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Antibiotic susceptibility profile of uropathogens in pregnant women with asymptomatic bacteriuria in tertiary care hospital: a cross-sectional study

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ABSTRACT

Urinary tract infections (UTIs) are common during pregnancy due to physiological and anatomical changes that predispose women to infections. One such condition, asymptomatic bacteriuria (ASB), if left undiagnosed and untreated, can lead to serious maternal complications such as pyelonephritis, postpartum UTI, and hypertensive disorders, as well as neonatal complications including preterm birth, low birth weight, and intrauterine growth restriction. This study aimed to determine the prevalence of ASB, identify the major uropathogens, and analyze their antibiotic susceptibility patterns in pregnant women, to guide effective antenatal care and treatment. This cross-sectional study was conducted on 100 midstream urine samples, which were cultured using standard microbiological techniques. The bacterial isolates obtained were identified, and their antibiotic susceptibility was determined following standard guidelines. Out of 100 samples, 14 (14%) were positive for significant bacteriuria. The most common isolates were *Staphylococcus aureus* (42.8%), followed by *Escherichia coli* (28.6%) and *Klebsiella* species (28.6%). ASB was most prevalent in women aged 21–30 years (64.3%), during the first trimester (64.2%), and among multigravida women (57.2%). The isolated organisms showed good susceptibility to Ceftazidime/Clavulanic acid, Ciprofloxacin, Vancomycin, Amikacin, Piperacillin–Tazobactam, Imipenem, Teicoplanin, and Linezolid. Early detection and treatment can significantly reduce adverse outcomes, making bacteriuria screening an essential part of routine antenatal care.

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1. INTRODUCTION

Urinary tract infections (UTIs) are more common in pregnancy due to physiological [1] and morphological changes. Progesterone-induced smooth muscle relaxation leads to ureteral dilatation, while mechanical compression of the lower ureters by the gravid uterus and vesicoureteral reflux contribute to urinary stasis, increasing the risk of infection. Immunological adaptations during pregnancy further compromise host defenses, making antenatal women more susceptible to UTIs [2].

Asymptomatic bacteriuria (ASB) is defined as significant bacteriuria ($\geq 10^5$ CFU/mL) in two consecutive clean-catch voided urine specimens yielding the same uropathogen, in the absence of symptoms such as dysuria, urgency, or frequency [3]. Though clinically silent, ASB can result in maternal complications, including pyelonephritis, postpartum UTI, and hypertensive disorders, and neonatal complications such as prematurity, low birth weight, intrauterine growth retardation, and perinatal death [4], [5].

Gram-negative bacteria are responsible for more than 85% of UTIs, predominantly *Escherichia coli*, originating from intestinal flora. Other uropathogens include Proteus, *Klebsiella*, Enterobacter, Streptococcus faecalis, and Staphylococcus species [4]. The prevalence of ASB in pregnancy ranges between 2% and 11% [6], and if untreated, 20%–40% of cases may progress to symptomatic infection such as pyelonephritis [7]. UTIs are therefore a significant cause of maternal morbidity with implications for both mother and fetus [8], [9].

Although maternal UTI rarely leads to direct fetal bloodstream infection, adverse fetal outcomes can occur through mechanisms such as uterine hypoperfusion, maternal dehydration, anemia, or bacterial endotoxin-mediated placental vascular damage, potentially resulting in fetal cerebral hypoperfusion [10]. UTIs are particularly common during the first 22 weeks of pregnancy, and untreated infections in this period have been linked to intrauterine growth restriction, preeclampsia, preterm delivery, and increased cesarean section rates [11], [12]. Both symptomatic and asymptomatic UTIs have been associated with adverse obstetric and neonatal outcomes, including stillbirth, sepsis, maternal anemia, amnionitis, and neonatal death, emphasizing the need for early diagnosis and treatment [13].

Several risk factors increase susceptibility to UTI during pregnancy, such as low socioeconomic status, diabetes mellitus, recurrent infections, early onset of UTI (<15 years), and structural abnormalities of the urogenital system [14], [15]. ASB is more commonly reported in parous women, and those from lower socioeconomic backgrounds, and women with sickle cell trait are also at higher risk [16]. Hence, screening and treatment of ASB should form an essential component of routine antenatal care, supported by health education, personal hygiene promotion, and nutritional counseling. Considering its asymptomatic nature and possible complications, this study aimed to determine the prevalence of ASB among pregnant women, identify the primary causative organisms, and evaluate their antibiotic susceptibility patterns.

