

# Prevalence of Asymptomatic Bacteriuria and Antimicrobial Resistance Profile among Pregnant Females in a Tertiary Care Hospital

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## Abstract

**Background and Objectives:** Urinary tract infection (UTI) is the most common clinical finding during pregnancy, and if it is asymptomatic bacteriuria (ASB), it can progress to pyelonephritis, leading to further complications. The present study aims to know the prevalence of ASB in pregnant females and the antimicrobial resistance pattern in our hospital setup. **Materials and Methods:** A total of 552 urine samples were collected from pregnant females (with no symptoms of UTI) both attending outpatient department and admitted in the wards of obstetrics and gynecology department. Urine culture was performed on blood agar, MacConkey agar, and UTI chromogenic agar. Antibiotic sensitivity test was done using Kirby-Bauer disc diffusion method, and the results were interpreted. **Results:** The prevalence rate of ASB in pregnant females was 17.4%. It was common in the age group of 25–33 years (60.4%). The infection rate was higher in the second trimester (43.7%) compared to the third (29.2%) and first (27.1%) trimester. Multiparity (60.4%) was a common finding in ASB during pregnancy. There was a significant finding of previous history of UTI (22.9%) and anemia (58.3%) associated with ASB in pregnant females. *Escherichia coli* (39.2%) was the most common microorganism isolated followed by *Staphylococcus aureus* (34.3%), *Enterococcus faecalis* (14.7%), *Klebsiella* (4.9%), coagulase-negative *Staphylococcus* spp. (2.9%), and *Citrobacter* and *Acinetobacter* (1.9%). Most sensitive drugs to be given in ASB during pregnancy were nitrofurantoin and fosfomycin. **Conclusion:** It was emphasized that urine culture should be done in early antenatal visit as routine screening to identify ASB in pregnant females as it can prevent fetal and maternal complications.

**Keywords:** Asymptomatic bacteriuria, *Escherichia coli*, urinary tract infection

## INTRODUCTION

Individuals of all age groups and genders are affected by urinary tract infection (UTI), but women are more susceptible than men, due to shorter urethra, easy fecal contamination of urinary tract, and various other reasons.<sup>[1]</sup> Asymptomatic bacteriuria (ASB) is the presence of actively multiplying bacteria in the urine of an individual without any symptoms of UTI. It is considered significant when bacteria are present in a quantity of  $\geq 10^5$  colony forming units (CFU)/ml.<sup>[2,3]</sup> ASB is more common in pregnant women because of factors such as stasis of urine, renal glycosuria, decreased immunity, and the effects of increased level of progesterone, which leads to relaxation of ureteric smooth muscles causing dilatation of ureters, aggravated due to pressure from the expanding uterus.<sup>[4,5]</sup> Various factors are known to be responsible for

ASB in pregnant females such as low socioeconomic status, multiparity, and personal hygiene.<sup>[6]</sup>

The prevalence of ASB ranges from 2% to 15% in the developing countries, while a lower prevalence of 2%–7% is seen in the developed countries.<sup>[5]</sup> However, some studies in India have also shown a higher prevalence rate of 17% and 25.3%.<sup>[7,8]</sup> The higher prevalence of ASB can also be attributed to illiteracy and a general lack of awareness and

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hygiene in the populations belonging to low socioeconomic group.

The most common microorganism causing ASB is *Escherichia coli* (80%–85%).<sup>[3,5,7,9,10]</sup> Other microorganisms causing ASB are *Klebsiella*, *Proteus*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus* (CoNS), and *Pseudomonas* spp.

The present study is conducted to know the prevalence of ASB in our hospital setup and the antimicrobial resistance profile which can help to start early treatment, thereby preventing maternal and fetal morbidity and mortality.

## MATERIALS AND METHODS

This is a cross-sectional study carried out in Bacteriology Laboratory in Microbiology Department on 552 pregnant females having no symptoms of UTI attending outpatient department (OPD) and inpatient department of obstetrics and gynecology in our hospital within the study duration of 6 months from June 1 to December 31, 2019.

