Original Article

Prevalence, Risk Factors and Antimicrobial Resistance of Asymptomatic Bacteriuria Among Antenatal Women

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ABSTRACT

Background: Asymptomatic bacteriuria (ABU) in antenatal women is microbiological diagnosis and if untreated have 20-30 fold increased risk of developing pyelonephritis during pregnancy. **Aim:** The prospective study was conducted to determine the prevalence, risk factors and antibiotic resistance related to ABU in antenatal women. **Subjects and Methods:** A total of 287 asymptomatic pregnant women who attended the antenatal clinic at a tertiary care hospital, Odisha, India from July 2012 to December 2012 were enrolled. Two consecutively voided urine specimens were collected by clean-catch midstream urine technique for culture. The urine samples were processed and microbial isolates were identified by conventional methods. Antimicrobial susceptibility testing was performed on all bacterial isolates by Kirby Bauer's disc diffusion method. Data were analyzed using GraphPad Quick Calcs Statistical Software Inc., USA. Inferential statistics was done by Chi-square (χ^2) test and a $P < 0.05$ was considered significant. **Results:** The prevalence of ABU in antenatal women was 11.5% (33/287). Lower socio-economic status and low level of education were significant risk factors related to ABU (P=0.02). Parity, maternal and gestational age was not significantly associated with ABU. *Escherichia coli* (54.5%, 18/33) were the most prevalent isolate followed by *Enterococcus faecalis* (15.2%, 5/33). Nitrofurantoin was the most effective antibiotic, showed resistance rate of 3% (1/33) for both Gram-negative and Gram-positive bacteria. **Conclusion:** Routine screening using urine culture method should be performed for ABU in early pregnancy. Specific guidelines should be issued and followed for testing antimicrobial susceptibility with safe drugs in antenatal women. Empirical treatment with nitrofurantoin can be recommended, which is a safe drug and active for both Gram-negative and Gram-positive bacteria.

KEY WORDS: Antenatal women, antibiotic resistance, asymptomatic bacteriuria, prevalence, risk factors

INTRODUCTION

Urinary tract infection (UTI) during pregnancy is classified as either symptomatic or asymptomatic. Symptomatic UTI are divided into lower tract (acute cystitis) and upper tract (acute pyelonephritis) infection. Asymptomatic bacteriuria (ABU), generally defined as true bacteriuria in the absence of specific symptoms of acute UTI. The prevalence of ABU among antenatal women varies between 2% and 10%.[1] The anatomical and physiological changes imposed on urinary tract by pregnancy, as well as pressure on ureters by the gravid uterus and the muscle relaxant effect of progesterone, predisposes women with ABU to UTI.[2] Women identified with ABU in early pregnancy have 20-30 fold increased risk of developing pyelonephritis during pregnancy, compared with women without bacteriuria.[3] These women also are more likely to experience premature delivery and to have infants with low-birth weight.

Escherichia coli remains the single most common organism isolated from bacteriuric women, other organisms including *Klebsiella pneumoniae, coagulase-negative Staphylococci, Enterococcus* spp., group B *Streptococci* and *Gardnerella vaginalis* are common as well.[4] Gestational diabetes, past history of UTI, multiparity, advanced maternal age, advanced gestational age, lower education level and lower socio-economic status have been documented as risk factors in some of the studies and conflicting results have been obtained from different studies.[5,6]

Quantitative urine culture is the gold standard for diagnosis of ABU. Prospective, comparative clinical trials have reported

Access this article online	
Quick Response Code	Website: www.jbcrs.org
	DOI: 10.4103/2278-960X.118647

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that antimicrobial treatment of ABU during pregnancy decreases the risk of subsequent pyelonephritis from 20-30% to 1-4% and decreases the frequency of low-birth weight infants and preterm delivery.[7,8] Therefore, all antenatal women requires screening for bacteriuria by urine culture at least once in early pregnancy so that they can be treated with appropriate antibiotics for 3-7 days.[9] Antimicrobial agents including selective ß-lactams, nitrofurantoin, quinolones and co-trimoxazole can be considered during pregnancy.[10] However, the emergence of drug resistance, limits the choice of antibiotics.

To the best of our knowledge, no information is available from Odisha state, India on the prevalence of ABU in antenatal women. Hence, this prospective study was designed to determine the prevalence of ABU, etiological agents, risk factors and antimicrobial resistance patterns in antenatal women who attended a tertiary care hospital, Odisha, India.

SUBJECTS AND METHODS

Study area

The present prospective study was carried out in the clinical Microbiology laboratory of a tertiary care hospital, which is located in southern Odisha, India. The duration of the study was 6 months period from July 2012 to December 2012.