2. METHOD

After obtaining the ethical clearance from the institutional ethical committee, a total of 100 midstream clean-catch urine samples were collected from asymptomatic antenatal women, aged 18 to 45 years, attending the Outpatient Department of Obstetrics and Gynecology at the District Hospital, Chamarajanagar, during a study period of two months (June and July 2023). Informed written consent was obtained from all participants after clearly explaining the purpose and procedure of the study. Relevant clinical and obstetric history was recorded for each participant.

2.1. Study design and sampling technique

This study employed a cross-sectional observational research design. The data collected were entered into and analyzed using Microsoft Word and Microsoft Excel. Descriptive statistics such as frequency, percentage, and mean were used for analysis and presentation of findings. Purposive sampling: Approximately 20 mL of midstream clean-catch urine was collected from each participant using a sterile universal container. The standard urine collection protocol was followed to minimize contamination. All specimens were appropriately labeled and transported to the Microbiology Laboratory within one hour of collection. If immediate processing was not feasible, samples were refrigerated at 4 °C for up to 24 hours to maintain their integrity. Each urine sample was initially subjected to macroscopic examination to assess color, turbidity, and the presence of deposits. Samples were then processed for urine culture using standard microbiological procedures to detect significant bacteriuria and to identify the uropathogens involved.

2.2. Inclusion criteria

- Pregnant women aged 18–45 years attending the antenatal clinic at any gestational age, without symptoms of urinary tract infection (e.g., dysuria, loin pain, or increased frequency of micturition).
- Participants not treated with antibiotics (oral, parenteral, or local) in the preceding one month before sample collection.

2.3. Exclusion criteria

- Patients presenting with vaginal discharge or per vaginal bleeding.
- Pregnant women with pre-existing medical conditions such as diabetes mellitus, hypertension, or congenital anomalies.

2.4. Screening procedures

A drop of well-mixed uncentrifuged urine was placed on a clean glass slide, air-dried, heat-fixed, and then Gram-stained for microscopic examination under oil immersion. The presence of at least one organism per oil immersion field (observed across 20 fields) was considered indicative of significant bacteriuria. For culture, urine specimens were inoculated using the standard loop technique on Cysteine Lactose Electrolyte Deficient (CLED) agar, blood agar, and MacConkey agar. A calibrated loop with a 3 mm internal diameter, capable of delivering 0.001 mL of urine, was used to ensure quantitative analysis.

The inoculated plates were incubated at 37 °C for 18–24 hours. Following incubation, colony counts were performed, and the bacterial concentration was calculated by multiplying the number of colonies by 1,000, expressing the result in colony-forming units per milliliter (CFU/mL). A urine sample was considered to show significant bacteriuria if the colony count exceeded 10^5 CFU/mL. Isolated colonies were further identified based on colony morphology, cultural characteristics, Gram staining, and a series of biochemical tests to confirm the bacterial species. The antimicrobial susceptibility of each isolate was tested using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, and results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [17].

The antibiotics were used in the antibiotic susceptibility testing were, Amikacin (AMK) (30 mcg), Ampicillin (AMP) (30 mcg), Ceftazidime (CAZ) (30 mcg), Ceftazidime/Clavulanic acid (CAZ/CLA) (30 mcg), Cephotaxime (CTX) (30 mcg), Cotrimoxazole (SXT) (30 mcg), Ciprofloxacin (CIP) (30 mcg), Imipenam (IPM) (10 mcg), Piperacillin/Tazobactam (TZP) (30 mcg), Tetracycline (TCY) (30 mcg), Levofloxacin (LE) (30 mcg), Cefixime (CFM) (30 mcg), Clindamycin (CLI) (30 mcg), Erythromycin (ERY) (30 mcg), Nitrofurantoin (NF) (30 mcg), Norfloxacin (NX) (30 mcg), Teicoplanin (TEC) (15 mcg), Vancomycin (VAN) (30 mcg), Linezolid (LNZ) (30 mcg), and Amoxyclav(AMC) (30 mcg).

3. RESULTS

Among the 100 urine specimens collected from asymptomatic pregnant women, 14 samples (14%) were positive for significant bacteriuria, indicating the presence of asymptomatic bacteriuria (ASB). The remaining 86 samples (86%) were sterile and showed no growth. Among the 14 uropathogens isolated, *Staphylococcus aureus* was the most frequently identified organism, accounting for 6 cases (42.8%), followed by *Escherichia coli* and *Klebsiella* species, each with 4 isolates (28.6%), shown in Figure 1.

