



Original Research Article

STUDY ON ASYMPTOMATIC BACTERIURIA AND ITS CORRELATION WITH IRON DEFICIENCY ANAEMIA AMONG PREGNANT WOMEN ATTENDING TERTIARY CARE CENTRE

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Received : 06/07/2026
Received in revised form : 05/08/2026
Accepted : 18/08/2026

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DOI: 10.70034/ijmedph.2026.3.958

Source of Support: Nil,

Conflict of Interest: None declared

Int J Med Pub Health

2026; 16 (3); 6203-6214

ABSTRACT

Background: Asymptomatic bacteriuria (ASB), defined as significant bacterial growth in urine without urinary symptoms, is a common condition during pregnancy and is associated with adverse maternal and fetal outcomes if left untreated. Iron deficiency anaemia (IDA), the most prevalent nutritional disorder in pregnancy, impairs immune function and may increase susceptibility to urinary tract infections. Understanding the relationship between ASB and IDA can facilitate early identification and timely intervention, thereby improving pregnancy outcomes. **Aim of the Study:** To study Asymptomatic bacteriuria and its correlation with iron deficiency anemia among pregnant women over 28 weeks of gestation.

Materials and Methods: This prospective observational study included 100 pregnant women over 28 weeks of gestation attending the antenatal clinic at Government General Hospital, Mahabubnagar.

All participants were asymptomatic for urinary tract infection at the time of enrollment. Pregnant women presenting to the antenatal clinic during the study period and fulfilling the inclusion criteria were consecutively enrolled after obtaining informed consent.

Results: A total of 100 asymptomatic pregnant women over 28 weeks of gestation were included in the study. Midstream urine samples were collected and processed for culture and sensitivity testing. Hemoglobin levels were measured and anemia was classified according to WHO criteria. The prevalence of asymptomatic bacteriuria was 19% with 19 out of 100 pregnant women demonstrating significant bacteriuria ($\geq 10^5$ CFU/mL). This indicates that nearly one-fifth of asymptomatic antenatal women harbored clinically significant urinary tract colonization. Moderate anemia was more frequently observed among ASB-positive women (74%) compared to ASB-negative women (44%). However, this association did not reach statistical significance ($p = 0.0696$). Pearson correlation analysis demonstrated a statistically significant moderate inverse correlation between hemoglobin levels and ASB ($r = -0.359$, $p < 0.0001$). Lower hemoglobin levels were significantly associated with increased likelihood of asymptomatic bacteriuria. *Escherichia coli* was the predominant isolate (8% of total cases and majority of culture-positive samples). Other organisms included *Staphylococcus aureus*, *Klebsiella* species, *Staphylococcus saprophyticus*, and *Enterococcus* species. Nitrofurantoin and Fosfomycin showed the highest sensitivity (95%) among isolates. Gentamicin and Piperacillin-Tazobactam demonstrated moderate sensitivity. High resistance was observed with Ciprofloxacin, Azithromycin, and Cefotaxime.

Conclusion: Iron deficiency anaemia is significantly associated with an increased risk of asymptomatic bacteriuria during pregnancy.

The present study concludes that asymptomatic bacteriuria (ASB) is common among pregnant women, with a prevalence of 19% in the study population. *Escherichia coli* was the most common organism isolated. Nitrofurantoin and Fosfomycin were found to be the most effective antibiotics. Routine screening for both anaemia and asymptomatic bacteriuria during antenatal care enables early diagnosis and appropriate treatment, reducing the risk of maternal complications such as pyelonephritis and adverse fetal outcomes including preterm birth and low birth weight. Combined preventive and therapeutic strategies can substantially improve maternal and neonatal health outcomes.

Keywords: Iron deficiency, *Escherichia coli*, Asymptomatic bacteriuria (ASB), Nitrofurantoin, Ciprofloxacin.

INTRODUCTION

Asymptomatic bacteriuria (ASB) is defined as the presence of actively multiplying bacteria in the urine at a colony count of $\geq 10^5$ colony-forming units (CFU) per milliliter in a properly collected midstream specimen, in the absence of clinical signs or symptoms of urinary tract infection.^[1] It is a well-recognized clinical entity during pregnancy and is of particular importance due to its potential association with adverse maternal and fetal outcomes if left untreated.

