## **Original Research Article**

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# Prevalence, risk factors and causative organisms of asymptomatic bacteriuria in pregnancy

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#### **ABSTRACT**

**Background:** Asymptomatic bacteriuria (ASB) is a relatively common condition occurring due to the morphological and physiological changes in the genitourinary tract during pregnancy. If left untreated, it may lead to acute pyelonephritis and adverse fetal and maternal outcomes. The objective was to determine prevalence, risk factors and etiological agents with susceptibility for ASB among pregnant women attending an antenatal clinic at a tertiary care hospital, Odisha, India.

**Methods:** A prospective study with 200 pregnant women was conducted, over a period of 4 months, starting from 1<sup>st</sup> April 2017 to 31<sup>st</sup> July 2017. The mid- stream clean catch urine specimen was collected and processed in all the cases and other data were collected from the questionnaire given to them. The isolates from all the cases of ASB were identified and antimicrobial susceptibility was tested by Kirby- Bauer disc diffusion method and interpreted.

**Results:** Prevalence of ASB in our study was 25.3%, with maximum prevalence among age group 21-30 yrs, during 3rd trimester, among multigravidae. Previous history of urinary tract infection (UTI), anaemia and diabetes have significant association with ASB. *Klebsiella spp.* was the predominant isolate in this study followed by *Escherichia coli*. Nitrofurantoin and Cefixime are safe and effective antibiotics against urinary pathogens in pregnancy.

**Conclusions:** Undiagnosed and untreated asymptomatic bacteriuria is associated with complications during pregnancy. Hence routine screening of antenatal women for ASB during all trimesters must be considered for preventing the adverse maternal and foetal outcomes particularly with known risk factors like increasing age, multiparity and previous history of UTI.

**Keywords:** Asymptomatic bacteriuria, *Klebsiella spp*, Pregnancy, Risk factors, Urinary tract infection

#### INTRODUCTION

Urinary Tract Infection (UTI) is a common problem in pregnancy due to the morphological and the physiological changes that take place in the genitourinary tract during pregnancy. Urinary tract infection during pregnancy could be either symptomatic or asymptomatic. Asymptomatic bacteriuria (ASB) is the presence of actively multiplying bacteria of more than  $10^5/\text{ml}$  of urine within the urinary tract excluding the

distal urethra, in the absence of any symptoms.<sup>2,3</sup> The risk associated with bacteriuria is that it does not always present with symptoms but if left untreated it may lead to adverse maternal and perinatal outcomes. Adverse maternal outcomes include symptomatic cystitis, development of pyelonephritis (in up to 30%), preterm labour and delivery.<sup>4</sup> The associated adverse foetal outcomes include prematurity, low birth weight and consequently increased perinatal mortality and morbidity. It is postulated that direct bacterial endotoxin is

responsible. Under normal circumstances, the relatively acidic pH, high osmolality and high urea concentration in urine is bacteriostatic to most bacteria.<sup>2</sup> The apparent reduction in immunity of pregnant women appears to encourage the growth of both commensal and noncommensal micro-organisms.<sup>5</sup> It has been demonstrated through randomized trials that antimicrobial treatment of asymptomatic bacteriuria during pregnancy will decrease the risk of subsequent pyelonephritis from 20-35% to 1-4% and the risk of having a low birth weight baby from 15% to 5%.<sup>6</sup>

Asymptomatic bacteriuria is a microbial diagnosis which depends on the isolation of specified count of bacteria in properly collected urine specimen from pregnant women. Semi-quantitative urine culture is the gold standard in screening and diagnosis of ASB.<sup>7</sup> Selection of mothers for screening with risk factors may reduce the necessity of urine culture for all pregnant women where cost is a constraint, and may be used as an appropriate alternative strategy of management and will be cost-beneficial.<sup>8</sup> Gestational diabetes, past urinary tract infection, multiparity, advanced maternal age, lower education level, advanced gestational age and lower socioeconomic status have been reported as risk factors in some of the studies.<sup>9</sup>

The predominant organism causing UTI in women is *Escherichia coli*, accounting for 90% of infections. However, the frequencies of isolated pathogens and their antimicrobial sensitivity are variable in different geographical regions.<sup>9</sup>

Based on these study results, the aim of this study was to estimate the prevalence of ASB and its associated risk factors among pregnant women attending the antenatal clinic in a tertiary care hospital. In addition, the bacterial pathogens implicated in causing ASB and their antimicrobial susceptibility was also evaluated.

