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Original Article

Microbiology Section

Asymptomatic Bacteriuria in Antenatal Cases: A Cross-sectional Study from Southern Rajasthan, India

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ABSTRACT

Introduction: Due to various anatomical and physiological changes during pregnancy, women are more prone to develop Urinary Tract Infections (UTI), which may progress to involve the upper urinary tract resulting in acute pyelonephritis, or may involve the lower tract resulting in acute cystitis.

Aim: To study Asymptomatic Bacteriuria (ASB) in antenatal cases and to isolate, identify, and establish the antimicrobial susceptibility pattern of the pathogens responsible for ASB.

Materials and Methods: This cross-sectional study was conducted from January 2020 to December 2020 on 363 antenatal cases attending Obstetrics Outpatient Department (OPD) in RNT Medical College, Udaipur, Rajasthan, India. The samples were processed within one hour of collection and tested (samples were cultured, and Gram's staining and wet mount were done) for the presence of significant ASB and the sensitivity pattern of the isolates. The statistical analysis of the variables was assessed using the odds ratio and a p-value <0.05 was considered significant.

Results: A total of 363 samples were evaluated; 44 samples showed significant bacteriuria in culture, with the most commonly isolated organism being *Enterococcus faecalis* 25 (56.8%), followed by *Escherichia coli* 14 (31.8%), *Klebsiella pneumoniae* 2 (4.5%), *Enterococcus faecium* 2 (4.5%), and *Pseudomonas* species 1 (2.3%). Gram-positive isolates were highly sensitive to amoxyclav, nitrofurantoin, vancomycin, linezolid, and imipenem, while showing reduced sensitivity to amoxicillin and nalidixic acid. Gram-negative isolates were highly sensitive to gentamicin, ceftaclav, cefoperazone-sulbactam, piperacillin-tazobactam, linezolid, vancomycin and imipenem and showed the least sensitivity to amoxicillin, cephalexin, and ceftriaxone.

Conclusion: To prevent serious complications in both the mother and the foetus, urine culture and antimicrobial sensitivity testing by Kirby-Bauer disk diffusion method in the first and second trimester in antenatal women should be done as a routine procedure for the early diagnosis of ASB.

Keywords: *Enterococcus* spp., Pregnant women, Urinary tract infection

INTRODUCTION

The UTI is the second most common microbial infection and affects about 10% of the population at some point in their lives [1]. UTIs are also the most prevalent infections associated with medical treatment. In comparison to the male urethra, the female urethra is shorter and is located close to the moist perirectal region, which harbours various microorganisms. Bacteria can easily access the bladder in the female host due to her narrower urethra, UTIs are more common in women [2]. The morphological and physiological changes that occur in the genitourinary tract during pregnancy further make pregnant women more susceptible to UTIs. Depending on the scenario, these infections can be symptomatic or asymptomatic [3]. ASB is defined as persistently and actively multiplying bacteria in significant numbers, i.e., $>10^5$ Colony Forming Unit (CFU)/mL in a culture of clean voided midstream urine of an antenatal woman without having fever or symptoms of UTI [4]. The majority of ASB patients does not have symptomatic UTIs and have no serious consequences. However, pregnant women's urinary tract undergoes structural and physiological changes, as well as alterations in their immune system, all of which raise the risk of ASB. In around 70% of cases, ASB is a substantial risk factor for UTIs in pregnant women [5]. ASB can cause acute pyelonephritis, postpartum UTI, hypertensive sickness, anaemia, preterm, low birth weight babies, and prenatal death in 2 to 10% of pregnant women if left untreated [1].

As a result, early detection and treatment are essential not only to avoid acute pyelonephritis and chronic renal failure in the mother but also to prevent premature birth and foetal mortality [6]. The aim was to study ASB in antenatal cases and to isolate, identify and establish the antimicrobial susceptibility pattern of the pathogens responsible for ASB.

