



Original Research Article

Evaluating the predictive value of screening and confirmatory tests for asymptomatic bacteriuria in pregnant women attending a tertiary care hospital in Mehsana, North Gujarat

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Abstract

Purpose: Asymptomatic bacteriuria (ASB) is a significant health concern among pregnant women, necessitating regular screening during all three trimesters of pregnancy. Diagnosis of ASB can be made through microscopy, urine culture, and rapid biochemical screening tests.

Methodology: In this cross-sectional study, midstream urine samples were collected in OPD from all pregnant women (20-40 years) attending the antenatal clinic for analysis. The samples were tested for biochemical markers, including leukocytes, nitrites, and other relevant parameters. Microscopic examination and urine culture were performed using standard methods. The results of all three diagnostic methods were compared, and the positive predictive value was assessed.

Results: ASB among study population was 8.33%. Positive predictive value (PPV) for microscopy, detection of leukocytes and nitrite was 54.29%, 21.05% and 69.23%. In this study, no significant difference (P value >0.05) was observed in development of ASB in different associated factors related categories.

Conclusion: In conclusion, prevalence rate of ASB among pregnant women was on average with different studies across the globe. It was observed that microscopy and nitrite detection is sensitive enough to screen primary cases of ASB up to some extent but detection of leukocyte is not having good correlation with true positive isolates.

Keywords: Asymptomatic bacteriuria, Biochemical analysis, Microscopy, Pregnant women, Urine culture.

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1. Introduction

ASB can be defined as presence of bacteria in urine; in which, bacteriological culture of urine shows a significant growth of organism which is greater than 10^5 bacteria/ml, but patient is not having any symptoms of urinary tract infection (UTI).¹ Pregnant women are at a higher risk of development of asymptomatic bacteriuria because of many factors e.g. increased in levels of progesterone, pressure by gravid uterus on bladder, changes in urine pH and osmolality, immunocompromised state, etc.^{2,3} ASB can progress to symptomatic stage and is responsible for various complications such as pyelonephritis, low birthweight and

preterm birth.⁴ Prevalence rate of asymptomatic bacteriuria among pregnant is generally lower in developed countries and same can be high in developing countries.⁵ So American College of Obstetricians and Gynaecologists, strongly recommends screening for ASB in all pregnant women.⁶ Many studies are being conducted on ASB worldwide but very few studies citing the importance of introduction of rapid test for identification of ASB. We focused on utilisation of rapid test and their evaluation in this study. In developing countries like India, especially in peripheral areas where diagnostic services are not readily available, rapid tests can provide primary screening for antenatal patients if deemed suitable in comparative evaluations. However, these tests

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have certain limitations, such as being unable to measure the extent of infection or provide guidance on antimicrobial therapy.

2. Aims and Objectives

This study was aimed at comparison of test result of available diagnostic methods for detection of ASB among pregnant women of 20-40 years age group. Primary objective of this study to know prevalence of ASB among pregnant female and to compare results of rapid urine tests, microscopy and culture. Secondary objective of this study was to analyse associated factors e.g. age, socioeconomic status, occupation, gestation age, hypertension, diabetes, parity and antimicrobial susceptibility patterns of isolated pathogens in the geographic location of north Gujarat region.

3. Materials and Methods

Study design and study period: An institutional based cross sectional study was conducted among pregnant women attending antenatal clinic in tertiary care centre in Mehsana from July 2022 to August 2022.

Sample size calculation and sampling technique: Sample size was calculated using a statistical formula taking a prevalence of ASB (18.6%) from latest study conducted by Harman M in 2021⁵ with degree of precision set at 0.05 and at 95% confidence interval & final sample size was 233. So in reference to this, total of 252 participants were enrolled in this study to enhance study's statistical power and robustness. Study participants were selected by a convenience sampling technique in which samples were selected based on ease and availability.

3.1. Inclusion criteria

Pregnant women without symptoms of urinary tract infection (UTI), and willing to provide urine samples.

3.2. Exclusion criteria

Pregnant women with symptoms of UTI, any history of antibiotics usage in the previous 2 weeks and not willing to provide consent.

