Original Article

Pakistan Journal of Public Health
www.pjph.org

An Assessment of Asymptomatic Bacteriuria during Pregnancy and Antimicrobial Resistance to its Common Bacterial Isolates in the Urine

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Abstract

Background: Asymptomatic bacteriuria (ASB) is common in pregnant women and can result in major consequences such as preterm labor and low birth weight. However, its identification is frequently neglected, and the rising frequency of antimicrobial resistance (AMR) complicates its treatment. This study examined the prevalence of Asymptomatic Bacteriuria and antimicrobial resistance trends in urinary pathogens in pregnant women who visit antenatal clinics.

Methodology: This cross-sectional study was conducted at Ali Fatima Hospital, Lahore, over six months. A self-designed questionnaire was used for data collection, with non-probability random sampling. One hundred and fifteen pregnant women were enrolled. Urine samples were grown to identify bacterial infections, and an antibiotic susceptibility test was carried out using standard microbiological techniques.

Results: The study found that 12.2% of pregnant women had ASB, with Escherichia coli (40.0%) followed by Klebsiella spp. (22.6%) being the most common pathogens. Antibiotic resistance was high, with Ampicillin showing 32.2% resistance, while Nitrofurantoin and Fosfomycin showed higher sensitivity. Age, education, and gestational age were all significantly associated with ASB prevalence.

Conclusion: Asymptomatic Bacteriuria is common in pregnant women, and there is high antibiotic resistance. E. coli was discovered as the most common pathogen that was resistant to ampicillin. The major indicators for ASB included a history of the trimester, parity, education, and low socioeconomic position.

Keywords: Asymptomatic bacteriuria; pregnancy; antimicrobial resistance; Escherichia coli; urinary tract infections; antibiotic resistance

How to cite this article: Sarfraz A, khurshid S, khurshid M, Ashraf A, Ahsan U, Hameed H. An Assessment of Asymptomatic Bacteriuria during Pregnancy and Antimicrobial Resistance to its Common Bacterial Isolates in the Urine. Pak J Public Health. 2026;16(2):61-7.
DOI: https://doi.org/10.32413/pjph.v16i2.1670

Introduction

Urinary tract infections (UTIs) are more common in pregnant women as compared to the general population (1). It is described as both invasion of urinary tract tissue and microbiological contamination of the urine (2).In the absence of symptoms, a condition known as asymptomatic bacteriuria (ASB) exists in which the urine contains a high number of colony-forming units per milliliter (CFU/mL) of bacteria.During pregnancy, ASB may occur due to mechanical compression, hormonal changes, and changes in the immune and renal systems (3). Unrecognized bacteriuria affects 11% to 14% of pregnant women (4). Another analysis showed it to be 17% (5).

Another study revealed that 45.3 percent of pregnant women had asymptomatic bacteriuria. Women who are pregnant and have untreated bacteriuria have a higher risk of having a baby who is born with a low weight at birth. Preterm delivery, recurrent abortions, IUGR, polyhydramnios, high blood pressure, oligohydramnios, premature membrane rupture, and labor induction are all associated with ASB (6). ASB may raise the risk of pyelonephritis during the postpartum phase. Accordingly, it has been suggested that all pregnant women, regardless of whether or not they are experiencing symptoms, undergo regular screening for ASB at the Antenatal clinic (7,8). Women who have asymptomatic bacteriuria in the first trimester but are not treated with antibiotics are more than twice as likely to develop pyelonephritis in their second or third trimester. Hospitalization is usually required for pyelonephritis caused by pregnancy (9).

These two adverse pregnancy outcomes—intrauterine development retardation and

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Submitted: 21-03-2025
Revised: 13-04-2026, 06-06-2026
Accepted: 23-06-2026
Published: 30-06-2026

early membrane rupture—have also been related to this disease, although bacteriuria may just be a coexisting disorder. When addressed, ASB may lead to acute pyelonephritis or acute cystitis in around 30%-50% of pregnancies (affecting 2%- 10% of all pregnant women) (10).Asymptomatic bacteriuria occurrence amongst pregnant women in Iran varied from 6.1% to 10.9%, according to several studies (11). According to studies, the prevalence of ASB amongst pregnant women in Asian countries including Pakistan, India, and Bangladesh ranges from 6 to 10.2% The detection and treatment of asymptomatic bacteriuria benefit greatly from the control of pyelonephritis risk (12).