MATERIALS AND METHODS

The present cross-sectional study was conducted at Rabindra Nath Tagore Medical College, Udaipur, Rajasthan, India over a period of 12 months from January 2020 to December 2020. Approval from the Institutional Ethics Committee (IEC) (RNT/Stat./IEC/2020/15) was obtained. A total of 363 asymptomatic antenatal female subjects in their first and second trimester, attending the OPD (Antenatal clinic) of Obstetrics at Rabindra Nath Tagore Medical College, Udaipur, were included in the study after obtaining written informed consent.

Inclusion criteria: All cases of ASB in the first and second trimesters attending OPD of Obstetrics and Gynaecology were included in the study.

Exclusion criteria: Participants with symptoms suggestive of a UTI, such as dysuria, frequency, and urgency, those with a history of antibiotic therapy in the previous two weeks, a history of fever, pregnancy-induced hypertension, diabetes mellitus, known congenital anomalies of the urinary tract, and antenatal cases in their third trimester, were excluded from the study.

Sample size calculation:

$n=z^{2*}p\hat{}(1-p\hat{})/\varepsilon^2$

Where n represents the sample size, z denotes the z-score, p^ represents the population proportion, and ε is the margin of error (confidence interval). A previous study by Vaghela HG and Ahir HR

showed that the proportion of patients with ASB was 11.97% [7]. Based on that, for the present study to have an acceptable error of 2.5% with a power of 80% and a type I error of <0.05, 331 patients were required. A total of 363 patients were recruited to compensate for possible dropouts and losses to follow-up.

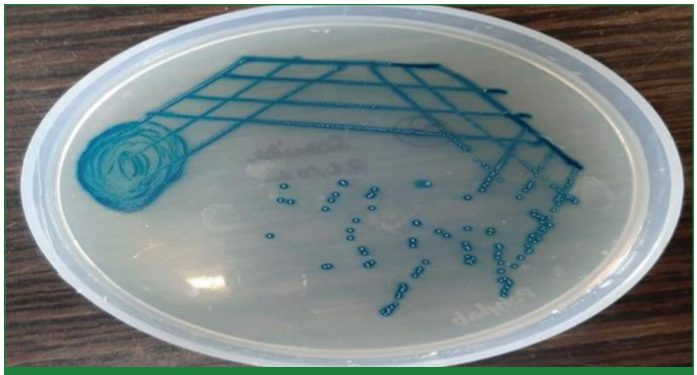
At the time of enrollment, detailed history was taken from the cases according to the predesigned form. Patients were advised to collect a "clean catch" mid-stream urine sample in a sterile, wide-mouthed container with a tight-fitting lid. The urine samples were processed using normal microbiological techniques (wet mount, Gram staining, culture, and antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method) within one hour of collection. Direct microscopy and Gram staining of the sample were performed, and subsequently, semiquantitative culture was conducted using a 1 µL calibrated loop (Nichrome loop-HiMedia, 1.45 mm ID and 70 mm length; 26 G; Multiplication factor is 1000) on Blood agar [Table/Fig-1], Hi-Chrome UTI agar, and Mac-Conkey agar. The cultures were then incubated at 37ºC for 24 hours.

[Table/Fig-1]: Shows colonies on blood agar plate by semiquantitative method.

A microscopic examination of a wet film of uncentrifuged urine was performed to look for pus cells, erythrocytes, microorganisms, casts, and other elements in the sample. Significant pyuria is indicated by the presence of one leukocyte per seven high-power fields [4]. Gram staining was done directly from the sample as well as from the culture to identify gram-positive or gram-negative bacteria in the sample. The presence of one bacterium per oil immersion field is significant and indicates a colony count of $\geq 10^5$ CFU/mL [2]. Pure isolated colony counts of $\geq 10^5$ CFU/mL were considered significant. The identification of the organism was done by colony characteristics and colour on McConkey and Hi-Chrome agar by *Escherichia coli* pink-purple [Table/Fig-2], *Enterococcus faecalis* blue small [Table/Fig-3], *Klebsiella pneumoniae* blue mucoid, *Pseudomonas aeruginosa* colourless with a diffusible greenish pigment, and through biochemical reactions, carbohydrate fermentation tests, and amino acid de-carboxylation tests.