Pregnancy predisposes women to the development of asymptomatic bacteriuria due to physiological and anatomical changes in the urinary tract. Increased progesterone levels lead to relaxation of smooth muscle, resulting in ureteric dilatation, decreased bladder tone, urinary stasis, and vesicoureteral reflux, all of which facilitate bacterial colonization and ascending infection.^[2] If untreated, asymptomatic bacteriuria can progress to symptomatic urinary tract infection and acute pyelonephritis in approximately 20–40% of pregnant women.^[3]

Several studies have demonstrated a significant association between asymptomatic bacteriuria during pregnancy and adverse obstetric outcomes such as preterm labour, premature rupture of membranes, low birth weight infants, postpartum hypertensive disorders, anaemia, and increased perinatal morbidity.^[4,5] Early detection and appropriate treatment of ASB have been shown to significantly reduce the incidence of pyelonephritis and improve pregnancy outcomes, emphasizing the importance of routine screening in antenatal care.^[3,4] Iron deficiency anaemia is one of the most common nutritional deficiencies affecting pregnant women, particularly in developing countries such as India. According to the World Health Organization (WHO), anaemia in pregnancy is defined as hemoglobin levels < 11 g/dL.^[6,7] Anaemia during pregnancy is associated with impaired immune function, including reduced neutrophil activity and diminished cellular immunity, which may predispose women to infections including asymptomatic bacteriuria. Several investigators have reported a higher prevalence of ASB among

anaemic pregnant women, suggesting that iron deficiency anaemia may act as a potential risk factor for urinary tract colonization.

Urine culture remains the gold standard for the diagnosis of asymptomatic bacteriuria. The most commonly isolated organism in pregnant women with ASB is *Escherichia coli*, accounting for nearly 70–90% of cases, followed by *Klebsiella pneumoniae*, *Staphylococcus aureus*, and other Gram-positive cocci.^[8] The antimicrobial susceptibility patterns of these organisms vary widely across geographical regions and time periods, with increasing resistance reported to commonly used antibiotics.^[4,9]

The prevalence of asymptomatic bacteriuria, the spectrum of causative organisms, and their antimicrobial resistance patterns show significant regional variation. Therefore, local epidemiological data are essential for formulating effective screening and treatment strategies.^[9] There is limited published data from the Mahabubnagar region regarding the prevalence of ASB and its correlation with iron deficiency anaemia among pregnant women.

This study was undertaken to determine the prevalence of asymptomatic bacteriuria, evaluate its correlation with iron deficiency anaemia, identify the causative organisms, and analyze their antimicrobial susceptibility patterns among pregnant women attending a tertiary care centre in Mahabubnagar. The findings of this study are expected to contribute to improved antenatal screening practices and reduction of preventable maternal and fetal complications.

Aims and Objectives

Aim of the Study

To study Asymptomatic bacteriuria and its correlation with iron deficiency anemia among pregnant women over 28 weeks of gestation.

Objectives

1. To determine the prevalence of asymptomatic bacteriuria among pregnant women over 28 weeks of gestation.
2. To study the correlation between asymptomatic bacteriuria and iron deficiency anemia in pregnant women.
3. To aid in the treatment with appropriate antibiotics.

MATERIALS AND METHODS

This study was conducted at Government General Hospital, Mahabubnagar, after obtaining approval from the Institutional Ethics Committee of Government Medical College, Mahabubnagar, and after obtaining written informed consent from all pregnant women who participated in the study.

Study Area

Antenatal Clinic, Department of Obstetrics and Gynaecology, Government General Hospital, Mahabubnagar, in collaboration with the Department of Microbiology.

Study Design

This was a prospective observational study.

Study Population

This prospective observational study included 100 pregnant women over 28 weeks of gestation attending the antenatal clinic at Government General Hospital, Mahabubnagar.