Study population

A total of 287 women in their first, second and third trimester of pregnancy, in the age group of 20-40 years who attended the antenatal clinic for the first time of our hospital were assessed for ABU. For asymptomatic pregnant women, bacteriuria is defined as two consecutive voided urine specimens with isolation of the same bacterial strain in quantitative counts $\geq 10^5$ colony forming units per milliliter (cfu/mL).[9] Inclusion criteria included those pregnant women who consented to give two consecutive urine samples on the first antenatal visit. Exclusion criteria included: (a) Non-pregnant women, (b) signs and symptoms of UTI and (c) antibiotics usage within week. Demographic data, medical and social information as well as gynecological and obstetrics history of the subjects were obtained from pre-tested, self-administered questionnaire. The study was conducted after approval from Institutional Ethical Committee.

Sample collection and processing

On each antenatal visit, two consecutive freshly voided clean-catch midstream urine samples were collected from antenatal woman in a sterile wide mouth screw-capped universal container with aseptic precautions in the antenatal clinic. The specimens were labeled and transported to the microbiology laboratory for processing within 2 h.

Semi-quantitative urine culture was done using a calibrated loop. A loopful (0.001 mL) of well mixed un-centrifuged urine was inoculated onto the surface of cysteine lactose electrolyte deficient medium. The culture plates were incubated aerobically at 37°C for 18-24 h and count were expressed as cfu/mL. For this study, significant bacteriuria was defined as culture of a single bacterial species from two consecutive urine samples at a concentration of $\geq 10^5$ cfu/mL.[11] Only patients with significant bacteriuria ($\geq 10^5$ cfu/mL) were included for microbiological analysis. The culture isolates were identified by standard microbiological methods.[12] All culture media were procured from HiMedia Laboratories, Mumbai, India.

Antimicrobial susceptibility testing

Isolates were tested for antimicrobial susceptibility testing by the standard Kirby-Bauer disc diffusion method according to Bauer *et al*.[13] Mueller-Hinton agar plates were incubated for 24 h after inoculation with organisms and placement of discs. After 24 h the inhibition zones were measured. The following standard antibiotic discs for the isolates were used; ampicillin 10 micrograms (mcg), amoxicillin (10 mcg), amoxicillin/clavulinic acid (20/10 mcg), nitrofurantoin (300 mcg), cephalexin (30 mcg), cefuroxime (30 mcg) ciprofloxacin (5 mcg) and norfloxacin (10 mcg). Antibiotic discs were obtained from HiMedia Laboratories, Mumbai, India. The results were interpreted according to Clinical and Laboratory Standards Institute guidelines.[14] The quality control strains used were *E. coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 for antimicrobial discs.

Statistical analysis

The data were analyzed using GraphPad Quick Calcs Statistical Software Inc., USA. Inferential statistics was done by Chi-square (χ^2) test and a $P < 0.05$ was considered significant.

RESULTS

The mean age of antenatal women who attended antenatal clinic and participated the study was 25.87 (5.2) years (median 24, minimum 20 and maximum 40 years). Out of total 287 antenatal women examined for ABU, 33 were positive for significant bacteriuria; thus showed a prevalence of 11.5% (33/287).

Table 1 shows the socio-economic characteristics of the study subjects by age, level of education, socio-economic status, estimated gestational age and parity. Majority (241/287,84%) of subjects were between the age group of 20 and 30 years, showed a prevalence of 11.2%(27/241). Similarly, majority (54.4%, 156/287) of the subjects were multipara and presented in 2nd and 3rd trimester of pregnancy (61%,

Table 1: Socio-demographic characteristics by the distribution of study subjects

Variables	Total no. of urine specimen collected from antenatal women			Chi-square value	*P* value
	No. tested (%)	UTI absent (%)	UTI present (%)		
Age in years					
20-30 years	241 (84)	214 (88.8)	27 (11.2)	0.01	0.91
31-40 years	46 (16)	40 (87)	06 (13)		(NS)
Level of education					
Up to primary level	195 (67.9)	167 (85.6)	28 (14.4)	4.05	0.04
College and higher	92 (32.1)	87 (94.6)	05 (5.4)		(S)
Socio-economic status					
Low	179 (62.4)	152 (84.9)	27 (15.1)	5.11	0.02
High	108 (37.6)	102 (94.4)	06 (5.6)		(S)
Gestational age					
<13 weeks (1st trimester)	112 (39)	104 (92.9)	08 (7.1)	2.76	0.09
≥13 weeks (2nd and 3rd trimester)	175 (61)	150 (85.7)	25 (14.3)		(NS)
Parity					
Primigravida	131 (45.6)	120 (91.6)	11 (8.4)	1.75	0.18
Multipara	156 (54.4)	134 (85.9)	22 (14.1)		(NS)