[Table/Fig-2]: Shows lactose fermented flat colonies of *Escherichia coli* on Mac-Conkey agar and purple colour colonies on Hi-Chrome agar.

The antibiotics susceptibility testing was done using the conventional Kirby-Bauer disc diffusion method and was interpreted using the CLSI Guidelines M100, 30th Edition 2020 [8]. The antibiotics tested will be amikacin (30 µg), gentamicin (10 µg), cephalexin (30 µg),



[Table/Fig-3]: Shows blue small colonies of *Enterococcus* sp. on chrome agar.

cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ceftazidime-clavulanic acid (30/10 µg), cefoxitin (30 µg), erythromycin (15 µg), amoxicillin (10 µg), ampicillin (10 µg), amoxicillin-clavulanic acid (30 µg), norfloxacin (10 µg), co-trimoxazole (25 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), meropenem (10 µg), piperacillin-tazobactam (100/10 µg), linezolid (30 µg), vancomycin (30 µg), Cefoperazone-sulbactam (75/30 µg).

STATISTICAL ANALYSIS

The data were entered into Microsoft Excel and interpreted. All frequencies were represented as absolute numbers and proportions. The central tendencies were represented as mean±SD. The statistical significance of the variables was assessed using p-value, odds ratio, and 95% confidence interval. A p-value of <0.05 was considered statistically significant. The performance evaluation of the test used in the study was assessed using sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV).

RESULTS

Among the 363 cases, 44 (12.12%) were culture positive with significant bacteriuria ($\geq 10^5$ CFU/mL), 77 (21.21%) were culture positive with an insignificant colony count ($<10^5$ CFU/mL), and 242 (66.67%) samples were sterile.

The age range of culture-positive cases was 18 to 36 years, and the mean age was 25.88±4.50 years [Table/Fig-4].

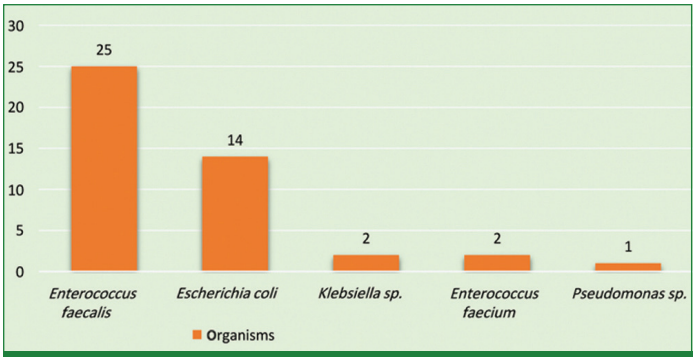
Age group (in years)	n (%)
<20	7 (15.9)
21-30	31 (70.5)
31-40	6 (13.6)

[Table/Fig-4]: Shows prevalence of ASB in different age groups.

Of the 44 culture-positive cases with significant bacteriuria, 28 (63.6%) in 2nd trimester and 16 (36.4%) in 1st trimester. In the present study, primigravida accounted for the highest number of cases, with 26 (59.1%), followed by primipara who accounted for the second-highest number of cases, with 11 (25%). Multipara accounted for 06 (13.6%) cases, and grand multipara accounted for 01 (2.3%) case.