All participants were asymptomatic for urinary tract infection at the time of enrollment. Pregnant women presenting to the antenatal clinic during the study period and fulfilling the inclusion criteria were consecutively enrolled after obtaining informed consent.

Study Period

After approval by the Institutional Ethics Committee, this study was started with protocol no: GMC/MBNR/IECBMR/AP/05/21/2024. Study was conducted over a period of 12 to 18 months (June 2024 to Aug 2025).

Inclusion Criteria

1. Pregnant women over 28 weeks of gestation attending the antenatal clinic.
2. Pregnant women without signs or symptoms of urinary tract infection.
3. Pregnant women willing to provide informed written consent.

Exclusion Criteria

1. Pregnant women currently on antimicrobial therapy or who had taken antimicrobials within the previous two weeks.
2. Pregnant women with symptomatic urinary tract infection, fever with chills, vaginal bleeding, recurrent urinary tract infections, diabetes mellitus, history of urolithiasis, urological surgery, or urogenital fistula.
3. Pregnant women unwilling to give informed written consent.

Sample Size and Sampling Technique

Sample Size: A total of 100 pregnant women were included in the study.

Sampling Technique: Consecutive sampling technique was employed.

Justification of Sample Size

Considering the duration of the study, patient inflow to the antenatal clinic, and feasibility within the study period, a sample size of 100 subjects was considered adequate for statistical analysis and to minimize sampling error.

Data collection technique

Primary data

- Demographic details
- Obstetric history
- Laboratory findings (urine culture, haemoglobin levels)

TOOLS

- Direct observation
- Interview method
- Laboratory investigations
- Standard microbiological protocols

Methodology

A prospective observational study was conducted on 100 asymptomatic pregnant women over 28 weeks of gestation attending the antenatal clinic at Government General Hospital, Mahabubnagar.

Urine Sample Collection

- Urine samples were collected by the standard mid-stream “clean catch” method in sterile, wide-mouthed containers with tightfitting lids.
- Samples were transported to the microbiology laboratory and processed without delay.

Microbiological Processing (Standard Loop Method)

Urine specimens were processed using the standard semi-quantitative calibrated loop technique. A sterile calibrated loop delivering 0.001 mL (1 μ L) of urine was used for inoculation onto CLED agar, MacConkey agar, and Blood agar plates. After incubation at 37°C for 18–24 hours, colonies were counted and multiplied by 1000 to obtain colony-forming units (CFU)/mL. This method is recommended for routine urine culture in standard microbiology practice.^{22–24} Urine specimens were inoculated onto MacConkey agar, Sheep Blood agar (incubated in 5–10% CO₂ atmosphere), and Cystine Lactose Electrolyte Deficient (CLED) agar using the standard calibrated loop method.

- Plates were incubated at 37°C for 18–24 hours.
- Culture results were interpreted as significant or insignificant bacteriuria based on standard quantitative criteria.
- Significant bacteriuria was defined as growth of $\geq 10^5$ CFU/mL of a single bacterial species in a properly collected midstream urine specimen, as described by Kass.²

Identification of Organisms: Organisms isolated from samples showing significant bacteriuria were identified using routine microbiological and biochemical methods.

Identification of Gram positive cocci

Staphylococcus aureus, Enterococci, and Micrococci were identified by colony morphology, Gram staining, and biochemical tests as per standard microbiological procedures.

1. Staphylococcus aureus, was identified based on the following characteristics i.e. gram-positive cocci in clusters on Gram's staining, golden yellow pigment on the Nutrient agar plate, positive for catalase and tube coagulase test

2. Staphylococcus aureus was confirmed with mannitol salt agar.
3. Staphylococcus aureus shows yellow colour colonies on Cled agar.