### Inclusion criteria

1. Antenatal females attending OPD and admitted in the obstetrics ward with varying ages, gestational periods, and parity (both nulliparous and multiparous)
2. No history of increased frequency of micturition, dysuria, loin pain, and fever
3. No history of antibiotics within 1 month
4. Pregnancy-induced diabetes mellitus, hypertension, and anemia.

### Exclusion criteria

1. Nonpregnant females
2. Patients with vaginal discharge or bleeding
3. Patients with underlying chronic renal disease.

A total of 552 nonduplicate urine samples (mid-stream urine) were collected from the pregnant females, and culture was done by semi-quantitative analysis using standard loop technique on blood agar, MacConkey agar, and UTI chromogenic agar. A bacterial colony count of  $\geq 10^5$  CFUs/ml was identified as significant growth. Further identification of organisms was performed as per the standard protocol.<sup>[11]</sup> Antibiotic susceptibility testing was performed using Kirby-Bauer disc diffusion method on Muller-Hinton agar (HiMedia), and the results were interpreted using the Clinical Laboratory Standards Institute 2018 guidelines.<sup>[12]</sup>

### Ethical clearance

Patient's consent was taken on informed consent form. The

study was ethically approved by the institutional ethical committee.

### Statistical analysis

All the data were entered in an Excel Sheet and presented in tables and charts. The results were statistically analyzed using Advanced Excel Software. Chi-square test was performed considering  $P < 0.05$  as statistically significant.

### Observations

Out of 552 nonduplicate urine samples collected from pregnant females, 96 (17.4%) had significant bacteriuria ( $\geq 10^5$  CFU/ml). While 356 samples (64.5%) were sterile, 97 samples (17.6%) had insignificant growth ( $< 10^5$  CFU/ml), and 3 samples (0.5%) were contaminated. The proportion of pregnant women developing ASB was 0.173 (96/552).

Based on the demographic features, the highest proportion of ASB was seen in women between 25 and 33 years of age (60.4%, 58/96) followed by 18–24 years (33.3%, 32/96) and  $\geq 34$  years (6.2%, 6/96). Considering the period of gestation, the high proportion of ASB was found in the second trimester (43.7%, 42/96) followed by the third trimester (29.2%, 28/96) and first trimester (27.1%, 26/96). When compared among parity, the higher proportion of ASB was in multiparous females (60.4%, 58/96) compared to nulliparous (39.6%, 38/96).

As shown in Table 1, out of 96 females having significant ASB, 56 presented with anemia (58.3%) compared to non-ASB (47.4%). There is a positive association of anemia in ASB with odd's ratio (OR) = 1.55. Eighty-eight pregnant females had previous history of UTI, out of which 22.9% (22/96) presented with ASB showing positive association (OR = 1.75). The association of diabetes mellitus and hypertension could not be determined as no history of diabetes mellitus and hypertension was seen in 96 pregnant females with ASB. Among the pregnant females with no ASB, 18 cases of hypertension and 9 cases of diabetes mellitus were reported.

Out of the total samples collected, 92 patients had ASB among 440 samples from OPD and 4 had ASB among 112 samples from admitted patients in the obstetrics ward. Out of 96 urine samples collected, 102 isolates were found, out of which 90 samples had single isolate while six isolates showed mixed growth (3 *E. coli* and *Enterococcus*, 2 *E. coli* and *Staphylococcus*, and 1 *Staphylococcus* and *Enterococcus*). The total number of Gram-positive bacteria was 53/102 (51.9%) and Gram-negative was 49/102 (48%). *E. coli* was the most common causative organism isolated (39.2%), followed by

**Table 1: Disease association in asymptomatic bacteriuria pregnant females**

Disease associated	Significant growth (exposed) (n=96), n (%)	Nonsignificant (nonexposed) (n=456), n (%)	OR
Hypertension	0	18 (3.9)	NS
Anemia	56 (58.3)	216 (47.4)	1.5
Diabetes mellitus	0	9 (1.9)	NS
Previous history of UTI	22 (22.9)	66 (14.5)	1.7

NS: Non significant, OR: Odds ratio, UTI: Urinary tract infection

*S. aureus* (34.3%) and other microorganisms as shown in Table 2.

