



## MICROBIAL PROFILE AND ANTIMICROBIAL SENSITIVITY PATTERN OF ASYMPTOMATIC BACTERIURIA AMONG PREGNANT WOMEN IN A TEACHING HOSPITAL FROM EASTERN INDIA

### Microbiology

**Ms. Basundhara Kar** Student of final year MSc Medical Microbiology, College of Medicine and JNM hospital, W.B.U.H.S, Kalyani, Nadia

**Dr. Priyanka Saha\*** Assistant Professor, Microbiology, College of Medicine and JNM hospital, W.B.U.H.S, Kalyani, Nadia \*Corresponding Author

**Dr. Sougata Kr. Barman** Assistant Professor, Gynae. & Obs, College of Medicine and JNM hospital, W.B.U.H.S, Kalyani, Nadia

### ABSTRACT

Asymptomatic bacteriuria or asymptomatic UTI is the isolation of a specified quantitative count of bacteria in an appropriately collected urine specimen obtained from a person without symptoms or signs of urinary infection. (1) This study was undertaken to find out prevalence, causative agents, and antimicrobial sensitivity pattern of asymptomatic bacteriuria (ASB) in pregnant women attending our center. Total 300 asymptomatic pregnant women were screened during April 2018 to September 2018. Isolates were identified by conventional methods and one *Streptococcus sp.* was identified by the Vitek 2 Compact system. Asymptomatic bacteriuria were more common in primigravidae & during 3<sup>rd</sup> trimester of pregnancy. *E.coli* was the commonest organism isolated (38.23%) followed by Coagulase negative *Staphylococcus* (CONS) (23.52%), *Klebsiella pneumoniae* (17.64%) and *Enterococcus sp.* (17.64%). Gram negative isolates were resistant to most of the common oral antibiotics. Hence, all pregnant women should be routinely screened for asymptomatic bacteriuria during antenatal check-up.

### KEYWORDS

Asymptomatic bacteriuria, pregnant women, significant bacteriuria

### INTRODUCTION :

Asymptomatic bacteriuria or asymptomatic urinary tract infection (UTI) is the isolation of a specified quantitative count of bacteria in an appropriately collected urine specimen obtained from a person without symptoms or signs of urinary infection. (1) Asymptomatic bacteriuria accounts for 2-10% pregnancies in affluent countries (2) incidence is much more in developing and underdeveloped countries. Pregnant women with asymptomatic bacteriuria (ASB) are at high risk of developing maternal as well as fetal complication. Complications like overt UTI later in pregnancy, post partum hypertension, anaemia may be seen in mother. On the other hand, serious problem in fetus like prematurity, low birth weight, even fetal wastage may arise due to ASB in mother. Various hormonal, mechanical changes lead to significant alterations in the urinary tract like dilatation, decrease in peristalsis in the ureter and decrease in bladder tone. In addition, the physiological increase in plasma volume during pregnancy, decrease urine concentration leading to decreased ability of the lower urinary tract to resist invading organisms (3,4). These changes have a profound impact on the acquisition of bacteriuria and lead to an otherwise healthy woman susceptible to serious infectious complication. Some other conditions like multiparity, and other medical disorders are also associated with increased incidence of ASB in pregnancy. (5) Symptomatic bacteriuria (UTI) can be easily diagnosed and treated as it presents with obvious symptoms. So it poses no problem. But ASB largely remains undiagnosed due to absence of symptoms. Thus to prevent untoward complications in mother & baby that may arise due to ASB, it has been suggested to do routine screening for all pregnant women attending ANC even in the absence of symptoms. (6) The main objective of this study was to find out the prevalence of asymptomatic bacteriuria in pregnant women, determine microbial profile, prepare antimicrobial sensitivity pattern of the isolate.

### MATERIALS & METHODOLOGY :

Our study was a cross-sectional observational study done from April 2018 to September 2018 among pregnant women in varying gestational periods of pregnancy visiting antenatal clinic at College of medicine and JNM hospital. After getting ethical clearance, this study was conducted among pregnant women having no signs and symptoms of UTI. A detailed history including the demography, complaints (symptoms of UTI), period of gestation, gravida, and medical history was taken after obtaining informed consent. Urine samples were collected by standard mid-stream "clean catch" method from all the pregnant women and immediately transported to the laboratory. Microscopic examination was done and pus cell count noted. Samples