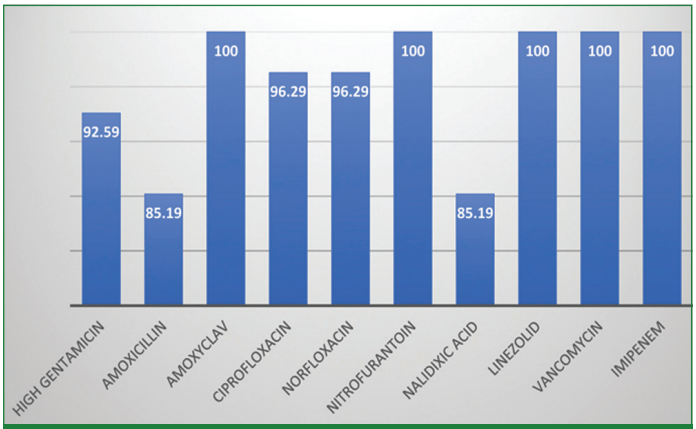
Further, among these 44 cases, 34 (77.3%) women residing in rural areas and 10 (22.7%) women residing in urban areas had ASB. Upon performing microscopy on these 44 samples, it was observed that significant pyuria was present in 38 (86.36%) samples in the wet mount examination; while 42 (95.45%) samples showed significant bacteriuria in Gram's staining. In the current study, it was noted that based on statistical analysis, Gram staining (Sensitivity- 90.9%, Specificity- 93.9%, PPV-95.2%, and NPV-88.5%) of urine samples was more accurate than wet mount examination (Sensitivity- 68.18%, Specificity- 75.75%, PPV-78.9%, and NPV- 64.1%) in comparison to the gold standard culture method. The p-value was 0.035; the odds ratio was 24.2, with a 95% confidence interval of 1.23 to 472.32.

In the present study, *Enterococcus faecalis* was the predominant species isolated, followed by others as shown [Table/Fig-5].



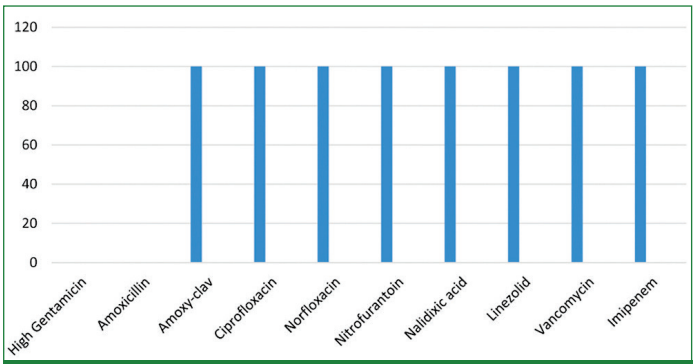
[Table/Fig-5]: Various organisms isolated from urine specimens.

In the current study, all isolates of *Enterococcus faecalis* were highly sensitive to most drugs such as amoxyclav, nitrofurantoin, vancomycin, linezolid, and imipenem [Table/Fig-6].

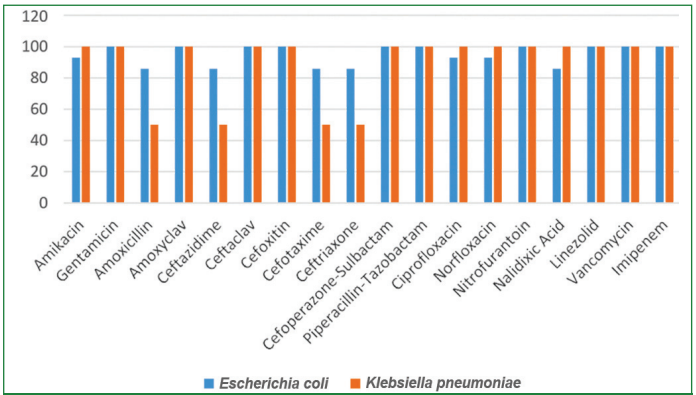


[Table/Fig-6]: Antibiotic susceptibility pattern of *Enterococcus faecalis*.

In the present study, two isolates of *Enterococcus faecium* were resistant to amoxicillin and high gentamicin and sensitive to amoxyclav, ciprofloxacin, norfloxacin, nitrofurantoin, nalidixic acid, linezolid, vancomycin, and imipenem [Table/Fig-7]. Similarly, the antibiotic susceptibility patterns of *Escherichia coli* and *Klebsiella pneumoniae* are shown in [Table/Fig-8].