Enterococci were identified based on microscopic morphology i.e. Grampositive cocci in diplos, Negative for catalase, Positive for bile esculin hydrolysis, heat tolerance property, and mannitol fermentation

Identification of Gram-negative Bacilli (GNB)

The gram-negative bacilli were identified based on the colony morphology, motility, catalase test, oxidase test, indole test, Methyl red, Voges Proskauer, triple sugar iron agar, citrate utilization, and urease production

Indole+, Citrate+, Urease+, TSI - A / A with Gas

Antibiotic Susceptibility Testing: Antimicrobial susceptibility testing was performed using the standardized Kirby–Bauer disc diffusion method, and results were interpreted according to Clinical and Laboratory

Standards Institute (CLSI-M100) guidelines.²⁰

ASSESSMENT OF IRON DEFICIENCY ANAEMIA

Haemoglobin levels were estimated for all participants using the automated hematology analyzer method (Cyanmethemoglobin method) in the laboratory of the hospital.

Anaemia was defined and classified according to the World Health Organization (WHO) criteria for anaemia in pregnancy.

According to WHO guidelines:

- a Normal: ≥ 11 g/dL
 - b Mild anaemia: 10.0 – 10.9 g/dL
 - c Moderate anaemia: 7.0 – 9.9 g/dL
 - d Severe anaemia: < 7.0 g/dL
- Pregnant women with haemoglobin levels < 11 g/dL were considered anaemic.^[21]
 - Correlation between asymptomatic bacteriuria and iron deficiency anaemia was analyzed.

Consent

Written informed consent was obtained from all participants. The nature of the study, purpose, benefits, and confidentiality were explained in detail.

Outcome Measures

1. Prevalence of asymptomatic bacteriuria among pregnant women
2. Identification of causative organisms
3. Antibiotic susceptibility patterns
4. Correlation between asymptomatic bacteriuria and iron deficiency anaemia

Statistical Analysis

Data were entered into Microsoft Excel and Statistical Package for the Social Sciences (SPSS) version 24.0

A 95% confidence interval was applied, and a p-value < 0.05 was considered statistically significant.

RESULTS

Prevalence of Asymptomatic Bacteriuria (ASB) in Pregnant Women

Table 1: Prevalence of Asymptomatic Bacteria (ASB) in Pregnant Women

ASB	No. of Cases	Percentage
Significant bacteriuria	19	19
No significant bacteriuria	81	81

Table 1: Prevalence of Asymptomatic Bacteria (ASB) in Pregnant Women

Among the 100 pregnant women evaluated, 19 (19%) were found to have significant bacteriuria, while 81 (81%) did not have significant bacteriuria. The prevalence of asymptomatic bacteriuria (ASB) in the study population was therefore 19%. This indicates that nearly one-fifth of pregnant women had bacteriuria without presenting any urinary symptoms. The findings highlight that ASB is

relatively common during pregnancy and supports the importance of routine screening, as early detection and treatment can help prevent maternal and fetal complications such as pyelonephritis, preterm labour, and low birth weight. This prevalence data serves as a basis for further analysis of the correlation between asymptomatic bacteriuria and iron deficiency anaemia among pregnant women.

Distribution of Age Based on ASB

Table 2: Distribution of Age Based on ASB

Age Interval (years)	Total No. of Cases (%)	Significant bacteriuria N(%)	No significant bacteriuria N (%)	p-value
<20	13(13)	01(05)	12(15)	0.0004*
20-30	73(73)	10(53)	63(78)	
>30	14(14)	08(42)	06(07)	

Table 2: Distribution of Age Based On ASB

In the present study, the majority of pregnant women belonged to the 20–30 years age group (73%), followed by women aged >30 years (14%) and <20 years (13%). Among the 19 women diagnosed with significant bacteriuria, 10 cases (53%) were in the 20–30 years age group, 8 cases (42%) were in the >30 years group, and only 1 case (5%) was in the <20 years group.

In contrast, among the 81 women without significant bacteriuria, the largest proportion (78%) belonged to the 20–30 years category, while 15% were <20 years and 7% were >30 years. Statistical analysis revealed a highly significant association between maternal age and asymptomatic bacteriuria ($p = 0.0004$).

