urine culture defined as diagnosis of ABU[7] after only one urine culture.

2. Double urine culture defined as diagnosis of ABU[7] after two urine cultures.

Results

Seven hundred fifty four pregnant women enrolled in the present study, 61 cases (8.1%) were positive on the first urine culture, and 30 (4%) were contaminated. Twenty cases (2.7%) were still positive on the second urine culture.

Table 1 shows the uropathogens responsible for ABU in these women. *Escherichia coli* was the most common uropathogen in single and double urine culture (27.9% and 40.0%, respectively).

Diagnostic performance of urine dipstick using single and double urine culture as gold standard for diagnosis of ABU is shown in Table 2. All diagnostic performances of double-urine culture were similar except slightly better negative predictive value (NPV) and worse positive predictive value (PPV) compared with single-urine culture. In term of sensitivity, either test positive was better than nitrite, leukocyte esterase, or both test positive in both single and double urine culture. However, in term of specificity, both test positive was better than the others. NPV was the best diagnostic performance in all tests while the PPV was the worst diagnostic performance in all tests.

Discussion

Prevalence of ABU in pregnant women in Rajavithi Hospital was markedly decreased about 66.7% from 8.1 to 2.7% (present study) when single and double urine culture were used as gold standard, respectively. In Spain, Gratacós et al[11] reported similar results of decreased prevalence of ABU (57%) using

Table 1. Microorganisms of asymptomatic bacteriuria in single urine culture and double urine culture

Organism	Single urine culture		Double urine culture	
	Number	Percent	Number	Percent
Escherichia coli	17	27.9	8	40.0
Viridans group streptococci	15	24.6	7	35.0
Gardnerella vaginalis	9	14.8	3	15.0
Staphylococcus aureus	8	13.1	2	10.0
Corynebacterium spp.	4	6.6	-	-
Group B streptococci	3	4.9	-	-
Coagulase negative staphylococci	2	3.3	-	-
Yeast	1	1.6	-	-
Enterococcus faecalis	1	1.6	-	-
Candida albicans	1	1.6	-	-
Total	61	100.0	20	100.0

Table 2. Summarizes the test statistics on the two dipstick tests compared with single and double urine culture

Test	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)		Accuracy (%)		LR positive		LR negative	
	Single	Double	Single	Double	Single	Double	Single	Double	Single	Double	Single	Double	Single	Double
Positive nitrite	29.5	35.0	87.3	86.5	17.0	6.6	93.4	98.0	82.6	85.2	2.3	2.6	0.8	0.8
Positive leukocyte esterase	50.8	50.0	52.4	52.2	8.6	2.8	92.4	97.5	52.3	52.1	1.1	1.0	0.9	1.0
Either test positive	62.3	65.0	45.9	45.5	9.2	3.1	93.3	97.9	47.2	46.0	1.2	1.2	0.8	0.8
Both test positive	18.0	20.0	93.8	93.2	20.4	7.4	92.9	97.7	87.7	91.3	2.9	2.9	0.9	0.9

PPV = positive predictive value; NPV = negative predictive value; LR = likelihood ratio

single and double culture from 8.7 to 4.7%, respectively. Even though they lost 6.9% of the positive first urine culture to repeat the second urine culture, the second urine culture were collected in every case of positive first culture in the present study. In a Filipino study[7], the prevalence of ABU decreased from 5.4 to 1.9% using single and double urine culture, respectively. However, only 54% of the positive first culture was followed-up for second culture[7]. This event will decrease the reliability of the study. When single urine culture was used as gold standard for diagnosis ABU, prevalence in the present study (8.1%) was similar to previous studies using the single culture such as Kovarisarach et al[6] 2005, Thailand (10%), Suntharasaj et al[4] 1993, Thailand (8.1%), Robertson and Duff[2] 1988, USA (8.3%), Kutlay et al[10] 2003, Turkey (10.6%), and Chongsomchai et al[9] 1994 to 1995, Thailand (11.2%). Lumbiganon et al[5] 1999, reported a quite higher prevalence of ABU in pregnant women (21.1%) in the same hospital as Chongsomchai et al[9]. *E. coli* was the most common uropathogen in the present study in both single and double urine culture (27.9% and 40%, respectively) similar to many previous studies varied from 69.4 to 94.8%[2,10,12] and 63.0 to 66.2%[7,11], in single and double urine culture, respectively.

Prevalence of *E. coli* in the present study using single urine culture (27.9%) was quite similar to 22.2%[6] in previous Rajavith Hospital's study using single culture. However, prevalence of *E. coli* was increased to 40% after double culture technique was used due to clearing of the contaminated pathogen.

The contaminated cases were not asked to collect the repeated urine culture because in previous study[6] 13.3% of all cases were contaminated and the repeated culture of all contaminated cases were negative culture in 68.75% and still contaminated in 31.25%[6]. In addition, the second culture was not collected in the first negative culture because the definition of ABU in the present study was present of the single and same pathogen in two consecutive mid-stream urine culture.

Previous studies[7,11] using double urine culture revisited for second culture if the first culture was positive, similar to the present study.

Staphylococcus aureus was not considered a pathogen by Bachman et al[13]. However, it could be identified in both double culture in Sescon et al's[7] and in the present study. Therefore, the authors believed that this organism should not be considered as contaminated in the present study.

Most diagnostic indicators of reagent strip testing in the present study were still not so good when both single-and double-urine culture were used as gold standard for diagnosis of ABU. However, they were slightly better in NPV and worse in PPV in every test.