E. coli was shown to be the most frequent pathogen discovered during culture investigation (13). The most frequent agent has been identified as E. coli, followed by Klebsiella spp In the US, a rapidly expanding ASB outbreak during the last ten years has identified Klebsiella pneumonia as the reason. In extensive research comparing Klebsiella pneumonia and E. coli, Streptococcus group B was shown to be a very prevalent agent (14).

The prevalence of bacteriuria caused by antimicrobial drug resistance is rising globally, and certain pathogenic bacteria can develop multidrug resistance to antimicrobials. Antimicrobial resistance rates are intimately correlated with the use and abuse of antibiotics, and they vary geographically. Antibiotic treatment resistance and the species that cause infections vary greatly, with different regions of the same country hosting different pathogens. However, resistant strains may cause asymptomatic infections in pregnant women. A study that examined 552 urine samples from pregnant women showed that the prevalence rate of ASB in pregnant females was 17.4%. It was common in the age group of 25–33 years which was (60.4%) (15).

The second trimester had a higher infection rate (43.7%) than the third (29.2%) and first trimesters (27.1%). Multiparity (60.4%) was a prevalent occurrence in ASB during pregnancy. Previous history of UTI (22.9%) and anemia (58.3%) were significantly linked with ASB in pregnant females. The most frequently isolated bacteria was Escherichia coli (39.2%), 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%). Nitrofurantoin and fosfomycin were the most sensitive medications administered in ASB during pregnancy. Another study in Pakistan showed that 13.2% was the prevalence rate of asymptomatic bacteriuria. E. coli was most prevalent pathogen, followed by Klebsiella pneumoniae and staphylococci. Urine pathogens showed resistance to ampicillin, amoxiclav, norfloxacin, and piperacillin/tazobactam (16).

The educational and socioeconomic level of pregnant women were shown to be risk factors for asymptomatic bacteriuria.This implies that instead of waiting for the culture result, these patients may be provided empirical medication, and some practical suggestions can be included in our regular practice guidelines for identification of asymptomatic bacteria in the urine during pregnancy.

The objectives of current study were to assess the frequency of asymptomatic Bacteriuria during Pregnancy and assess Antimicrobial Resistance to Collective Bacterial Isolates in the Urine during Pregnancy

Operational Definitions:

1. Asymptomatic Bacteriuria:

The presence of a significant number of bacteria in the urine without clinical symptoms of urinary tract infection such as urinary urgency, frequency, dysuria, or flank pain.

2. Significant Bacteriuria:

The presence of more than 100,000 colony-forming units per ml ($\geq 10^5$ CFU/mL) in a voided midstream urine sample.

Common Bacterial Isolates:

Common bacterial isolates which were studied are

- Ecoli
- Klebsiella
- Staphylococcus aureus
- Streptococcus group B
- Enterococcus fecalis

Common Antibiotics:

- Nitrofurantoin
- Fosfomycin
- Imipenem
- Cefixime
- Cefoparazone
- Ampicillin
- Amoxiclave
- Norfloxacin
- Azithromycin
- Piperacillin tazobactum

Antibiotic Resistance:

The ability of bacteria to resist an antimicrobial agent to which they were at first sensitive as assessed by urine culture and sensitivity. Every antibiotic has its own sensitivity zone and disc difference according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Methodology

Study design: The current study was a cross-sectional study.

Study Setting: The study was conducted at the Antenatal Clinic, Ali Fatima Hospital, Lahore.

Study duration: The study duration was six months from the date of approval of the synopsis.