UTI – Urinary tract infection; *P*<0.05 (statistically significant); S – Significant; NS – Not significant; (*n*=287)

Table 2: Prevalence of uropathogens among asymptomatic antenatal women in a tertiary care hospital, Odisha, India

Gram reaction	Microorganism	Number	Percentage
Gram-negative bacteria	*Escherichia coli*	18	54.5
	Klebsiella pneumoniae	02	6.1
	Citrobacter freundii	02	6.1
	Proteus mirabilis	01	3
Gram-positive bacteria	*Enterococcus faecalis*	05	15.2
	Staphylococcus saprophyticus	04	12.1
	Staphylococcus epidermidis	01	3
Total number of bacteria (both Gram-negative and Gram-positive)		33	100

(*n*=33)

175/287), had revealed prevalence of 14.1% (22/156) and 14.3% (25/175) respectively. These variables did not show statistically significant results. Evaluation of significant bacteriuria in relation to the level of education and socio-economic status showed significant association with low level of education and lower socio-economic status.

The frequency of microorganisms isolated is shown in Table 2. From total 33 significant bacteriuria isolates, Gram-negative bacteria accounted for 69.7% (23/33), while Gram-positive bacteria accounted for 30.3%(10/33). *E. coli* (54.5%, 18/33) was the most frequently isolated bacteria, followed by *E. faecalis* (15.2%, 5/33), The antibiotic resistance profiles of the bacterial isolates are summarized in Table 3. Overall, Gram-negative isolates showed higher resistance pattern in comparison to Gram-positive. Nitrofurantoin was the most effective antibiotic for both Gram-negative and Gram-positive bacteria, showed resistance rate of 3% (1/33), followed by ciprofloxacin 30.3% (10/33) and amoxicillin/clavulinic acid 36.4% (12/33).

DISCUSSION

ABU in antenatal women is a microbiologic diagnosis determined with a gold standard urine culture for significant bacteriuria during their 1st antenatal visit preferably at the end of 1st trimester.[10] This present study provides valuable laboratory data to know the prevalence of ABU among antenatal women, to study their socio-demographic profiles, to monitor the status of antibiotic resistance in uropathogens and to improve treatment recommendations in a specific geographic region. This study also allows comparison of the situation in Odisha state with other regions within and outside India.

From total 287 urine samples collected from asymptomatic antenatal women and tested, 33 yielded significant uropathogens thus showed a prevalence of 11.5% (33/287). This correlates with the global prevalence of ABU among antenatal women, which varies between 2% and 10%. Similar prevalence of ABU 9.8%, 11.2%, 13.7% and 16% among antenatal women was reported by Marahatta *et al*. in Kathmandu, Nepal, Chitralekha *et al*. in Chennai, India, Saeed and Tariq in Karachi, Pakistan and Ansari and Rajkumari in Hyderabad, India respectively.[15-18] Low prevalence rate of 6.1% and 7.5% was observed by Ahmad *et al*. in Kashmir, India and Saraswathi and Aljabri in Hyderabad, India.[19,20] High prevalence of 29.1%, 38.3% and 45.3% was revealed by Rahimkhani *et al*. in Tehran, Iran, Rizvi *et al*. in Aligarh, India and Imade *et al*. in Benin city, Edo state, Nigeria respectively.[21-23] Geographical location and varied distribution of microorganisms may be the reason for this wide difference in prevalence.

In our study, majority of the bacteriuric women belonged to lower socio-economic status and they studied up to primary level. The ABU was significantly associated among them ($P < 0.05$). The close association between ABU, low socio-economic status and low level of education has been documented by various researchers.[24-27] This association may be due to poor knowledge and practice of personal hygiene in pregnancy. Another reason could be as a result of poor genital hygiene practices by antenatal women who may find it difficult to clean their anus properly after defecating or clean their genital after passing urine.[23]

Table 3: Resistance patterns of *Escherichia coli*, Gram-negative isolates and Gram-positive isolates