Trimester-wise distribution of ASB revealed that most cases occurred in the first trimester, with 9 women (64.2%) affected. This was followed by 3 cases (21.6%) in the third trimester and 2 cases (14.2%) in the second trimester. Gravida status analysis showed that ASB was more prevalent among multigravida women, comprising 8 out of 14 cases (57.2%), while the remaining 6 cases (42.8%) were in primigravida women. Age-wise distribution indicated that 5 women (35.7%) with ASB were between 18–20 years of age, whereas the majority, 9 women (64.3%), were between 21–30 years old.

Antibiotic susceptibility pattern shown in Figures 2-4. *Staphylococcus aureus* isolates were uniformly sensitive to Amikacin, Vancomycin, and Linezolid, with moderate resistance to fluoroquinolones and macrolides, and higher resistance (50%) to Cefixime. *E. coli* showed complete sensitivity to Amikacin, Imipenem, Piperacillin/Tazobactam, and Nitrofurantoin, but high resistance to Ceftazidime and Amoxicillin-Clavulanate (75%). *Klebsiella* species were fully sensitive to most antibiotics except for complete resistance to Ampicillin and moderate resistance to Amoxicillin-Clavulanate. Overall, Amikacin and Imipenem were the most consistently effective agents across isolates.

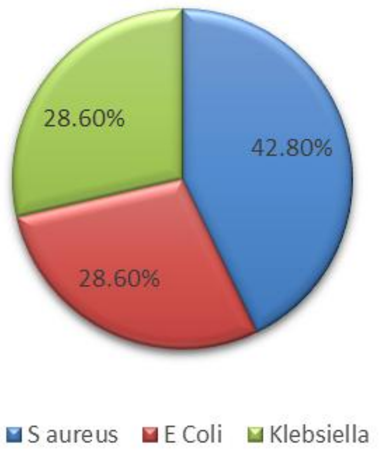

Figure 1. Prevalence of uropathogens causing asymptomatic bacteriuria

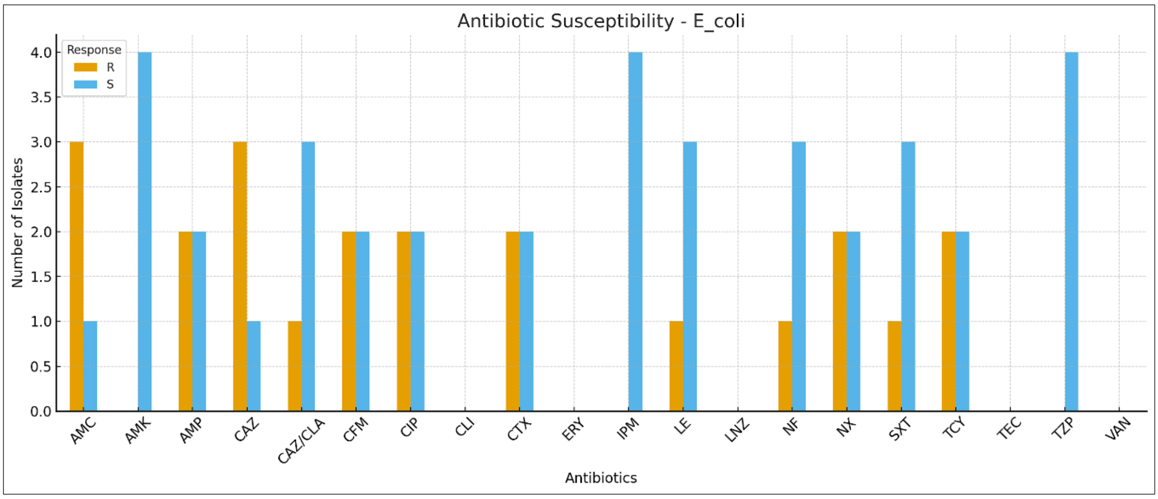

Figure 2. Antibiotic susceptibility pattern of *E. coli*

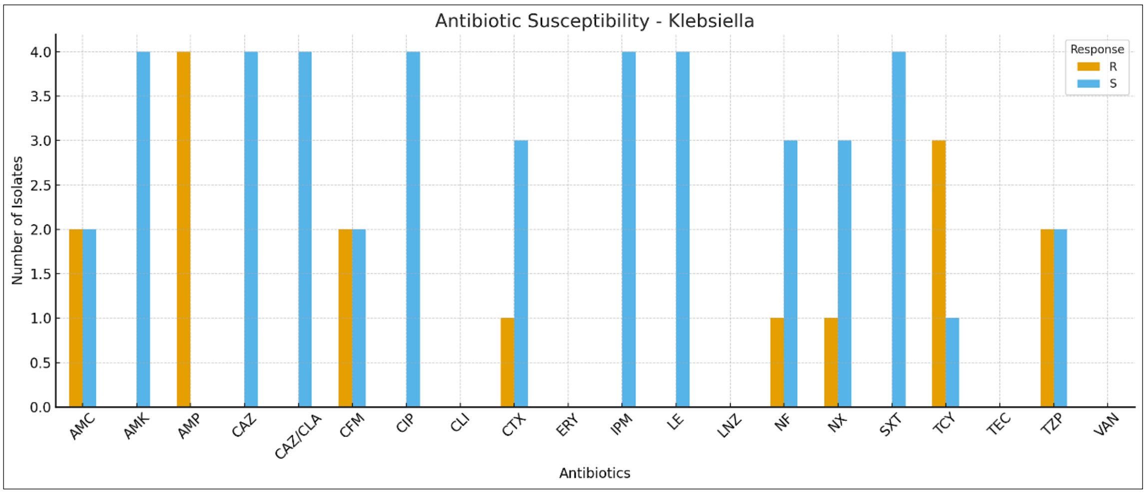

Figure 3. Antibiotic susceptibility pattern of *Klebsiella* species

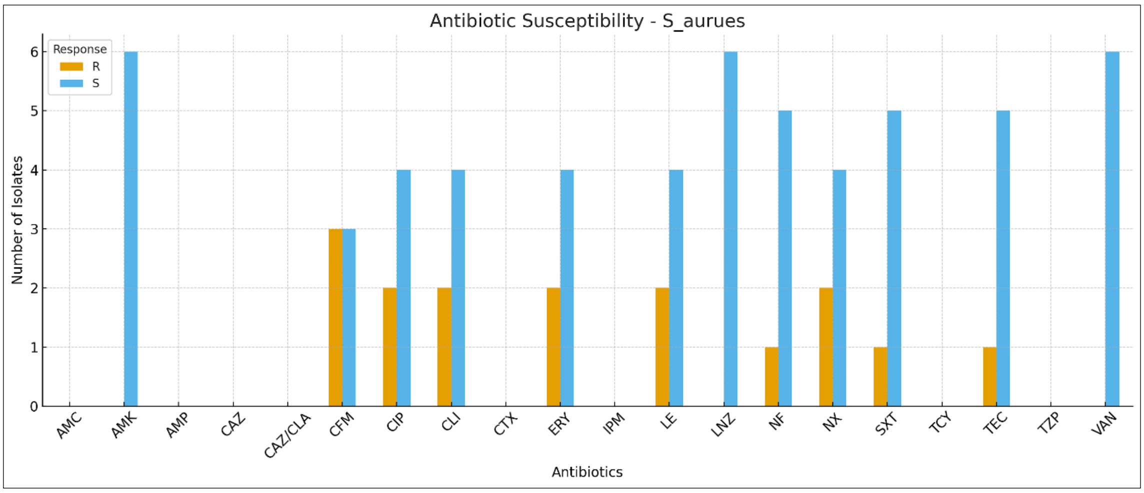

Figure 4. Antibiotic susceptibility pattern of *S. Aureus*

4. DISCUSSION

4.1. Prevalence of asymptomatic bacteriuria

In the present study, ASB was identified in 14% of pregnant women. This prevalence is comparable to that reported by Prasanna *et al.* (17%) [18] and another study documenting 19% [19]. Lower prevalence rates have been documented in several studies, including Mukherjee *et al.* (8.4%) [7], Jambhule and Dey (8.28%) [20], Alemu *et al.* (10.4%) [21], Gowda and Rajini (10.8%) [6], Shubhasini and Vani [22], Talukdar *et al.* (10%) [23], and as low as 4.68% (26 cases) in another report [24].