#### **METHODS**

This is a prospective study conducted in the Department of Obstetrics and Gynaecology and Department of Microbiology in Kalinga institute of Medical Sciences and PBMH, over a period of 4 months, starting from 1st April 2017 to 31<sup>st</sup> July 2017. A total number of 200 pregnant women who attended antenatal clinic were included in this study. Pregnant women between 18-40 years of age, irrespective of parity and socioeconomic status, willing to participate in the study were included in the study group.

Pregnant women with symptoms of UTI (dysuria, frequency and urgency, pain lower abdomen), history of antibiotic therapy taken in the previous two weeks and catheterized patients were excluded from this study.

Sample collection: Informed consent was taken for participation in the study. A questionnaire was used to

collect the obstetric data and data on possible risk factors. A urine routine microscopy and culture and sensitivity test was advised to all pregnant women willing to participate in the study at their first visit to the ante natal clinic. These patients were counseled and instructed about the collection of "clean catch" mid-stream sample of urine into sterile wide mouthed container that was covered with a tightly fitted lid. The samples were transported immediately to the laboratory and processed without delay.

Investigations: The specimens were subjected to routine microscopy and culture by the standard loop technique on Cystine Lactose Electrolyte Deficient (CLED) medium using a calibrated wire loop, incubated at 37° C, aerobically for 24 hrs. Prolonged incubation was done for further 24 hours if there was no growth. The identification of organisms was done by Gram staining, motility test, catalase test, oxidase test, coagulase test and routine biochemical tests. The growth was interpreted as sterile if no growth was obtained after 48hrs of incubation. It was interpreted as Significant if the number of colonies corresponded to 10<sup>5</sup> colonies forming units (CFU) per ml. Insignificant growth was be reported when colony count was less than 10<sup>5</sup> CFUs per ml.

The standardized Kirby-Bauer disc diffusion method on Muller Hinton agar plate as per recommendations of Clinical Laboratory Standard Institute (CLSI) was used for antibiotic sensitivity testing. All the asymptomatic bacteriuric pregnant women were advised to take proper medication as per antibiotic sensitivity test report and asked to come back after 7-10 days for repeat culture and susceptibility testing, which was done as a part of follow-up.

#### **RESULTS**

The present study included 200 pregnant women attending the antenatal OPD, KIMS and PBMH Bhubaneswar. Among them 51 (25.3%) cases were found to have culture positive with significant bacterial growth (Asymptomatic bacteriuria). So, a prevalence rate of 25.30% was found in this study. 119 (59.33%) of the urine samples were sterile while 21 (10.66%) showed insignificant bacteriuria, and contaminants were grown in 9 (4.6%) specimens.

Table 1: Distribution of cases according to age.

Age (yrs)	Number of cases	%	No. of ASB	%
18- 20 yrs	21	10.5	4	19.04
21-30 yrs	126	63	48	37.73
31-40 yrs	53	26.5	11	21.42
Total	200	100	51	25.3%

Among 200 pregnant women who participated in the study, maximum no. of cases 126 (63%) were in the age group of 21-30 years, followed by the age group 31-40

yrs 53 (26.50%). Asymptomatic bacteriuria cases were also most common (37.73%) in the age group of 21-30 yrs (Table 1).

It was seen that most of our patients were second gravida (47%) and Asymptomatic bacteriuria cases were also high among 2<sup>nd</sup> gravida (39.85%). ASB cases in gravida 3 and more were 23.07% and the lowest was in primigravida (5.97%) (Table 2).

Table 2: Distribution of cases according to gravid.