were cultured on MacConkey agar, Blood agar and Hi-chrome UTI agar plates. A colony count of  $10^5$  or more colony forming unit (CFU) /ml of urine of a single type bacteria indicates 'significant bacteriuria'. (7) The identification of the isolates were done by standard biochemical methods (1) & Vitek 2 Compact system was used to identify one *Streptococcus sp.*. Antimicrobial susceptibility test were performed using Kirby-Bauer disc diffusion test against Ampicillin (10µg), Piperacillin-tazobactam (100/10µg), Cephalexin (30µg), Cefixime (30µg), Ceftriaxone (30µg), Meropenem (10µg), Amikacin (30µg), Levofloxacin (5µg), Nitrofurantoin (300µg), Fosfomycin (200µg), Penicillin-G (10 units), Vancomycin (30µg), Teicoplanin (30µg), Linezolid (30µg), High level Gentamicin (120µg) according to Clinical Laboratory Standard Institute (CLSI) guidelines (8). Extended spectrum betalactamases (ESBLs) were screened for enterobacteriaceae (cut-off zone sizes were  $\leq 27$  mm for cefotaxime,  $\leq 22$  mm for ceftazidime,  $\leq 25$  mm for ceftriaxone,  $\leq 27$  mm for aztreonam). These isolates were further subjected to combined disc diffusion test for ESBL detection. A disc of Ceftazidime (CAZ) alone and a disc containing Ceftazidime clavulanic acid (CAC) (30/10µg) was put up at least 20 mm apart from each other. An increase in zone size of  $> 5$  mm in CAC was considered to be ESBL positive. Cefoxitin (30µg) was used as a surrogate for oxacillin resistance. All strains of *Staphylococcus sp.* resistant to cefoxitin were considered resistant to all other beta-lactams antimicrobials including cephalosporins and carbapenems. The above tests were done as per CLSI guidelines. (8) The control strains used were *Escherichia coli* American Type Culture Collection (ATCC) 25922, *Pseudomonas aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923. For ESBL test control strain used was *Klebsiella pneumoniae* ATCC (700603).

Descriptive statistical analysis: Data was tabulated and analysed using the Statistical Package for Social Sciences SPSS software using mean, median and chi-square test. P value was calculated to assess the association between the variables tested. P value less than 0.05 was taken as significant.

### RESULTS

Out of 300 samples were screened, 34 women had significant bacteriuria giving a prevalence rate of 11.33%. Among them, 28 women (82.35%) were in the age group of 15-25 years and rest 6 (17.64%) were in the age group of 26-35 years. The p-value between age and ASB was found to be 0.290, no significant association was found. (table.1)

**Table 1. Distribution of the cases in the study population based on age**

Age group in years	Total number of culture positive women	Total number of women screened
15-25 years	28 (82.35%)	220
26-35 years	06 (17.64%)	80
Total	34	300

p-value= 0.29

As seen in Table 2, 22 women (64.70%) were primigravidae & 12 (35.29%) were multigravidae among culture positive isolates. The p-value between parity and ASB was found to be 0.435, statistically no significant association was found.

**Table 2 : Distribution of cases in the study population based on parity**

Parity	No. of cases with significant bacteriuria	No. of cases without significant bacteriuria	Total no. of pregnant women
Primigravidae	22 (64.70%)	149	171
Multigravidae	12 (35.29%)	117	129
Total	34	266	300

p-value = 0.435

Table 3 depicts that out of 34, 7 women (20.58%) were in 1<sup>st</sup> trimester, 11 (32.35%) were in 2<sup>nd</sup> trimester(13-24 week) and 16 ( 47.05%) were in 3<sup>rd</sup> trimester (25-36 week) of pregnancy. The number of positive cases vs gestation gave a p-value of 0.309 which was not significant statistically.

**Table 3 : Distribution of cases in the study population based on Trimester**

Trimester	No. of cases with significant bacteriuria	No. of cases without significant bacteriuria	Total number of pregnant women screened
1st (1-12 week)	07(20.58%)	35	42
2nd (13-24 week)	11(32.35%)	118	129
3rd (25-36 week)	16(47.05%)	113	129
Total	34	266	300

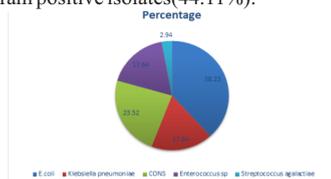
p-value = 0.309

Table 4 shows out of the 34 culture positive samples 25 (73.52%) showed a pus cell count of <5/HPF and 9 (26.47%) showed a pus cell count of >5/HPF. ( Table 4)

**Table 4 : Distribution of cases in the study population based on Pyuria**

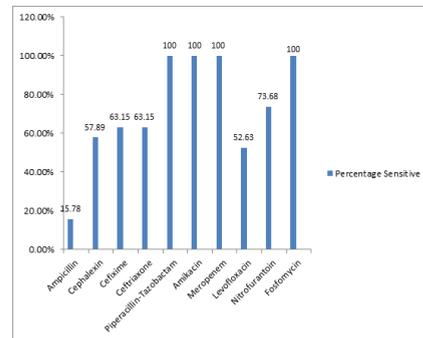
PYURIA	No. of cases with significant bacteriuria	No. of cases without significant bacteriuria	Total pregnant women
>5 Pus cells/HPF	9(26.47%)	4	13
<5 Pus cells/HPF	25(73.52%)	262	287
Total	34	266	300