The antibiotic resistance profile for Gram-negative bacteria is shown in Table 3. It was found that *E. coli*, *Klebsiella*, and *Citrobacter* were completely sensitivity to fosfomycin. In *Acinetobacter*, norfloxacin, nitrofurantoin, and fosfomycin are

not tested. The antibiotic resistance profile for Gram-positive bacterial isolates is depicted in Table 4. All *S. aureus*, CoNS, and *Enterococcus faecalis* were completely sensitive to vancomycin and linezolid. High-level gentamicin and fosfomycin is used only for *Enterococcus*. Novobiocin is used in CoNS species to differentiate between *S. saprophyticus* and *S. epidermidis*.

**Table 2: Frequency of different bacterial isolates in the urine collected from pregnant females causing asymptomatic bacteriuria**

Microorganism	Frequency (n=102), n (%)
<i>Escherichia coli</i>	40 (39.2)
<i>Citrobacter</i> spp.	2 (1.9)
<i>Staphylococcus aureus</i>	35 (34.3)
<i>Enterococcus faecalis</i>	15 (14.7)
<i>Klebsiella</i> spp.	5 (4.9)
Coagulase-negative <i>Staphylococcus</i>	3 (2.9)
<i>Acinetobacter</i> spp.	2 (1.9)

## DISCUSSION

UTI is a globally prevalent disease with higher incidence in pregnant females, owing to decreased immunity and various physiological effects of increased progesterone levels.<sup>[4,5]</sup> In our study, the prevalence of ASB was 17.4% which is similar to a study conducted in Uttar Pradesh with a prevalence of 16.9%<sup>[13]</sup> and another study in Andhra Pradesh with a prevalence of 17%.<sup>[7]</sup> A study conducted in Odisha showed higher prevalence of 25.3%.<sup>[8]</sup> In Southern India, studies conducted in the past few years reported a prevalence rate between 11% and 14%.<sup>[5,6,14,15]</sup> Lower prevalence was seen in studies in Kolkata (8.4%) and

**Table 3: Antibiotic resistance profile for Gram-negative bacterial isolates**

Drugs	<i>Escherichia coli</i> (n=40), n (%)	<i>Citrobacter</i> (n=2), n (%)	<i>Klebsiella</i> (n=5), n (%)	<i>Acinetobacter</i> (n=2), n (%)
Ampicillin	19 (47.5)	2 (100)	5 (100)	2 (100)
Ceftriaxone	12 (30)	1 (50)	3 (60)	2 (100)
Ceftazidime	12 (30)	1 (50)	3 (60)	2 (100)
Cefepime	18 (45)	1 (50)	2 (40)	2 (100)
Aztreonam	22 (55)	2 (100)	5 (100)	2 (100)
Ticarcillin-clavulanate	20 (50)	2 (100)	3 (60)	2 (100)
Piperacillin-tazobactam	5 (12.5)	1 (50)	1 (20)	0
Gentamicin	9 (22.5)	1 (50)	2 (40)	1 (50)
Amikacin	14 (35)	0	1 (20)	1 (50)
Ciprofloxacin	16 (40)	1 (50)	4 (80)	2 (100)
Levofloxacin	10 (25)	0	2 (40)	1 (50)
Norfloxacin	20 (50)	1 (50)	4 (80)	-
Meropenem	6 (15)	0	2 (40)	0
Ertapenem	8 (20)	0	3 (60)	1 (50)
Imipenem	5 (12.5)	0	1 (20)	0
Tetracycline	15 (37.5)	0	1 (20)	0