Gravida	Number of cases	% (n=200)	No. of ASB	%
1	67	33.5	4	5.97
2	94	47	38	39.85
>= 3	39	19.5	9	23.07
Total	200	100	51	25.3

Among the study population, number of pregnant women in the first trimester were 44.5%, in second trimester 35% and in third trimester 20.5%. Most of the cases of asymptomatic bacteriuria were found in 3rd trimester of pregnancy (53.65%) (Table 3).

Table 3: Distribution of cases according to trimester of pregnancy.

Trimester	Number of cases	% (n=200)	No. of ASB	%
1 <sup>st</sup>	89	44.50%	13	(14.60)
2 <sup>nd</sup>	70	35%	16	(22.85)
3 <sup>rd</sup>	41	20.50%	22	(53.65)
Total	200	100%	51	(25.30)

Among the risk factors, a maximum number of women had a past history of UTI (36.5%) and higher number of ASB (43.83%) cases were found among them. Anaemia was present in 59/200 (29.5%) pregnant women and these patients had ASB rate of 33.89%.

Gestational diabetes was present in 35 (17.5%) of cases, out of which 31.42% had ASB. 29.03% of cases of ASB were admitted with Preterm labour and 23.07% cases of ASB had Pre- eclampsia (Table 4).

Table 4: Association of risk factors in ASB.

Risk factors	Number of cases	% (n=200)	No. of ASB	%
Past h/o UTI	73	36.5	32	43.83
Anaemia	59	29.5	20	33.89
Pre- eclampsia	26	13	6	23.07
Diabetes	35	17.5	11	31.42
Preterm labour	31	15.5	9	29.03

Out of 51 significant bacterial isolates from urine samples, *Klebsiella spp*. was found to be most common 23 (45%) isolate, followed by *Escherichia coli* 12 (23.5%), *Staphylococcus aureus* 7 (13.72%), *Enterococcus spp* 5 (9.80 %), *Citrobacter spp*. 2 (3.92%) and *Pseudomonas aeruginosa* 2 (3.92%) (Table 5).

Table 5: Distribution of bacterial isolates among culture positive samples.

Types of bacterial isolate	Number of isolates (n=51)	%
Klebsiella spp.	23	45
Escherichia coli	12	23.52
Staphylococcus aureus	7	13.72
Enerococcus spp.	5	9.80
Citrobacter	2	3.92
Pseudomonas aeruginosa	2	3.92
Total	51	100

All the gram-negative isolates of this study exhibited 100% sensitivity to Imipenem and Meropenem. Sensitivity of Klebiella spp. to different antibiotics was found as: Cefepime (95.65%), Gentamicin (78.26%), Nitrofurantoin (78.26%),Norfloxacin (60.86%),Cefopodoxime Cefixime (52.17%),(47.82%),Cefuroxime (47.82%) and Cotrimxazole (43.47%), Amoxyclav (39.13%), Cefadroxil (0%). Sensitivity of Escherichia coli isolates was found as 100% to Cefepime, Nitrofurantoin and Cotrimxazole. Sensitivity pattern of Escherichia coli to other antibiotics were as follows: Gentamicin (75%), Norfloxacin (75%), Cefixime (66.66%), Cefopodoxime (50%), Cefuroxime (50%), Amoxyclav (41.60%) and Cefadroxil (33.3%). Citrobacter spp were 100% sensitive to all the above antibiotics. Pseudomonas aeruginosa isolates had shown 100% sensitive to all the cephalosporin group of antibiotics (Cefixime, Cefopodoxime, Cefuroxime, Ceftrixone, Ceftazidime, Cefepime and Piperacillin-Tazobactam), Gentamicin, Tobramycin and Nitrofurantoin, 50% sensitive to Norfloxacin and Cotrimaxazole.