Conversely, higher prevalence has been reported in other studies, with rates as high as 24.7% [25] and 31% [26]. The relatively higher prevalence observed in the present study may be attributed to multiple factors, including poor personal hygiene, low socioeconomic status, inadequate genital hygiene, certain sexual practices, and unfavorable environmental conditions. These factors are well-recognized contributors to increased susceptibility to urinary tract colonization and subsequent ASB.

4.2. Etiological agents and shifting trends

The current study identified *Staphylococcus aureus* as the predominant uropathogen, followed by *Escherichia coli* and *Klebsiella* species. Similar observations were made by Jojan *et al.* [8]. However, most earlier studies consistently reported *E. coli* as the most common causative organism in pregnancy-related UTIs [4], [8], [18], [21], [27]. This indicates a possible shift in etiological patterns, with *S. aureus* emerging as a significant uropathogen alongside *E. coli*. This changing trend emphasizes the need for continuous local epidemiological surveillance to guide empirical treatment and promote antibiotic stewardship in antenatal care.

4.3. Trimester-wise distribution

ASB was most prevalent in the first trimester (64.2%), followed by the third trimester (21.6%) and the second trimester (14.2%). Prasanna *et al.* [18] reported the highest prevalence in the third trimester, followed by the first and second. Kalaivani *et al.* [4] also documented predominance in the third trimester. Gowda *et al.* [6] reported the highest prevalence in the second trimester (37%), followed by the third (33%) and first (30%). The variation in trimester-wise prevalence may be explained by physiological changes occurring early in pregnancy. The risk of UTIs increases from the first six weeks of gestation and peaks between 22–24 weeks due to progesterone-induced smooth muscle relaxation, vesicoureteral reflux, and mechanical compression of the urinary tract [4], [8].

4.4. Gravidity-wise distribution

This study found ASB to be more common in multigravida women, a finding consistent with Prasanna *et al.* [18]. However, Gowda and Rajini [6] reported a higher prevalence in primigravidae (67%) compared to multigravidae (33%). Parity-wise distribution in another study showed the highest prevalence among nulliparous women (57.69%), followed by para 1 (30.77%), para 2 (7.69%), and para 3 (3.85%) [24]. The highest proportion of women with UTI in another study was in the third trimester of pregnancy (47.54%), with multigravida (72.31%) and multiparity (50.82%) [28].

The higher incidence of ASB among multigravida may be attributed to repeated hormonal changes and cumulative alterations in the urinary tract from previous pregnancies. It may also be related to changes in genital tract flora. Additionally, the increasing uterine size and hormonal milieu as pregnancy advances create a more favorable environment for microbial colonization [8].

4.5. Age-wise distribution

In the present study, ASB was most frequently observed among women aged 21–30 years (64.29%), followed by those aged 18–20 years (35.71%). These findings are consistent with the reports of Kalaivani *et al.* [4] and Jojan *et al.* [8], who also documented a higher incidence of ASB among women in the active reproductive age group. Similarly, Dhanya *et al.* [26] reported that 55.8% of cases occurred in women aged 18–25 years, 32.6% in the 26–35 year group, and 11.6% in women over 35 years. In another study, the highest proportion of pregnant women with UTI belonged to the 20–25 year age group (42.62%), with the majority being homemakers (55.74%), illiterate (69.67%), and residing in rural areas (67.21%) [28]. Likewise, another study documented the highest prevalence in the 18–25 year age group (63%) [19].

A related study found most culture-positive pregnant women were aged 26–30 years (48%), followed by 21–25 years (30%). A significant proportion belonged to low socioeconomic backgrounds (78%) and were illiterate (49%), with 74% and 67% of culture-positive cases occurring in these respective groups [6], [24]. Another study reported participant ages ranging from 17 to 39 years, with a mean age of 25.8 years. Most were in the 20–24 year age group (43.9%), and their educational levels varied widely [5]. Although the present study noted higher ASB prevalence in younger women, advancing maternal age has also been reported as a risk factor. The age-related decline in glycogen deposition and reduced vaginal lactobacillus colonization are believed to increase bacterial adherence and pathogen invasion, thereby enhancing susceptibility to UTIs [18].

4.6. Microbiological profile of isolates

In this study, *Staphylococcus aureus* accounted for 42.8% of isolates. *Escherichia coli* and *Klebsiella* species each accounted for 28.6%. In other reports, *E. coli* was the most frequent isolate (47.8%), followed by *Klebsiella* pneumoniae (17.4%), *Staphylococcus aureus* (8.7%), and other pathogens such as *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Citrobacter koseri*, *Enterococcus faecalis*, and *Candida tropicalis* [24], [29].