Graph 1 shows that out of the 34 positive isolates, the isolated organisms were *E.coli* (13)38.23%, *Klebsiella pneumoniae* (6)17.64%, Coagulase negative *Staphylococcus*(CONS) (8)23.52%, *Enterococcus sp.*(6)17.64% and *Streptococcus agalactiae*(1)2.94% as seen in table.5. Gram negative isolates were more common (55.88%) compared to Gram positive isolates(44.11%).



**Graph 1 : Frequency(%) of different isolates**

Table 6 depicts the gram negative isolates (N=19) were 100% sensitive to Piperacillin-tazobactam, Meropenem, Amikacin and Fosfomycin . Nitrofurantoin showed high sensitivity to gram negative isolates (73.68%); followed by 63.15% sensitivity to Ceftriaxone & Cefixime, 57.89% sensitivity to Cefalexin . Levofloxacin showed a sensitivity of 52.63%. Ampicillin was 15.78% sensitive. Gram negative bacilli showed the presence of Extended spectrum beta-lactamases (ESBLs) in 7( 36.84%) out of 19 isolated enterobacteriaceae.



**Graph 2 : Antibiotic sensitivity pattern (% Sensitive) in Enterobacteriaceae(N=19)**

As shown in table 5. *E.coli* isolates showed less sensitivity(<55%) to oral cephalosporin & levofloxacin than *Klebsiella pneumoniae* (sensitivity >83%). But *E.coli* isolates(84.61%) were more sensitive to nitrofurantoin than *Klebsiella pneumoniae*(50%). ESBL production rate was more in case of *E.coli*(46.15%) compared to *Klebsiella pneumoniae*(16.66%)

**Table 5. Sensitivity pattern of Klebsiella pneumoniae & E.coli**

ANTIBIOTIC	Klebsiella pneumoniae % Sensitive (N = 6)	E. coli % Sensitive (N=13)
Ampicillin	0	3 (23.07%)
Cephalexin	5 (83.33%)	6 (46.15%)
Cefixime	5 (83.33%)	7 (53.84%)
Ceftriaxone	5 (83.33%)	7 (53.84%)
Piperacillin-Tazobactam	6 (100%)	13 (100%)
Amikacin	6 (100%)	13 (100%)
Meropenem	6 (100%)	13 (100%)
Levofloxacin	5 (83.33%)	5 (38.46%)
Nitrofurantoin	3 (50%)	11 (84.61%)
Fosfomycin	6 (100%)	13 (100%)
ESBLs	1 (16.66%)	6 (46.15%)

CONS showed 100% sensitivity to Vancomycin, Teicoplanin, Linezolid, Amikacin . Methicillin resistance in CONS was low(12.5%). Ceftriaxone showed a high sensitivity of 87.5%. levofloxacin & Ampicillin showed low sensitivity( 37.5% & 25% respectively). *Enterococcus sp.* were 100% sensitive to Pen-G, Ampicillin, Vancomycin, Teicoplanin, Linezolid, Nitrofurantoin, high dose Gentamicin and Fosfomycin. Levofloxacin showed a sensitivity of 66.66% to *Enterococcus sp.* Only 1 *Streptococcus agalactiae* was isolated which was sensitive to Pen-G, Ampicillin, Ceftriaxone, Vancomycin, Teicoplanin, Linezolid, Levofloxacin, Nitrofurantoin.

**Table 6 : Antibiotic sensitivity pattern (% Sensitive) in Gram Positive Cocci**

ANTIBIOTICS	CONS (% Sensitive) (N = 8)	Enterococcus sp. (% Sensitive) (N= 6)	Streptococcus agalactiae (% Sensitive) (N=1)
PEN-G	2(25%)	6(100%)	1/1(100%)
Ampicillin	2(25%)	6(100%)	1/1(100%)
Cefoxitin	7(87.5%)	-	-
Ceftriaxone	7(87.5%)	-	1/1(100%)
Vancomycin	8(100%)	6(100%)	1/1(100%)
Teicoplanin	8(100%)	6(100%)	1/1(100%)
Linezolid	8(100%)	6(100%)	1/1(100%)
Gentamicin(HD)	-	6(100%)	-
Amikacin	8(100%)	-	-
Levofloxacin	3(37.5%)	4(66.66%)	1(100%)
Nitrofurantoin	8(100%)	6(100%)	1(100%)
Fosfomycin	-	6(100%)	-

**DISCUSSION :**

The prevalence of ASB in the present study was 11.33% which lies within the range of global prevalence of ASB which varies from 4% to 23.9% in various studies. It was similar to study conducted by Guntoory *et al* (9), Lavanya *et al* (10) and Lata *et al*(11). The rate of prevalence in Prasanna *et al* (5) was found to be 17%, a little higher than our study. This prevalence rate varies due to difference in socio-economic status & personal hygiene practices by pregnant women in different regions.