Antibiotic (μg)	Number (%) of isolates resistant among *Escherichia coli* (*n*=18)	Number (%) of isolates resistant among all Gram-negative isolates (*n*=5)	Number (%) of isolates resistant among all Gram-positive isolates (*n*=10)
Ampicillin (10)	17 (94.4)	05 (100)	06 (60)
Amoxicillin (10)	17 (94.4)	05 (100)	06 (60)
Amoxicillin/clavulinic acid (20/10)	08 (44.4)	02 (40)	02 (20)
Nitrofurantoin (300)	01 (5.6)	0	0
Cephalexin (30)	10 (55.6)	02 (40)	03 (30)
Cefuroxime (30)	09 (50)	01 (20)	03 (30)
Norfloxacin (10)	16 (88.9)	04 (80)	07 (70)
Ciprofloxacin (5)	07 (38.9)	01 (20)	02 (20)

In the present study, prevalence of 11.2% was recorded in the age group of 20-30 years and 13% among 31-40 age groups. No relationship between prevalence of ABU and patient's age group was found ($P = 0.91$). Similar findings were obtained in previous studies.[24,26,28] Advanced maternal age (≥35 years) was reported as risk factor for ABU in pregnancy.[5]

There was no significant difference in the prevalence of ABU with respect to trimester and parity in our study ($P = 0.09$ and 0.18 respectively). This agrees with earlier studies.[23,27] It has been reported that advanced gestational age and multipara are risk factors for acquiring ABU in pregnancy.[18,24]

In our study, Gram-negative aerobic bacteria predominated (69.7%), among which *E. coli* was the most prevalent uropathogens, followed by *E. faecalis* (15.2%) and *S. saprophyticus* (12.1%). There is increase in prevalence of *Enterococcus spp*. and *S. saprophyticus* have been reported by various authors in different studies.[21,29-31] The data collected from different places around the world showed that *E. coli* and *Klebsiella spp*. are still commonest pathogens in ABU.[15,16,19,22] Gram-negative aerobic bacteria including *Enterobacteriaceae* have several factors responsible for their attachment to uroepithelium. They colonize in the urogenital mucosa with adhesin, pili, fimbriae and P-1 blood group phenotype receptor.[32]

Treatment of ABU has been shown to reduce the rate of pyelonephritis in later part of pregnancy and therefore regular screening for and appropriate treatment of ABU has become a standard of obstetrical care.[33] The antibiotic chosen for antenatal women should have a good maternal and fetal safety profile, excellent efficacy and low resistance rate. United States food and drug administration, category B drugs including ampicillin, amoxicillin, amoxicillin/clavulinic acid, cephalexin, cefuroxime and nitrofurantoin and category C drugs including ciprofloxacin, norfloxacin, levofloxacin and co-trimoxazole should be used as empirical therapy for both ABU and symptomatic UTI during pregnancy.[10] However, local antibiotic susceptibility patterns must be taken into account before choosing an agent due to increasing antibiotic resistance prevalent for the population in question. In this present study, Gram-negative isolates showed higher resistance pattern in comparison to Gram-positive isolates. Gram-negative isolates including *E. coli* showed high level of resistance pattern to ampicillin, amoxicillin, norfloxacin, cephalexin, cefuroxime, amoxicillin/clavulinic acid and ciprofloxacin. Nitrofurantoin was found to be single most effective drug for both Gram-negative and Gram-positive bacteria, showed resistant rate of 3%. Similar resistance pattern was reported from studies conducted in different parts of India and its neighboring countries.[15,30,31,34] Irrational prescription of antibiotics which are available over-the-counter in India and indiscriminate use has created has created a high level of drug resistance. Our findings thus suggest that empirical treatment with commonly used antibiotics except nitrofurantoin should no longer be appropriate.

This study was limited by less sample size and some of the study subjects attended antenatal clinic late, i.e., in their 2nd and 3rd trimester of pregnancy was included.

CONCLUSION

Our study showed prevalence rate of ABU among antenatal women was 11.5%. Nitrofurantoin was the most effective antibiotic for both Gram-negative and Gram-positive bacteria with resistance rate of <10%. Therefore, it is important to screen all antenatal women with gold standard urine culture for significant bacteriuria during their 1st antenatal visit preferably at the end of 1st trimester. This should be followed with antibiotic susceptibility for determining therapy as inappropriate or no therapy has been responsible for recurrences of ABU and subsequent development of acute pyelonephritis. Thus empirical treatment of ABU may not apply for specific geographical regions, where decreased susceptibility rates to commonly used antibiotics have been documented for uropathogens.

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How to cite this article: Dash M, Sahu S, Mohanty I, Narasimham MV, Turuk J, Sahu R. Prevalence, risk factors and antimicrobial resistance of asymptomatic bacteriuria among antenatal women. J Basic Clin Reprod Sci 2013;2:92-6.

Source of Support: Nil, **Conflict of Interest:** None declared