3.3. Data collection

Data was collected using case record form after taking reference from different studies. The form contained two parts: clinical and laboratory part. Clinical part contained sociodemographic characteristics, clinical history and pregnancy related factors. Laboratory part contained results of rapid screening test, microscopic examination and culture and sensitivity results of collected samples.

3.4. Sample collection procedure

All pregnant women attending antenatal clinic in gynecology OPD were screened for signs and symptoms of UTI. After taking informed consent, clinical and other required

information were collected and incorporated in case record form by nurse. The study participants were instructed to collect 10–15mL of midstream urine by “clean catch” method.

3.5. Laboratory investigations

Collected samples were sent to microbiology laboratory immediately after collection. In laboratory, urine samples were being separated into three portions. From 1st portion, detection of leukocyte and nitrite was performed by “Reckon U stik” reagent strips. 2nd portion was used for microscopic examination; in which focus was given to detect presence of bacteria, motility and pus cells. From 3rd portion, aerobic culture and antimicrobial susceptibility was performed on Cystine Lactose Electrolyte Deficient (CLED) agar, blood agar and MacConkey agar by semiquantitative streaking method using a calibrated loop wire with capacity of containing 0.001 ml of urine.⁷ Sample was labelled as positive if significant growth of organism $> 10^5$ bacteria/ml is detected after 24hr aerobic incubation at 37°C and results were compared with microscopy and rapid test. Antimicrobial susceptibility test was performed on Muller Hinton agar using lawn culture technique with recommended antibiotic disc panel as per guidelines of clinical and laboratory standard institute (CLSI).⁸

3.6 Data analysis

Results of all three methods were compared and analysed. Qualitative or categorical variables were expressed in terms of frequency and percentage. PPV was measured on the basis of test results. Data of associated factors were entered in Microsoft excel 2016 and analysis was done by MedCalc v 12.5.0 for descriptive statistics, comparison and means and diagnostic test evaluation e.g. PPV. Chi-square test was used for statistical analysis. $p < 0.05$ was considered as statistically significant value at 95% confidence interval.

4. Results

Total 252 samples were collected from Gynaecology OPD. Out of which 21 samples showed microbiological growth. So total prevalence rate of ASB was 8.33% in this study. Details are summarised in **Table 1**.

Table 1: Prevalence rate of asymptomatic bacteriuria (ASB) among pregnant women

Parameters	Results
Total samples	252
Culture Positive	21
Prevalence rate	8.33%

As a part of primary objective, comparative analysis was done between available laboratory methods for detection of ASB e.g. detection of leukocyte, nitrite by rapid method; presence of pus cells by microscopy and standard culture method. Total 57 out of 252 samples, detected leukocyte by

rapid method, amongst 12 shows actual growth in culture. This shows PPV of this test was 21.05%. Out of 252 samples tested, total 13 samples detected nitrite by rapid method, amongst 9 shows growth by culture method. This shows PPV of this test was 69.23%. Out of 252 samples tested, total 35 samples showed presence of pus cells by microscopy method. Amongst these 19 shows culture growth. This shows PPV of this test was 54.29%. **Table 2** shows results of comparison between different methods.

Table 2: Comparison of the results of rapid urine tests, microscopy and culture

Laboratory indicators	Results	value
Rapid leukocyte	True Positive	12
	Total No. of Positive (True + False)	57
	PPV	21.05%
Rapid Nitrite	True Positive	9
	Total No. of Positive (True + False)	13
	PPV	69.23%
Microscopy (Presence of Pus cells)	True Positive	19
	Total No. of Positive (True + False)	35
	PPV	54.29%

As a part of secondary objective of this study, information regarding different associated factors was collected and summarized in **Table 3**. Categorisation of patients was done into different groups in relation to the available information such as age, occupation, education status, gestational age, parity, h/o previous UTI and catheterisation, Haemoglobin (Hb) level and hypertension.