*E. coli*: *Escherichia coli*

**Table 4: Antibiotic resistance profile for Gram-positive bacterial isolates**

Drugs	<i>Staphylococcus aureus</i> (n=35)	CoNS (n=3)	<i>Enterococcus</i> (n=15)
Penicillin	20 (57.1)	2 (66.7)	3 (20)
Ampicillin	13 (37.1)	2 (66.7)	2 (13.3)
Cefoxitin*	5 (14.3)	2 (66.7)	-
Ampicillin-sulbactam	2 (5.7)	2 (66.7)	0
Ciprofloxacin	20 (57.1)	2 (66.7)	8 (53.3)
Gentamicin	1 (2.8)	1 (33.3)	-
Norfloxacin	18 (51.4)	2 (66.7)	8 (53.3)
Levofloxacin	11 (31.4)	1 (33.3)	5 (33.3)
Teicoplanin	3 (8.6)	2 (66.7)	0
Nitrofurantoin	1 (2.9)	1 (33.3)	0
Tetracycline	8 (22.9)	1/3 (33.3)	1 (6.7)
High-level gentamicin**	-	-	1 (6.7)

\*Cefoxitin is used in *Staphylococcus* and CoNS, \*\*High-level gentamicin is used in *Enterococcus*. CoNS: Coagulase-negative staphylococci

Tamil Nadu (5%).<sup>[16,17]</sup> Since the majority of females attending our tertiary care hospital belong to the rural areas nearby, the high prevalence can be attributed to lack of awareness regarding maternal health and personal hygiene. Apart from India, the study conducted in Dhaka showed the prevalence of 26%.<sup>[18]</sup>

In our study, high proportion of ASB was found between 25 and 33 years of age (60.4%) which may be due to more number of pregnant females of this age group attended hospital. There are studies conducted which showed that higher infection rate of ASB was between 26 and 35 years of age.<sup>[6,7]</sup> Early marriages are common in our Indian society, especially in rural area, so females  $\geq 25$  years of age are usually multiparous which is an important risk factor for ASB.<sup>[6,7,10]</sup> The advancement of age is also a risk factor to acquire ASB in pregnancy as glycogen deposition and reduction in *Lactobacillus* occur in an aging process, which enhances bacterial adherence and invasion and make them more vulnerable.<sup>[6,7]</sup> Our study had reported high proportion of ASB in the second trimester (43.7%) which is in support with other studies.<sup>[5,6]</sup> Sujhata *et al.* showed higher infectivity ASB in the first trimester which may be due to the hormonal changes in early pregnancy.<sup>[19]</sup> However, some studies reported higher infection rate in the third trimester due to urine stasis in advancing gestational age compared to first and second trimester.<sup>[7,18]</sup> In the present study, higher proportion of ASB was observed in multiparous (60.4%) compared to nulliparous females (39.6%). There are studies suggesting that, in multiparous females, there is increased colonization of urinary tract by pathogenic organisms due to repeated exposure to urinary stasis or previous UTI.<sup>[3,6,7]</sup>

In our study, we have seen that the presence of anemia is a more frequent finding in pregnant females having ASB (58.3%) when compared to non-ASB (47.4%). Further, there is a positive association with OR = 1.55 (OR > 1) [Table 1]. Other studies have also shown association of ASB with anemia, but its etiopathogenesis in anemia cannot be determined as there are various factors responsible for anemia in pregnancy.<sup>[6,13]</sup> The presence of previous history of UTI in ASB is 22.9% compared to non-ASB (14.5%), reflecting positive association with OR = 1.75 (OR > 1). UTI is a recurrent infection; our findings support previous studies conducted.<sup>[14,15]</sup>

Different bacterial isolates are responsible for ASB in pregnant females. In our study, the most common microorganism causing ASB is *E. coli* (39.2%) as seen in most of the studies.<sup>[3,7,9]</sup> In pregnancy, it is difficult to maintain personal hygiene and easier fecal contamination of the urethra helps the motile bacteria to easily ascend into the urinary tract.<sup>[10,15]</sup> *S. aureus* (34.3%) was the second most common causative microorganism in our study, similar to a study reported.<sup>[5]</sup> However, another study reported *S. aureus* as the most common microorganism isolated.<sup>[2]</sup> Other microorganisms isolated in ASB were *E. faecalis* (14.7%), *Klebsiella* (4.9%), CoNS (2.9%), *Citrobacter* (1.9%), and *Acinetobacter* (1.9%), respectively, which is similar to the other studies conducted<sup>[8,15]</sup> [Table 2]. Mixed culture growth

was observed in six urine samples in our study. A similar result with two urine cultures containing mixed growth was reported in a previous study.<sup>[20]</sup>