Sample size: The sample size was 115 using level of significance 0.05%, 95% confidence level, an error margin of 5% with a 17% frequency of asymptomatic bacteriuria during pregnancy(6)

Sampling technique: A non-probability convenience sampling technique was used.

Sample selection:

Inclusion criteria: All the pregnant women of any gestation irrespective of age who attend the Antenatal Clinic, Ali Fatima Hospital, Lahore on the routine antenatal visit, were enrolled in the current study.

Exclusion criteria: Pregnant women weren't included if they had a history of urinary tract infections, were taking antibiotics within the previous two weeks, had a history of being diabetic or suffering from renal illness, or had a catheterization within the previous two weeks.

Ethical approval and informed consent: Ethical approval for this research work was taken from the hospital ethical and research committee. Each participant got short description of the research before the data collecting procedure begins, and their signed consent was collected.

Data collection procedure: Data was gathered via a self-designed questionnaire with two components. The first portion consisted of demographic data like age, maternal history, parity, educational level, socioeconomic situation. The data gathered via cultural examination of the urine was provided in the second part. Antibiotic resistance and common pathogen prevalence rates was recorded. During the analysis of the urine samples, each sample was separated into two parts for analysis. Firstly each urine sample was checked by microscopic examination, If the findings of the urine microscopy were positive for bacteriuria then the other sample would be maintained in a cold, dry area and used for the cultural investigation to find out the type of bacteria and its antibiotic resistance.

Statistical analysis:

All the collected data was analyzed by using IBM SPSS version 23. Quantitative variables were documented in the form of means and standard deviation while qualitative variables such as asymptomatic bacteriuria, common bacterial isolates, antimicrobial resistance, educational status, and socioeconomic status were documented in the form of frequencies and percentages. The presentation of data was done in the form of figures and tables. Data was stratified for Effect modifiers such as age, parity, gestational age, educational, and socioeconomic status, and a post-stratification Chi-square test was applied. A P-value of ≤ 0.05 was considered as significant.

Results

The study's 115 participants had a variety of demographic, socioeconomic, clinical, and microbiological features. It included with most aged 20–30 years (48.7%) and 52.2% being illiterate. Socioeconomic status showed 60% in the low-income group (<50,000 Rs./month). Regarding gestational age, 43.5% were in the second trimester, and 53.9% were multigravida. Asymptomatic bacteriuria was found in 12.2%. The most common organism isolated was E. coli (40%), followed by Klebsiella (22.6%) and Streptococcus Group B (14.8%). Sensitivity was highest for nitrofurantoin (32.2%) and fosfomycin (27.8%), while resistance was most notable for amoxiclav (33.9%) and ampicillin (32.2%). These findings underscore significant patterns in bacterial etiology and antimicrobial resistance.

The study analyzed the association of asymptomatic bacteriuria with various demographic, socioeconomic, and clinical variables. Results showed no significant association with age (p=0.56). Education level was borderline significant (p=0.05), with asymptomatic bacteriuria more common in illiterate participants (50%). Socioeconomic status was significantly associated (p=0.01), with the highest prevalence in the low-income group (20.8%).

Gestational age had a strong association (p=0.000), with all cases of asymptomatic bacteriuria occurring in the second trimester. Gravidity was also significant (p=0.002), with higher prevalence in primigravida (22.6%). Among organisms, E. coli was most common (p=0.04), followed by Klebsiella. These findings highlight key factors influencing asymptomatic bacteriuria in the study population.

Drug sensitivity showed a significant association with bacterial organisms (p=0.000). E. coli had the highest sensitivity to nitrofurantoin (25/46), while Klebsiella was more sensitive to fosfomycin (10/26). Resistance patterns (p=0.01) revealed E. coli was most resistant to ampicillin (20/46) and amoxiclav (14/46). Enterococcus faecalis showed notable resistance to ampicillin (8/14) and piperacillin-tazobactam (3/14). These findings underscore variations in sensitivity and resistance among bacterial species.