Table 3: Analysis of associated factors

Associated factors	Category	Total	Positive	P value
Age group	<25	126	11	0.8424
	25-30	98	8	
	30-35	20	2	
	>35	8	0	
Occupation	Housewife	164	13	0.8947
	Farming	70	6	
	Working	18	2	
Education level	Uneducated	30	4	0.5498
	Partially educated	176	13	
	Educated	46	4	
Gestational age	1st trimester	30	2	0.3015
	2nd trimester	104	12	
	3rd trimester	118	7	
Parity	Primigravida	94	8	0.8752
	Multigravida	158	13	

Most common age group of pregnant women was < 25 years of age followed by age group of 25-30 years. As this is peripheral area, most of the women were house wife and some were doing farming also. Information regarding education status was also collected for analysis. Women completing 10th standard were considered educated, 5th to 10th were considered as partially educated and rest were considered uneducated. Most of the women were partially educated in this study. Majority (158 of 252) women in this study were multigravida and rest were primigravida. 10 of the participants were having history of previous UTI and none of the participants had history of catheterisation during pregnancy. 74 of the 252 participants were having low Hb level while 14 of 252 participants having hypertension. 6 of the women in study were having diabetes while 246 were having normal sugar level. Also in this study no any significant difference (P value >0.05) was observed in development of ASB in different associated factors related categories.

Table 4 describes frequency of organisms isolated from pregnant women responsible for asymptomatic bacteriuria. Highest numbers of organisms isolated were *E. coli* followed by *Klebsiella* spp., *Candida* spp., *Proteus* spp. and *Staphylococcus aureus*.

All isolated organisms in this study were showing good susceptibility to available antibiotics. Urine panel of antibiogram was used for testing of isolated pathogens by culture. As this was OPD based study, all isolated pathogens were considered community acquired pathogens. Among all gram negative bacteria, *Enterobacteriaceae* (*E. coli*, *Klebsiella* and *Proteus* spp.) showed very good sensitivity to Ampicillin/sulbactam, meropenem followed by quinolones. *Staphylococcus aureus* as single pathogen among gram positive bacteria, showed good sensitivity to Linezolid, Co-trimoxazole and quinolones.

Table 3 Continued...				
H/o UTI	Yes	10	2	0.4364
	No	242	19	
H/O of Catheterization	Yes	0	0	--
	No	252	21	
Hb Level	>10	178	13	0.5046
	<10	74	8	
Hypertension	Yes	14	2	0.7401
	No	238	19	
Diabetes	Yes	6	1	1.0
	No	246	20	

Table 4: Distribution of organisms causing asymptomatic bacteriuria

Organism isolated	Total N=21
<i>E. coli</i>	9
<i>Klebsiella spp.</i>	5
<i>Candida spp.</i>	4
<i>Proteus species</i>	2
<i>Staphylococcus aureus</i>	1

Table 5: Antimicrobial susceptibility patterns of isolated pathogens

Antibiotic name	Code	Strength (mcg)	<i>E-coli</i> (n=9)	<i>Klebsiella</i> spp. (n=5)	<i>Proteus</i> spp. (n=2)	<i>S. aureus</i> (n=1)
Ampicillin/sulbactam	AS	20	9 (100)	4 (80)	2(100)	NT
Co-trimoxazole	BA	25	7 (77.77)	4 (80)	2(100)	1 (100)
Ceftizoxime	CI	30	6 (66.66)	3 (60)	1 (50)	0(0)
Chloramphenicol	CH	30	8 (88.88)	3 (60)	1 (50)	0(0)
Cephalexin	PR	30	3 (33.33)	2 (40)	1 (50)	0(0)
Tetracycline	TE	30	5 (55.55)	4 (80)	NT	1 (100)
Meropenem	MP	5	9 (100)	5 (100)	2(100)	NT
Nitrofurantoin	FD	300	9 (100)	4 (80)	1 (50)	1 (100)
Levofloxacin	GF	5	8 (88.88)	3 (60)	2(100)	1 (100)
Norfloxacin	NX	10	8 (88.88)	3 (60)	2(100)	1 (100)
Amikacin	AK	30	7 (77.77)	4 (80)	NT	1 (100)
Linezolid	LZ	30	NT	NT	NT	1 (100)