Amongst the gram-positive cocci, Staphylococcus aureus was isolated most frequently (13.72%), followed by Enterococcus faecalis. All the gram-positive isolates were 100% sensitive to Nitrofurantoin, Cotrimoxazole, Linezolid and Vancomycin. Sensitivity pattern of Staphylococcus aureus to other antibiotics were: Gentamicin (85.7%), Ciproflaxacin and Norfloxacin (71.42%), 60% sensitive to Cephalosporins (Cefixime, Cefopodoxime, Cefuroxime and Cefadroxil), Amoxyclav (57.14%), Penicillin G (42.85%), Azithromycin (42.85%)

Sensitivity pattern of *Enterococcus spp.* was: Gentamicin (80%), Ciproflaxacin and Norfloxacin (60%), 40% sensitive to Cephalosporins (Cefixime, Cefopodoxime, Cefuroxime and Cefadroxil), Amoxyclav (60%), Penicillin G (40%) (Table 6).

Table 6: Antimicrobial sensitivity pattern of isolated organisms.

	Organisms isolated					
Antimicrobials	Gram Negative Isolates			Gram Positive Isolates		
	Klebsiella spp. n=23	Escherichia. Coli n=12	Citrobacter spp. n=2	Pseudomanas aeruginosa, n=2	Staph. aureus n=7	Enterococcus Spp. n=5
Penicillin G	ND	ND	ND	ND	3 (42.85%)	2 (40%)
Amoxyclav	9 (39.13%)	5 (41.6%)	2 (100%)	ND	4 (57.14%)	3 (60%)
Gentamicin	18 (78.26%)	9 (75%)	2 (100%)	2 (100%)	6 (85.71%)	4 (80%)
Ciprofloxacin	11 (47.82%)	8 (66.66%)	2 (100%)	1(50%)	5 (71.42%)	3(60%)
Cefadroxil	Nil (0%)	4 (33.33%)	2 (100%)	ND	6 (85.71%)	2 (40%)
Cefopodoxime	11 (47.82%)	6 (50%)	2 (100%)	2 (100%)	6 (85.71%)	2 (40%)
Cefixime	12 (52.17%)	8 (66.66%)	2 (100%)	2 (100%)	6 (85.71%)	2 (40%)
Cefuroxime	11 (47.82%)	6 (50%)	2 (100%)	1 (50%)	6 (85.71%)	2 (40%)
Norfloxacin	14 (60.86%)	9 (75%)	2 (100%)	2 (100%)	5 (71.42%)	3 (60%)
Nitrofurantoin	18 (78.26%)	12 (100%)	2 (100%)	1 (50%)	7 (100%)	5 (100%)
Cotrimoxazole	10 (43.47%)	12 (100%)	2 (100%)	2 (100%)	7 (100%)	5 (100%)
Azithromycin	ND	ND	ND	ND	3 (42.85%)	2 (40%)
Linezolide	ND	ND	ND	ND	7 (100%)	5 (100%)
Vancomycin	ND	ND	ND	ND	7 (100%)	5 (100%)
Tobramycin	ND	ND	ND	2 (100%)	ND	ND
Piperacillin- tazobactum	ND	ND	ND	2 (100%)	ND	ND
Ceftriaxone	ND	ND	ND	2 (100%)	ND	ND
Cefepime	22 (95.65%)	12 (100%)	2 (100%)	2 (100%)	ND	ND
Ceftazidime	ND	ND	ND	2 (100%)	ND	ND
Imipenem	23 (100%)	12 (100%)	ND	2 (100%)	ND	ND
Meropenem	23 (100%)	12 (100%)	ND	2 (100%)	ND	ND

ND: Not done.

#### **DISCUSSION**

Asymptomatic bacteriuria during pregnancy is a cause of serious maternal and perinatal morbidity. Women who have bacteriuria have a 20-50-fold increased risk of developing pyelonephritis as compared to women who do not have bacteriuria. Prevalence of ASB also increases with higher parity and advanced maternal age. This morbidity can be reduced with routine screening and giving treatment as per antimicrobial sensitivity report of the bacteria which are isolated from the urine samples.