Table 1: Demographic and Clinical Characteristics of Participants, Organisms, and Antibiotic Sensitivity/Resistance Patterns

Categories	Frequency (N=115)	Percent 100%
Age		
<20	19	16.5%
20-30	56	48.7%
30-40	32	27.8%
>40	8	7.0%
Education		
Illiterate	60	52.2%
Primary	24	20.9%
Middle	17	14.8%
matric & above	14	12.2%
socio Economic status		
low(<50,000Rs./m)	69	60.0%
middle(50,000-100,000Rs./m)	19	16.5%
high(>100,000Rs./m)	27	23.5%
Gestational age		
1st trimester (0-13 weeks)	38	33.0%
2nd trimester(14-27 weeks)	50	43.5%
3rd trimester (28 -40 weeks)	27	23.5%
Gravidity		
Primigravida	62	53.9%
Multigravida	53	46.1%
Asymptomatic Bacteriuria		
Yes	14	12.2%
No	101	87.8%
Organisms		
E. coli	46	40.0%
Klebsiella	26	22.6%
Staphylococcus aureus	12	10.4%
Streptococcus group B	17	14.8%

Categories	Frequency (N=115)	Percent 100%
Enterococcus fecalis	14	12.2%
Sensitivity To Which Drugs		
Nitrofurantoin	37	32.2%
Fosfomycin	32	27.8%
Imipenem	20	17.4%
Cefixime	13	11.3%
Cefoparazone	13	11.3%
Resistant To Which Drugs		
Ampicillin	37	32.2%
Amoxiclave	39	33.9%
Norfloxacin	18	15.7%
Azithromycin	14	12.2%
Piperacillin tazobactum	7	6.1%

Table 2: Factors Associated with Asymptomatic Bacteriuria Among Participants

Variables	Categories	Asymptomatic Bacteriuria		total	P value
		yes	no		
Age	<20	2	17	19	0.56
	20-30	9	47	56	
	30-40	2	30	32	
	>40	1	7	8	
Total		14	101	115	
Education	illeterate	7	53	60	0.05
	primary	6	18	24	
	middle	0	17	17	
	matric & above	1	13	14	
Total		14	101	115	
socio Economic status	low(<50,000Rs./m)	11	42	53	0.01
	middle (50,000-100,000Rs./m)	2	34	36	
	high(>100,000Rs./m)	1	25	26	
Total		14	101	115	
gestational age	1st trimester (0-13 weeks)	0	38	38	0.000
	2nd trimester(14-27 weeks)	14	36	50	
	3rd trimester (28 -40 weeks)	0	27	27	
Total		14	101	115	
Gravidity	primigravida	12	41	53	0.002
	multigravida	2	60	62	
Total		14	101	115	
Organisms	E. coli	8	38	46	0.04
	Klebsiella	4	22	26	
	Staphylococcus aureus	1	11	12	
	Streptococcus group B	1	16	17	
	Enterococcus fecalis	0	14	14	
Total		14	101	115	

Table 2 shows the association between asymptomatic bacteriuria (ASB) and selected variables. Chi-square tests were applied at a 95% confidence level (α = 0.05). Significant associations were found between ASB and education (p = 0.05), socioeconomic status (p = 0.01), gestational age (p < 0.001), and gravidity (p = 0.002), while no significant association was observed with age (p = 0.56).

Table 3: Sensitivity and Resistance Patterns of Isolated Organisms to Various Antibiotics in Participants with Asymptomatic Bacteriuria

Variable	Categories	sensitivity To Which Drugs					Total	P vale
		Nitrofurantoin	Fosfomycin	Imipenem	Cefixime	Cefoparazone		
organisms	E. coli	25	12	3	2	4	46	0.000
	Klebsiella	6	10	5	4	1	26	
	Staphylococcus aureus	2	5	3	2	0	12	
	Streptococcus group B	3	4	5	3	2	17	
	Enterococcus fecalis	1	1	4	2	6	14	
Total		37	32	20	13	13	115	
Variable	**Categories**	**Resistant To Which Drugs**					**Total**	**P value**
		Ampicillin	Amoxiclave	Norfloxacin	Azithromycin	Piperacillin tazobactum		
organisms	E. coli	20	14	7	4	1	46	0.01
	Klebsiella	6	6	5	5	3	25	
	Staphylococcus aureus	4	5	2	1	0	12	
	Streptococcus group B	4	4	5	4	1	18	
	Enterococcus fecalis	8	0	3	0	3	14	
Total		42	29	22	14	8	115	