*NT: Not tested

5. Discussion

In this study, prevalence rate of ASB among pregnant women was 8.33%. Total 21 organisms isolated during two months of period from urine samples of pregnant women attending antenatal clinic in tertiary care centre. Most common organism isolated was *Escherichia coli* followed by *Klebsiella* spp., *Candida albicans*, *Proteus* spp. and *Staphylococcus aureus*. Prevalence rate in this study was consistent with previous study of Sujatha R (prevalence rate of 7.3%) at Kanpur,⁹ Trupthi G (prevalence rate of 10.8%) at Bangalore¹⁰ and Prabhavathi V (prevalence rate of 11.33%) at Andhrapradesh.⁵ *Escherichia coli* was the most common organisms responsible for ASB in previous studies of Paul

E,¹ Mekuria E,⁶ Sujatha R,⁹ Trupthi G,¹⁰ Harman M¹¹ and Mangalgi S.¹² *Escherichia coli* and other *Enterobacteriaceae* are the normal flora of gastrointestinal tract (GIT) and due to close proximity to the urogenital tract in women there are increased chances of contamination of urinary tract with these normal flora. This may be the reason why *Escherichia coli* and other *Enterobacteriaceae* were the most common isolated pathogens responsible for ASB in different studies all over the globe.¹³

Prevalence rate among different groups related to various associated factors were analysed and no any significant differences were observed in prevalence rate related to occupation, education level, parity, UTI or catheterization, Hb level, Blood pressure and diabetes. In the

previous study of Mekuria E and Harman M, incidence of ASB was higher in women having past history of catheterisation and UTI.^{6,9,11,14} But in this study none of participant was having past history of catheterization and only 10 women were having past history of UTI, so there might be large scale studies are required to detect proper significance with these associated factors.

Comparisons for results of different available methods were done and predictive value was finalized against gold standard culture test in **Table 2**. PPV of the detection of leukocyte and nitrite in urine sample by rapid test was 21.05% and 69.23% respectively. Detection of leukocyte by rapid test has very poor correlation with positive culture test hence found not effective in implementing as satisfactory screening test. Detection of nitrite by rapid has more predictive value as compared to detection of leukocyte but out of total 21 isolates, only 9 showed positive reactions in strip. So this data suggest lack of reliability of nitrite test as most of isolates failed to detect by nitrite test in rapid strip method. Presence of pus cells by light microscopy has a PPV of 54.29% when we compared with positive culture results. Positive point we noted in this method that out of 21 isolates, 19 showed presence of pus cells in the urine which can be detected easily by using very common consumables e.g. slides, cover slips and microscope. Detection of pus cells in urine showed very good sensitivity but due to lack of specificity PPV was less (54.29%). Still separate study with large dataset is required to validate the sensitivity if these rapid tests in identifying ASB. Antibigram of isolated organism shows good sensitivity to available antibiotics. So treatment might not be issue in pregnant women if ASB is detected early¹⁵ and also early detection can reducing maternal and obstetric complications associated with pregnancy.^{16,17} But using any king of drug including antimicrobials is matter of concern due to any possible unwanted side effects on foetus.

6. Conclusion

In conclusion, prevalence rate of ASB among pregnant women was average (8.33%) as compared to different studies across the globe. In this study it was observed that microscopy is sensitive enough to screen primary cases of ASB but detection of leukocyte and nitrite was not having good correlation with true positive isolates. So in the in the diagnostic centres, rapid screening by strip methods cannot be used as screening tools for detection of ASB among pregnant women. Because, it is possible that many positive cases can be missed out if we relied on this rapid method alone for detection of asymptomatic bacteriuria. There is a need of encouragement to the researchers for development of rapid screening tool for screening of ASB among pregnant women. Also present study showed that various associated factors analysed here, did not have any significant impact in prevalence rate of ASB during the pregnancy.

7. Source of Funding

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8. Conflict of Interest

We authors declare no conflicts of interest.

9. Compliance with Ethics Guidelines

Ethical Permission was obtained from institutional ethics committee (IEC/NMCRC/Approval/49/2022) before initiation of the study.

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