Based on our knowledge, the present study is the only study that compared the diagnostic performance of reagent strip test for screening of ABU in pregnant women using double urine culture criteria. Therefore, the diagnostic performance of reagent strip test in the previous studies were considered under single urine culture criteria. All previous studies reported varied sensitivity of the both test positive by using single urine culture criteria from 38.7[10] to 50%[13], while in the present study, it was 18.0% and 20.0% when single and double urine culture criteria were used respectively.

In the present study, reagent strip testing was not a good screening tool for ABU in pregnant women because of poor sensitivity (20 to 50%) in every test even though it had a fair sensitivity in either test positive (65%) when double urine culture technique was performed.

It means that after exclusion of the contaminated organism by second urine culture, the diagnostic performance was still unjustified to screen for ABU in pregnant women.

The excellent NPV in both single and double urine culture of all test of the present study (92.4 to 93.4%, and 97.5 to 98%, respectively) were similar from 91.5 to 96.1% in single urine culture technique in previous Rajavithi Hospital's study[6]. These results

may help the physicians to rule out ABU when all tests were negative.

Conclusion

Prevalence of ABU was decreased from 8.1 to 2.7% using single and double urine culture as gold standard. Reagent strip testing is not sensitive for screening of ABU in pregnant women.

What is already known on this topic?

Prevalence of ABU in pregnancy using single urine culture varied from 5.4 to 21.1%. Many Thai studies reported that using single or double culture as gold standard was not accurate for screening of ABU while many western studies had opposite results.

What this study adds?

Prevalence of ABU in pregnant women was decreased 66.7% from 8.1 to 2.7% using single and double culture as gold standard. Reagent strip testing is still not sensitive for screening of ABU. Either single or double urine culture were used as gold standard.

Potential conflicts of interest

None.

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ความสามารถของแถบทดสอบตรวจปัสสาวะในการคัดกรองภาวะติดเชื้อแบคทีเรียในปัสสาวะขณะตั้งครรภ์แบบไม่มีอาการ เปรียบเทียบระหว่างการเพาะเชื้อในปัสสาวะหนึ่งและสองครั้ง

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วัตถุประสงค์: *เพื่อหาความชุกของภาวะ ABU ในหญิงตั้งครรภ์และความสามารถของการใช้แถบทดสอบตรวจปัสสาวะในการคัดกรอง ภาวะ ABU โดยการใช้เกณฑ์เพาะเชื้อจากการตรวจปัสสาวะเองและเก็บส่วนกลางอย่างสะอาดหนึ่งและสองครั้ง*

วัสดุและวิธีการ: *หญิงตั้งครรภ์ที่มาฝากครรภ์ครั้งแรกที่ห้องฝากครรภ์ โรงพยาบาลราชวิถี ตั้งแต่วันที่ 22 สิงหาคม พ.ศ. 2554 ถึง วันที่ 4 พฤศจิกายน พ.ศ. 2554 ที่ได้รับการคัดเลือกเข้าศึกษา ได้เก็บปัสสาวะที่ถ่ายเองและเก็บส่วนกลางอย่างสะอาด เพื่อตรวจโดย แถบทดสอบจุ่มปัสสาวะ และเพาะเชื้อโดยใช้อาหารเลี้ยงเชื้อที่เป็นเลือดและแม็คคองกี้ ให้เก็บปัสสาวะอีกครั้งเฉพาะในหญิงตั้งครรภ์ ที่ผลตรวจเป็นบวกจากการตรวจปัสสาวะครั้งแรกเท่านั้น*

ผลการศึกษา: *หญิงตั้งครรภ์ 61 ราย ในทั้งหมด 754 ราย (ร้อยละ 8.1) ให้ผลเพาะเชื้อในปัสสาวะเป็นบวก มีเพียง 20 ราย ใน 61 ราย ที่ยังให้ผลเพาะเชื้อในปัสสาวะเป็นบวกในการเพาะเชื้อครั้งที่สอง ดังนั้นความชุกของ ABU โดยการใช้ผลการเพาะเชื้อ หนึ่งและสองครั้งเป็นเกณฑ์ เท่ากับร้อยละ 8.1 และ 2.7 ตามลำดับ เอสเชอริเชียร์ โคไล เป็นเชื้อก่อโรคที่พบได้บ่อยที่สุดในการ เพาะเชื้อทั้งครั้งที่ 1 และ 2 (27.9% และ 40%) การตรวจแถบทดสอบสำหรับไนไตร์ท ลูโคไซท์เอสเตอเรส และทั้งสองวิธีได้ผลบวก ร่วมกัน มีความไวเท่ากับร้อยละ 35.0, 50.0 และ 20.0 ความจำเพาะเท่ากับร้อยละ 86.5, 52.2 และ 93.2 ค่าทำนายผลบวกเท่ากับ ร้อยละ 6.6, 2.8 และ 7.4 ค่าทำนายผลลบเท่ากับ 98.0, 97.5 และ 97.7 ตามลำดับ ความสามารถของการใช้แถบทดสอบในการ คัดกรองภาวะ ABU โดยการใช้ผลการเพาะเชื้อสองครั้งในเกณฑ์ได้ผลพอ ๆ กับการใช้ผลเพาะเชื้อหนึ่งครั้ง ยกเว้นค่าทำนายผลบวก ที่ต่ำกว่า*

สรุป: *ความชุกของภาวะ ABU ในหญิงตั้งครรภ์ลดลงจากร้อยละ 8.1 เป็น 2.7 เมื่อใช้เกณฑ์เพาะเชื้อจากการตรวจปัสสาวะหนึ่ง และสองครั้ง การใช้แถบทดสอบตรวจปัสสาวะไม่ไวพอสำหรับการคัดกรองภาวะ ABU ไม่ว่าจะใช้เกณฑ์การเพาะเชื้อจากการตรวจ ปัสสาวะหนึ่งและสองครั้ง*