Table 3 presents the antimicrobial sensitivity patterns of different organisms. Chi-square tests were applied to assess differences in drug sensitivity and resistance at a 95% confidence level (α = 0.05). Significant differences were observed among the organisms, with E. coli showing the highest sensitivity to Nitrofurantoin (p < 0.001) and the highest resistance to Ampicillin (p = 0.01). Other organisms, including Klebsiella and Staphylococcus aureus, exhibited variable degrees of sensitivity and resistance to the antibiotics tested.

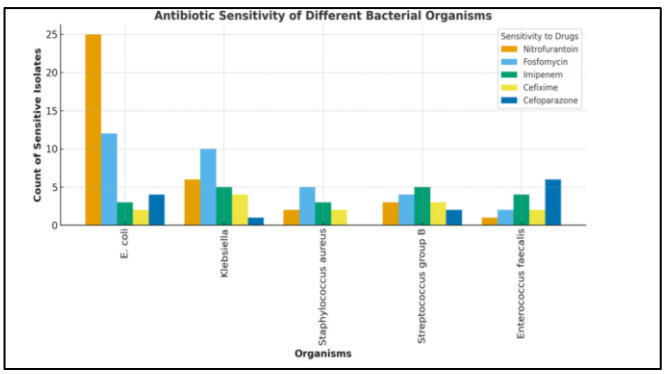

Figure 1: The bar chart illustrates the sensitivity of different organisms to various antibiotics, emphasizing the variable levels of response among bacterial strains.

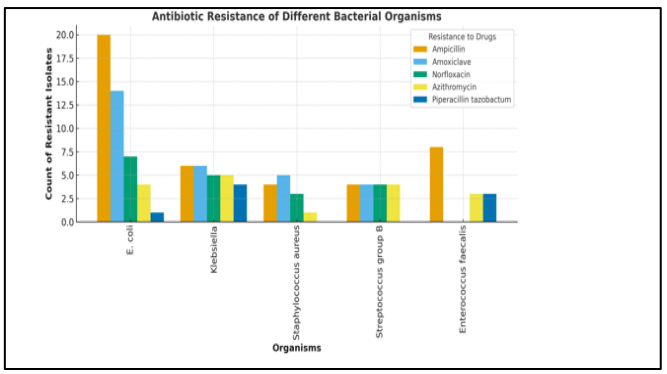

Figure 2: The bar chart shows the resistance patterns of bacterial organisms to several antibiotics, highlighting considerable variances in resistance across medication categories for each organism.