In this study, the prevalence of asymptomatic bacteriuria was 25.3%. Which was similar to those seen in various other studies. Neupane et al (26.5%), Rohini et al.<sup>12,9</sup> But it is significantly high when compared with findings of Sujatha R et al (7.2%) and Annie Rajaratnam et al (13.2%) [14] and less when compared with Imade et al (45.3%).<sup>13,14,5</sup> This variation may be explained by the fact that there were differences in the environment, social habits of the community, socio-economic status, standards of personal hygiene and education of the patients who were studied.

The highest prevalence of asymptomatic bacteriuria was found in the age group of 21-30 yrs (37.73%) and least in the younger age group < 20 yr (19.04%). Similar results are also found by Rohini UV et al, Alghalibi et al

(55.62%), and Sujatha R et al (72.72%). 9,15,13 Some studies have found significant association with age, showing that ASB is common with advancing age. 16,17 Few other studies also reported a higher prevalence in the age group of 20-25 years. 10,15 The observed trend of bacteriuria in this study and reports from other studies showed that the age range of 21-40 years served as the high risk group for the development of UTI in pregnant women.

Our study showed prevalence of asymptomatic bacteriuria was highest among 2<sup>nd</sup> gravida (29.85%), followed by Primigravida (26.33%) and gravida 3 and above (23.07%). Similar association was seen in study by Ramalingam K et al.<sup>18</sup> Like our study more cases of ASB in multigravidae (51.1%) than in primigravida was seen in the studies of Sujatha R et al and Roy et al.<sup>13,19</sup>

In this study, a higher rate of infection was detected in 3<sup>rd</sup> trimester of pregnancy (53.65%), followed by second trimester (22.85%) and 1<sup>st</sup> trimester (14.6%) which is similar to the results of the study by Rohini UV et al, Ramalingam K et al, and Saeed et al. <sup>9,18,20</sup> But Yashodara et al. reported incidence of ASB was highest in 1<sup>st</sup> trimester, while studies done by Roy et al and Nath et al. <sup>21,19,22</sup> reported higher incidence of ASB during 2nd trimester. Findings from all the studies concluded that screening for asymptomatic bacteriuria should be done in

all the three trimesters of pregnancy which is necessary to prevent the dangerous complications associated with asymptomatic bacteriuria in pregnancy. Other studies also conclude with the same recommendations. <sup>18,21</sup>

Among the risk factors associated with ASB, highest prevalence was seen in patients with previous history of UTI (43.83%). Similar association is reported in studies of Rohini UV et al, Rajaratnam A et al. 9,14 Lindsay E. Nicolle and many others.<sup>23</sup> The association between ASB and anaemia during pregnancy is varying. In our study, among 59 (29.5%) anaemic pregnant women, 33.89% cases had ASB. Since anaemia tends to be a feature when the bacteria are resistant to treatment, ASB in anaemia should be treated promptly.24 Anaemia was recorded as an important risk factor for asymptomatic bacteriuria. 11 (21.15%) of anaemic and only 4 (8.33%) of non-aneamic showed asymptomatic bacteriuria in the study by Ramalingam K et al. 18 Lavanya and Joagalakshmi found that bacteriuria in pregnancy was associated with maternal anaemia. But Fatima and Ishrat did not find any association between bacteriuria and anaemia.<sup>6</sup> Thus increased prevalence of ASB in anaemia among pregnant women needs further evaluation.

Diabetes mellitus is an independent risk factor and pyelonephritis occurs more frequently among diabetics than non-diabetics.<sup>25</sup> In our study 35 (17.5%) cases had diabetes (both Type2 and Gestational Diabetes). Preeclampsia has been reported to increase susceptibility to infection. A significant difference (p-value<0.005) in the rate of ASB was found in patients with pre-eclampsia (19%) in a study done by JA Hill et al.<sup>26</sup> In our study we had 6 (23.07%) cases pre-eclampsia who were positive for ASB.