Gram-negative isolates were highly sensitive to nitrofurantoin, meropenem, imipenem, piperacillin-tazobactam, and fosfomycin in our study, which is similar to other studies.<sup>[10,21-23]</sup> In *E. coli*, norfloxacin is 50% resistant similar to other studies.<sup>[24]</sup> Ampicillin is nearly 50% sensitive in *E. coli* similar to another study.<sup>[23]</sup> Most *E. coli* and *Klebsiella* isolates in our study were found nearly 80%–85% sensitive to nitrofurantoin which is concordance with other studies.<sup>[21,23]</sup> However, another study showed 100% sensitivity of nitrofurantoin to *E. coli* and *Klebsiella*<sup>[2]</sup> [Table 3].

All the Gram-positive isolates were 100% sensitive to vancomycin which is similar to other study conducted.<sup>[22,25]</sup> High sensitivity to gentamicin, nitrofurantoin, linezolid, and teicoplanin was seen in our study which is similar to other studies.<sup>[2,16,20,23]</sup> *S. aureus* showed nearly 50% resistance to penicillin, ciprofloxacin, and norfloxacin. *E. faecalis* ( $n = 15$ ) showed high sensitivity to penicillin, ampicillin-sulbactam, teicoplanin, and fosfomycin which is concordance with some previous studies conducted.<sup>[19,23,25]</sup> CoNS isolates were sensitive to novobiocin [Table 4].

Very limited drugs can be prescribed to pregnant females due to their adverse effects. Our study reports high sensitivity to drugs such as nitrofurantoin and fosfomycin which are safe to be used in pregnancy. Some studies have quoted nitrofurantoin/fosfomycin as the drug of choice in UTIs in pregnancy.<sup>[3,10,23]</sup> Further, ampicillin is a good oral drug in pregnancy.<sup>[3,4]</sup> Carbapenems are highly sensitive which can be used in extended-spectrum beta-lactamases-producing microorganism.<sup>[10]</sup>

Urine culture is considered the gold standard test identification of ASB in pregnant females.<sup>[5,10]</sup> It should be done early in pregnancy since ASB can occur as early as the 6<sup>th</sup> week of gestation and peaks around 22<sup>nd</sup>–24<sup>th</sup> weeks.<sup>[6,18,19]</sup> ASB occurs without any apparent symptoms of UTI, so it becomes important to detect any undiagnosed bacterial infection present in the urinary tract during pregnancy as it can progress to symptomatic bacteriuria, which further leads to maternal and fetal complications such as pyelonephritis, spontaneous abortion, anemia, preeclamptic toxemia, postpartum endometritis, maternal and neonatal sepsis, low birth weight (LBW), intrauterine growth retardation, premature preterm rupture of membrane, preterm labor, and higher fetal mortality rates.<sup>[3,6,7,10,13]</sup>

According to a study by the WHO for global burden of disease, LBW and perinatal causes are the leading causes of death and disability. Therefore, it is always better to screen and treat ASB during antenatal period to avoid further complications.<sup>[9]</sup> It will be the cost-effective interventions at primary healthcare for safe motherhood and newborn care in developing countries.<sup>[5,13]</sup> The present study is also useful in promoting awareness among



pregnant females regarding personal and environmental hygiene as counseling was done at the time of interview for sample collection.

## CONCLUSION

Through the present study, we wish to emphasize that urine culture testing should be implemented in the regular antenatal checkup in pregnant females for early diagnosis and treatment of ASB as it helps prevent its progression to symptomatic UTI, which can lead to maternal and fetal complications. Furthermore, the antibiotic resistance pattern of ASB can help to decide treatment profile that can be given to the pregnant females with ASB before the culture sensitivity report. There is a need for health education regarding awareness of personal and environmental hygiene during pregnancy which will reduce the risk of infection and comorbidities.

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