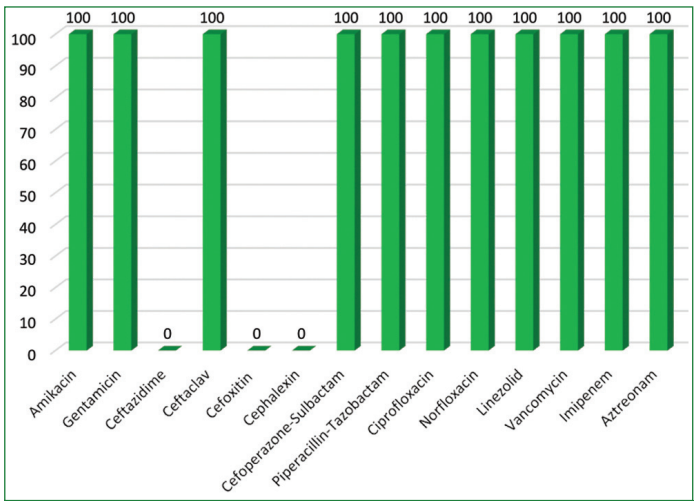


[Table/Fig-7]: Antibiotic susceptibility pattern of *Enterococcus faecium*.



[Table/Fig-8]: Antibiotic susceptibility pattern of *Escherichia coli* and *Klebsiella pneumoniae*.

The susceptibility pattern of the *Pseudomonas* spp, which was isolated in only one patient, was resistant to ceftazidime, cefoxitin, and cephalexin, and sensitive to the rest of the antibiotics [Table/Fig-9].



[Table/Fig-9]: Antibiotic susceptibility pattern of *Pseudomonas* sp.

DISCUSSION

Out of 363 samples, 44 (12.12%) were found to be culture positive with significant bacteriuria, with a colony count of $\geq 10^5$ CFU/mL, while the rest were either sterile (66.67%, n=242) or had an insignificant colony count (21.21%, n=77). These results were in agreement with those of Muthuprabha P and Ramalakshmi S [9] and Vaghela HG and Ahir HR [7], who reported a culture-positive prevalence rate of 10.8% and 11.97%, respectively. On the other hand, the prevalence was quite high in studies conducted by Sonkar N et al., [5] (16.7%) in Lucknow, Akpan NG et al., (19%) in Nigeria [10], and Tadesse S et al., (21.2%) in Ethiopia [11]. These differences may be attributable to factors such as demographic variation in the study population, social habits within the community, and health practices in the population. The different levels of ASB across different states within the country and different countries might be due to differences in related factors, such as the sample size, geographical differences, social habits prevalent in the community, and health-related practices. This study was done to find out the occurrence of significant bacteriuria in antenatal cases so that timely intervention can be done to prevent further complications. Similar to studies done by Sujatha R and Nawani M in Kanpur and by Chandel LR et al., in Shimla, the majority (70%) of culture-positive cases in pregnant women were in the age range of 21 to 30 years [12,13]. In a study conducted by Imade PE et al., in Nigeria, the maximum numbers of patients with ASB were found in the age group of 26-30 years, followed by patients aged 36 to 40 years [14]. This finding was in accordance with the current study in which the maximum numbers of patients were in the age group 21-30 years.

Similar findings were found in another study by Khan S et al., in Nepal, where 64% of patients were in the age group 21-30 years [15]. In the study conducted by Turpin CA et al., in Ghana, women in the age group of 35-39 years accounted for the highest number of ASB cases [16]. In this study, the prevalence of significant bacteriuria in antenatal cases was higher among women residing in rural areas (77.3%). This could have been due to the unavailability of constructed toilets in rural areas. Similar results were found in a study conducted by Tadesse S et al., in Ethiopia in which the highest number of cases were residing in rural areas (70.8%) [11].

Out of the 44 culture-positive cases, 42 (95.45%) had significant bacteriuria in Gram staining, while 38 (86.36%) had significant pyuria in wet mount examination. In the current study, it was observed that Gram staining of urine samples was found to be more accurate than wet mount examination of the urine samples in

relation to the culture method [7]. Similar findings were observed in another study by Bose AM et al., in Kerala, where they found that the sensitivity and specificity of Gram staining were higher than wet mount [17].