In this study, *Klebsiella spp.* was found to be commonest (45%) isolate, followed by Eschericia coli (23.5%). Similar results are reported by Ramalingam K et al as 50% of isolates are Klebsiella spp. 18 Muharram SH et al, and Olamijulo JA et al also reported Klebsiella spp. as the commonest micro-organism. 27,28 Garnizov TM et al reported cases of ASB due to Klebsiella pneumoneae in pregnant woman.<sup>29</sup> But many of the studies from India and abroad have reported Escherichia coli as the commonest isolate and there is increasing trend towards Klebsiella spp. as most potent urinary pathogen. 12,30-32 Causative agents of asymptomatic bacteriuria in females are usually the commensal bacteria of the female genital tract and of the bowel. Different determinants of virulence such as presence of adhesins, stasis produced by gravid uterus etc. play a role in the causation of UTI. Other authors have also reported Staphylococcus aureus as the most common urinary pathogen among the grampositive cocci. 9,33,34 In the present study, there were similarities in the organisms isolated as in other studies, i.e. members of family Enterobacteriaceae predominated. Although the spectrum of agents causing UTI in pregnant constant, their antibiotic women is relatively are different in different susceptibility patterns

geographical locations which is because of the emergence of resistant strains due to indiscriminate usage of antibiotics.

All the gram-negative isolates of this study exhibited 100% sensitivity to Imipenem and Meropenem. In the present study, the isolates were highly susceptible to Nitrofurantoin, Cotrimoxazole, Gentamicin. Moderatly sensitive to Cefixime, Cefuroxime, Cefopodoxime and lesser sensitivity was seen for Cephadroxil and Amoxyclav. The susceptibility patterns of antibiotics in the present study slightly varies with that of other studies. K Ramalingam in his study showed gram negative bacteria from ASB cases were highly susceptible to gentamicin, cefotaxime followed by Nitrofurantoin and Cotrimoxazole. Marked degree of resistance was seen for Cephalexin and Ceftriaxone. Lavanya and Jogalakshmi identified maximum susceptibility to cephalexin (35.7%) followed by nitrofurantoin (28.5%).

Amongst the gram-positive cocci, Staphylococcus aureus was isolated most frequently (13.72%), followed by Enterococcus faecalis. In this study, the gram-positive isolates were 100 % sensitive to Nitrofurantoin, Cotrimoxazole, Linezolid and Vancomycin, highly sensitive to Gentamicin, Ciproflaxacin and Norfloxacin, Cephalosporins (Cefixime, Cefopodoxime, Cefuroxime and Cefadroxil), Amoxyclav (57.14%) and less sensitive to Penicillin G and Azithromycin. Gram positive microorganisms were found to be important causes of ASB in some studies. Enayat et al reported that Enterococus spp. is the most common Gram-positive microorganism (4.5%).<sup>35</sup> Isolates were highly resistant to ampicillin, cloxacillin and erythromycin, moderately resistant to Gentamicin, Ciprofloxacin, Cotrimoxazole and 100 % sensitive to Nitrofurantoin, Cotrimoxazole, Linezolid and Vancomycin. Antibiogram of our study correlated with those of other studies. 9,35,36

The upsurge in antibiotic resistance patterns could have been caused by antibiotic abuse and self-medication. Low costs and easy availability of drugs could be other factors contributing to antibiotic resistance. Based on susceptibility patterns, drug regimens were suggested for 7-10 days to prevent the risk of re-infection from vaginal and faecal reservoirs.

### **CONCLUSION**

This study revealed omit 25.3% prevalence of asymptomatic bacteriuria among pregnant women is 25.3%. Which is a matter of concern. So first antenatal visit should include urine culture sensitivity testing as a routine procedure. The omit predominant organisms isolated were *Klebsiella spp. and Escherichia coli*. Nitrofurantoin and Cefixime are safe and effective against urinary pathogens in pregnancy. Routine testing of urine of antenatal women during all trimesters must be done, particularly in women with known risk factors. In view of changes observed in the trend of bacterial isolates

and the antibiotic resistance pattern among the pathogens to common drugs, Obstetricians should be guided by the empirical management of cases of ASB.

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institutional ethics committee

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