4.7. Antibiotic susceptibility patterns

Staphylococcus aurei are highly susceptible: Amikacin, Teicoplanin, Linezolid, Cotrimoxazole, Nitrofurantoin, Vancomycin. Moderate resistance to Cefixime (50%), Clindamycin (33.3%), Norfloxacin (33.3%), Erythromycin (33.3%). These findings are in line with Jojan *et al.* [8], who also reported the effectiveness of carbapenems against *S. aureus*. *Escherichia coli* is highly susceptible: Amikacin, Imipenem, Piperacillin-Tazobactam. Resistant to Amoxyclav (75%), Ampicillin (50%), Tetracycline (50%), Norfloxacin (50%), Cotrimoxazole (25%), Levofloxacin (25%), Nitrofurantoin (25%). Mukherjee *et al.* [7] found similar resistance to commonly used oral agents, though Ciprofloxacin was reported as effective. *Klebsiella* species are sensitive to Amikacin, Ceftazidime-Clavulanic acid, Cotrimoxazole, Ciprofloxacin, Imipenem, and Levofloxacin. Resistant to Ampicillin (100%), partial resistance to Amoxyclav (50%), Tetracycline (75%), Norfloxacin (25%), Nitrofurantoin (25%). Comparable results were documented by Bose *et al.* [24] and Olufadi-Ahmed *et al.* [30]. *Other Isolates:* Citrobacter koseri are completely resistant to Cephalexin, Nitrofurantoin, and Amoxyclav; fully sensitive to aminoglycosides, third-generation cephalosporins, cotrimoxazole, fluoroquinolones, and higher-generation injectables [24]. Gram-negative organisms are highly susceptible to Imipenem (84.54%), Meropenem (81.84%), Amikacin (80.80%), and Gentamicin (78.74%), with low susceptibility to Ciprofloxacin, Cefixime, and Cotrimoxazole (30–35%). Gram-positive bacteria: Fully sensitive to Vancomycin, with high susceptibility to Linezolid (88.9%) and Teicoplanin (94.4%). Nitrofurantoin showed 50% efficacy against GPB, while lower sensitivity was noted to penicillins, Ciprofloxacin, and Aminoglycosides [2].

4.8. Implications for antenatal care

The present study underscores a changing etiological trend, with *Staphylococcus aureus* emerging as a significant pathogen in asymptomatic bacteriuria among pregnant women. Alarmingly high resistance was noted to commonly prescribed antibiotics such as Ampicillin, Amoxyclav, and Norfloxacin. These findings emphasize the critical importance of routine urine culture and sensitivity testing as part of antenatal care, coupled with continuous local surveillance of microbial prevalence and resistance patterns. Judicious antibiotic use and the tailoring of treatment protocols to local resistance profiles are essential to ensure effective management, reduce the risk of complications, and safeguard maternal and fetal health outcomes.

5. CONCLUSION

The present study reveals an increasing prevalence of asymptomatic bacteriuria (ASB) among pregnant women. While previous research has commonly identified *Escherichia coli* as the predominant uropathogen, this study highlights the emergence of *Staphylococcus aureus* as a significant causative agent, indicating a shifting microbial pattern. ASB was found to be more common among women aged 21–30 years, with higher prevalence observed in multiparous women and with advancing maternal age. The study also demonstrated rising antibiotic resistance among uropathogens. Routine screening for ASB in each trimester of pregnancy is strongly recommended. Early detection and appropriate treatment can significantly reduce the risk of maternal and fetal complications. Incorporating bacteriuria screening into standard antenatal care protocols can enhance maternal health outcomes.

This study was conducted on a relatively limited sample size, which may not fully represent the larger antenatal population. Being hospital-based, the results may not reflect the prevalence and microbial profile in community settings. Additionally, molecular characterization of resistant strains and extended-spectrum β-lactamase (ESBL) detection were not performed, which could have provided deeper insights into resistance mechanisms. Future studies should: i) Include larger, community-based cohorts to enhance generalizability; ii) Employ molecular methods for pathogen identification and resistance gene profiling; iii) Explore the impact of preventive strategies such as hygiene education, nutritional interventions, and antibiotic stewardship programs; and iv) Assess the long-term maternal and neonatal outcomes following ASB treatment.

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