Table 4: Correlation among study variables

		Correlation									
		Age	Education	socio Economic status	gestational age	gravidity	comorbidity	asymptomatic Bacteriuria	organisms	Sensitivity to Which Drugs	Resistant To Which Drugs
age	Pearson's r	1	.085	.012	-.080	.003	-.119	-.088	.303**	.168	-.018
	Sig. (2-tailed)		.369	.900	.396	.977	.205	.348	.001	.072	.851
	N	115	115	115	115	115	115	115	115	115	115
education	Pearson's r	.085	1	.166	-.464**	.214*	-.007	.079	.434**	.081	-.069
	Sig. (2-tailed)	.369		.076	.000	.022	.940	.401	.000	.391	.467
	N	115	115	115	115	115	115	115	115	115	115
socio Economic status	Pearson's r	.012	.166	1	-.279**	.086	-.104	.223*	.317**	.167	.092
	Sig. (2-tailed)	.900	.076		.003	.360	.267	.016	.001	.075	.330
	N	115	115	115	115	115	115	115	115	115	115
gestational age	Pearson's r	-.080	-.464**	-.279**	1	-.165	.106	-.048	-.488**	-.248**	.080
	Sig. (2-tailed)	.396	.000	.003		.077	.259	.612	.000	.008	.393
	N	115	115	115	115	115	115	115	115	115	115
gravidity	Pearson's r	.003	.214*	.086	-.165	1	-.219*	.296**	.223*	.067	-.079
	Sig. (2-tailed)	.977	.022	.360	.077		.019	.001	.016	.478	.400
	N	115	115	115	115	115	115	115	115	115	115
comorbidity	Pearson's r	-.119	-.007	-.104	.106	-.219*	1	.056	-.099	-.107	.006
	Sig. (2-tailed)	.205	.940	.267	.259	.019		.549	.292	.256	.950
	N	115	115	115	115	115	115	115	115	115	115
asymptomatic Bacteriuria	Pearson's r	-.088	.079	.223*	-.048	.296**	.056	1	.187*	.156	.018
	Sig. (2-tailed)	.348	.401	.016	.612	.001	.549		.045	.096	.849
	N	115	115	115	115	115	115	115	115	115	115
organisms	Pearson's r	.303**	.434**	.317**	-.488**	.223*	-.099	.187*	1	.437**	.100
	Sig. (2-tailed)	.001	.000	.001	.000	.016	.292	.045		.000	.288
	N	115	115	115	115	115	115	115	115	115	115
sensitivity To Which Drugs	Pearson's r	.168	.081	.167	-.248**	.067	-.107	.156	.437**	1	.166
	Sig. (2-tailed)	.072	.391	.075	.008	.478	.256	.096	.000		.076
	N	115	115	115	115	115	115	115	115	115	115
Resistant To Which Drugs	Pearson's r	-.018	-.069	.092	.080	-.079	.006	.018	.018	.100	.166
	Sig. (2-tailed)	.851	.467	.330	.393	.400	.950	.849	.849	.288	.076
	N	115	115	115	115	115	115	115	115	115	115
**. Correlation is significant at the 0.01 level (2-tailed).											
*. Correlation is significant at the 0.05 level (2-tailed).											

Pearson's r = Pearson Correlation Coefficient

Table 4 shows the correlation analysis revealed several significant relationships among the studied variables. Age was positively correlated with the type of organisms isolated ($r = 0.303$, $p < 0.01$), while education showed a negative correlation with gestational age ($r = -0.464$, $p < 0.01$) and a positive correlation with gravidity ($r = 0.214$, $p < 0.05$) and organisms ($r = 0.434$, $p < 0.01$). Socioeconomic status was negatively associated with gestational age and positively associated with

asymptomatic bacteriuria and organisms. Gravidity was positively linked to both asymptomatic bacteriuria and organisms. Gestational age showed negative correlations with both organisms and drug sensitivity. Asymptomatic bacteriuria was positively correlated with organisms ($r = 0.187$, $p < 0.05$), and the organisms isolated were significantly associated with drug sensitivity ($r = 0.437$, $p < 0.01$). No significant correlations were found between drug resistance and the other variables.

Discussion

This study presents a comprehensive review of the demographic, clinical, microbiological, and antibiotic sensitivity characteristics of asymptomatic bacteriuria (ASB) in pregnant women, with major public health and therapeutic implications. The prevalence rate of ASB in pregnancy is different due to the varied cultures and geographic regions. In our study the prevalence rate was 12.2 %. A study revealed that unrecognized bacteriuria affects 11% to 14% of pregnant women who had asymptomatic bacteriuria (17).

The prevalence of ASB in the study (25.2%) is within the range reported in other studies from low- and middle-income countries (15-30%), including who found a 21.2% prevalence in Ethiopia. However, this study indicated a higher incidence among younger adults (20-30 years), in contrast to Fatima et al. (2023) in Pakistan, who found higher rates among older participants (>30 years) (18). This disparity could be due to population-specific risk factors, gaps in healthcare access, or societal differences, all of which warrant additional investigation.