In this study, the most common organism isolated was *Enterococcus faecalis* 25 (56.8%), followed by *Escherichia coli* 14 (31.8%), *Klebsiella pneumoniae* 2 (4.5%), and *Pseudomonas* species 1 (2%). This was consistent with previous studies where the most commonly isolated organism was *Enterococcus* species, followed by *Proteus mirabilis* and *Escherichia coli* [18,19]. However, a study conducted in Telangana also found that *Enterococcus faecalis* (42.1%) and *Escherichia coli* (42.1%) were the most commonly isolated organisms, followed by *Staphylococcus aureus* and *Klebsiella* species [20].

The isolates of *Enterococcus* species were highly sensitive to most drugs such as amoxyclav, nitrofurantoin, vancomycin, linezolid, and imipenem, while showing reduced sensitivity to amoxicillin and nalidixic acid. The majority of *Escherichia coli* isolates were sensitive (100%) to gentamicin, ceftaclav, cefoperazone-sulbactam, piperacillin-tazobactam, linezolid, vancomycin, and imipenem, and showed the least sensitivity to amoxicillin, cephalexin, and ceftriaxone. The isolates of *Klebsiella pneumoniae* are highly sensitive to the majority of drugs and least sensitive to only a few drugs like amoxicillin, cephalexin, and ceftriaxone. The single isolate of *Pseudomonas* species was sensitive to most drugs such as amikacin, gentamicin, and resistant to ceftazidime, cefoxitin, and cephalexin. Various studies documented in the literature have explored sensitivity patterns among isolates causing ASB in pregnancy [20-23]. In a study by Radha S et al., it was found that the majority of isolates causing ASB were sensitive to nitrofurantoin [20]. Similar findings regarding the sensitivity of nitrofurantoin were also reported in previous studies [21-23], which align with the current study. Sonkar N et al., found that *Escherichia coli* was the most common Gram-negative organism isolated, while Coagulase-negative Staphylococci and *Staphylococcus aureus* were the most common Gram-positive organisms isolated [5]. Effective antibiotics against Gram-negative bacteria included piperacillin-tazobactam, cefepime, nitrofurantoin, and meropenem. For Gram-positive bacteria, ampicillin, vancomycin, linezolid, and nitrofurantoin were effective. In a study conducted by Imade PE et al., it was revealed that *Escherichia coli* (27.1%) and *Staphylococcus aureus* (24.4%) were commonly isolated among the isolates causing ASB in pregnancy [14]. Antibiotic susceptibility testing was performed for all isolates using ampicillin, cloxacillin, erythromycin, nitrofurantoin, gentamicin, amoxyclav, ceftriaxone, and ciprofloxacin. The isolates showed maximum sensitivity to ciprofloxacin, ceftriaxone, amoxyclav, gentamicin, and nitrofurantoin [1,6].

Women in their second trimester accounted for the highest number of culture-positive cases (63.6%), followed by women in their first trimester (36.4%). This finding was consistent with studies conducted by Arumugam V et al., in Tamil Nadu [23], Mukherjee M et al., in Kolkata [24], and Imade PE et al., in Nigeria [14], where the prevalence was higher in the second trimester.

Of the 44 positive cases with significant bacteriuria, primigravida accounted for the highest number of cases (59.1%), followed by the second para, who accounted for the second-highest number of cases (25%), followed by multipara (13.6%), and grand multipara (2.3%). In studies conducted by Muthuprabha P et al., in Tamil Nadu, Bose AM et al., and Mukherjee M et al., in Kolkata, the prevalence of ASB was higher in primigravida compared to multipara, which was consistent with the present study [9,17,24].

Limitation(s)

This study specifically addresses a population that is predominantly rural; hence, the findings apply to a group that may not necessarily have easy access to quality healthcare. The patient population consisted of patients attending the hospital; hence, it may not be completely representative of all pregnant women. Therefore, the external validity needs to be re-evaluated in a further study.

CONCLUSION(S)

The prevalence of ASB in pregnant women was found to be 12.12%. Therefore, there is a necessity to maintain strict guidelines that would implement urine culture as a routine part of check-ups for pregnant females during their antenatal visits. This is essential to prevent serious complications for both the mother and the foetus.

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