Women in the younger age group( 15-25 years) had a higher incidence(82.35%) of ASB in our study than older age group (26-35 years). This data could be compared to study conducted by Guntoory *et al* (9). This may be due to this group being highly sexually active. The association between age group and ASB was statistically not significant in our study.

In our study 64.70% with significant bacteriuria were primigravidae. This could very well be compared with the studies of *Sudha et al* (12) and *Lavanya et al* (10). This could be so because the women were becoming mother for the first time and they were not experienced enough to take care of their personal hygiene during pregnancy. P-value of parity and ASB was calculated which showed no significant association.

In our study majority of the women with ASB were in the 3<sup>rd</sup> trimester of pregnancy (47.05%). This correlated well with the study of *Prasanna et al* (5) and *Ansari HQ et al* (6). The incidence of ASB was more pronounced in the 3<sup>rd</sup> trimester probably because of the anatomical and physiological changes related to advancing gestational age. This probably leads to stasis of urine and encourage bacterial multiplication. P- value showed no statistical relevance with ASB.

In our study the most common isolate was of *E.coli* (38.23%). This data could very well be compared to the findings of *Prasanna et al* (5), *Sudha et al* (12), *Lavanya et al* (10), *Jeyaseelan et al* (13). *E.coli* possess ability to colonize and infect the urinary tract, hence it is a commonly isolated organism in the community acquired infections.(5)This was followed by CONS (23.52%), *Klebsiella pneumoniae*(17.64%), *Enterococcus sp.*(17.64%), & *Streptococcus agalactiae* (2.94%).

In our study, in gram negative isolates were 100% sensitive to Piperacillin-tazobactam, Meropenem, Amikacin, Fosfomycin . This data was comparable to the data of *Prasanna et al* (5), *Sabharwal et al* (14), *Rao Mallikarjun et al* (15).The reason for this high sensitivity rate for these many antibiotics could be because of the fact that these patients were all from the community and therefore not exposed to highly resistant bacterial flora. The next most sensitive antibiotic was Nitrofurantoin (73.68%). Nitrofurantoin has been used for more than five decades for the treatment of uncomplicated cystitis and has been found to remain effective against both ESBLs and non-ESBL producers.(16,17) The drug has minimal side effects and can be safely used for the treatment of uncomplicated cystitis even during pregnancy.(16,18) The cephalosporins showed a comparatively less sensitivity ( 57.89% to 1<sup>st</sup> & 2<sup>nd</sup> generation & 63.15% to 3<sup>rd</sup> generation)to gram negative isolates . This could be due to overuse of cephalosporin in our community and this is of real concern because beta lactam group of antimicrobials are considered the traditional drugs safe in pregnancy. Therefore administration of cephalosporins empirically would not be advisable. Other oral drugs like Fluoroquinolones also showed less sensitivity ( 52.63%) to enterobacteriaceae due to overuse empirically. In our study the sensitivity for Fosfomycin was 100% and this antibiotic was found to be promising oral drug for treatment which should be reserved for resistant cases.

Among the gram positive isolates Coagulase negative *Staphylococcus* (CONS) was the most common isolate. Most of them(85%) were methicillin sensitive. Vancomycin, Teicoplanin, Linezolid and Nitrofurantoin were most sensitive drugs among gram positive cocci. Cephalosporins in CONS showed a pretty good sensitivity (87.5%) and this was a good finding as cephalosporins are considered to be safe in pregnancy. Fluoroquinolones showed less sensitivity to gram positive isolates.

**CONCLUSION:**

Since the ASB prevalence rate in our hospital was found to be 11.33%.

it is imperative to make it a routine practice to screen all pregnant women visiting the antenatal clinic for asymptomatic bacteriuria by doing a simple urine culture to prevent untoward complications both in mother & baby. In our study ASB was more commonly seen in primigravidae, during 3<sup>rd</sup> trimester, and in the younger age group (15-25 years). We found no association between ASB and age group, trimester, parity of women. *E.coli* was the commonest organism isolated like in the cases of symptomatic bacteriuria. Gram negative isolates showed high resistance to commonly used oral beta-lactams. Fosfomycin was only oral drug which reported 100% sensitivity, so it should be used judiciously to prevent drug resistance in near future. Meropenem & Nitrofurantoin also showed good sensitivity to gram negative bacteria.

**Source of Fund :** Nil

**Conflict of interest :** Nil

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