Educational attainment emerged as a critical factor, with illiterate individuals showing the highest prevalence (52%). This finding aligns with Ahiatrogah et al. (2021) in Ghana, where low health literacy correlated with higher ASB rates (19). Education's protective role underscores the importance of health literacy and targeted educational interventions to reduce ASB risk.

Socioeconomic status also showed an important control, with low-income participants having the highest prevalence .These findings are consistent with a 2019 study that related poverty to increased infection risk due to inadequate sanitation and restricted healthcare access (20). Addressing socioeconomic disparities through focused public health interventions is critical to reducing ASB prevalence. Gestational age was significantly associated with ASB prevalence, with the second trimester having the highest incidence. This tendency corresponds to the findings of Rohini et al. (2017) in Nigeria, who reported a 35% frequency in late pregnancy. (21). Physiological changes in late pregnancy, such as urine stasis caused by uterine growth, are believed to contribute to this elevated risk. Primigravida women had a higher ASB prevalence (53%) than multigravida women (46%), which is consistent with Dalawaiet al.'s (2019) finding of that prevalence in primigravida communities. Hormonal and structural changes during a first pregnancy may increase susceptibility to bacterial colonization (22).

In the current study, E. coli was found to be the strongest pathogen discovered using urine culture as Escherichia coli was the most prevalent (40.0%), followed by Klebsiella (22.6%), Staphylococcus aureus (10.4%), Streptococcus group B (14.8%), and Enterococcus faecalis (12.2%). A study concluded that E. coli was detected in 58.9% of patients, with a 2.5% incidence rate (11). According to an Indian study, E. coli was the most prevalent isolate (23). Another study found that E. coli was the leading pathogen, followed by Staphylococcus aureus. Another study identified E. coli in 43.75% and the second most common Staphylococcus aureus in 31.25%.(14) The study also showed a significant change in the dominance of bacteria responsible for ASB from place to place, highlighting the need for urine culture in identifying the particular causal organism (24).

Many pathogens isolated in urine are sensitive to antibiotic treatment in the present study. Screening at early stages would give useful grounds to the antibiotic treatment(25)in the present study Nitrofurantoin and Fosfomycin show efficacy against E. coli and Klebsiella, respectively. However, high resistance rates for Ampicillin and Amoxiclav raise concerns about their continued use. This study supports global AMR trends and calls for region-specific surveillance systems. Some studies strongly recommended screening at an early stage of pregnancy or at the first antenatal visit at a medical center (25).

This study provides strong data about the risk factors, prevalence, microbiological trends, and antibiotic resistance in ASB during pregnancy. The findings underline the importance of focused screening, improved healthcare access, and antibiotic stewardship in reducing the incidence of ASB and its sequelae. Addressing socioeconomic determinants of health and implementing evidence-based treatment regimens was critical for improving maternal and fetal outcomes.

Conclusion

This study found high prevalence of asymptomatic bacteriuria (ASB) in pregnant women, which had strong associations with age, education, and socioeconomic status. Escherichia coli and Klebsiella were the most common bacteria, and they were growing increasingly resistant to traditional medicines like ampicillin and amoxiclav. Nitrofurantoin and fosfomycin showed higher sensitivity. The findings highlight the importance of routine ASB screening, particularly in high-risk groups, as well as targeted antibiotic therapy and improved healthcare access.

Ethical Approval:
This study was approved by the Research Ethics Committee of the Green International University, Lahore, Pakistan.
Ref. REC/GIU/FMAHS/02/25/003 Dated: 19-02-2025

Data Availability: Data supporting the findings are available upon reasonable request.

Financial support and sponsorship: No external funding was received for the conduct of this study.

Conflict of interest: The authors declare that they have no conflict of interest.

Authors' Contribution:

AS: Concept and design of the study, data collection and interpretation

SK: Analysis of data, drafting of the article

MK: Drafting the article, major revision of the intellectual data

AA: Concept and design of the study and interpretation of data

UA: Analysis of data, drafting of the article

HH: Data collection and interpretation

All the authors approved the final draft of the manuscript.

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