Distribution of Gestational Age Based on ASB

Table 3: Distribution of Gestational Age Based on ASB

Gestational age (weeks)	Total No. of Cases (%)	Significant bacteriuria N (%)	No significant bacteriuria N (%)	p-value
25-29	25(25)	02(11)	23(28)	0.2472
30-34	41(41)	10(53)	31(38)	
35-39	34(34)	07(37)	27(33)	

Table 3: Distribution of Gestational Age Based on ASB

In the present study, 41% of pregnant women were between 30–34 weeks of gestation, followed by 35–39 weeks (34%) and 25–29 weeks (25%). Among the 19 women with significant bacteriuria, the majority (53%) were in the 30–34 weeks group, while 37% were between 35–39 weeks and 11% were between 25–29 weeks. Among the 81 women without significant bacteriuria, 38% were in the 30–34 weeks group, 33% were between 35–39 weeks, and 28% were between 25–29 weeks. Although a

relatively higher proportion of asymptomatic bacteriuria cases was observed in the 30–34 weeks gestational age group, statistical analysis revealed that the association between gestational age and asymptomatic bacteriuria was not statistically significant ($p = 0.2472$). This indicates that gestational age did not have a significant influence on the occurrence of asymptomatic bacteriuria in the present study, suggesting that susceptibility to bacteriuria persists throughout late pregnancy rather than being confined to a specific gestational period.

Anaemia level Based on ASB

Table 4: Anaemia Level Based On ASB

Hemoglobin	Total No. of Cases (%)	Significant bacteriuria (%)	N	No significant bacteriuria N (%)	p-value
Mild anaemia	49(49)	05(26)		44(54)	0.0696
Moderate anaemia	50(50)	14(74)		36(44)	
Severe anaemia	01(01)	0		01(01)	

Table 4: Anaemia Level Based On ASB

In the present study, among the 100 pregnant women evaluated, 49% had mild anaemia, 50% had moderate anaemia, and 1% had severe anaemia. Among the 19 women diagnosed with significant bacteriuria, the majority (74%) had moderate anaemia, while 26% had mild anaemia, and none had severe anaemia. In contrast, among the 81 women without significant bacteriuria, 54% had mild anaemia, 44% had moderate anaemia, and 1% had severe anaemia. Although a higher proportion of asymptomatic bacteriuria was observed among women with moderate anaemia compared to those

with mild anaemia, statistical analysis revealed that the association between hemoglobin category and asymptomatic bacteriuria was not statistically significant ($p = 0.0696$). However, the noticeable predominance of moderate anaemia among ASB-positive women suggests a possible clinical trend indicating increased susceptibility to bacteriuria with lower hemoglobin levels. While statistical significance was not achieved, the distribution pattern supports a potential association between anaemia severity and the occurrence of asymptomatic bacteriuria in pregnancy.

Table 5: Distribution of Bacterial Isolates Among Pregnant Women Table

Organisms	No. of Cases	Percentage
Staphylococcus aureus	04	04
E. coli	08	08
Klebsiella species	04	04
Enterococcus species	01	01
Staphylococcus saprophyticus	02	02
No bacterial growth	81	81

In the present study, out of 100 urine samples analyzed, 81% showed no bacterial growth, while 19% demonstrated significant bacteriuria. Among the culture-positive cases, *E. coli* was the most frequently isolated organism, accounting for 8% of the total cases and forming the majority of isolates among the 19 positive samples. *Staphylococcus aureus* and *Klebsiella* species were each identified in 4% of cases, followed by *Staphylococcus saprophyticus* (2%) and *Enterococcus* species (1%). The predominance of *E. coli* indicates that Gram-negative organisms are the principal causative

agents of asymptomatic bacteriuria in pregnant women, with Gram-positive organisms contributing to a smaller proportion of cases. This microbial distribution pattern is consistent with established epidemiological trends of urinary tract infections in pregnancy. The findings emphasize the importance of routine urine culture and antimicrobial susceptibility testing in antenatal care to ensure accurate diagnosis and selection of appropriate antibiotic therapy, thereby preventing potential maternal and fetal complications.

Table 6: Antibiotic Susceptibility Pattern of the Bacterial Isolates

Drugs	Organisms, N (%)					
	<i>Staphylococcus aureus</i> (n=4)	<i>E. coli</i> (n=8)	<i>Klebsiella</i> species (n=4)	<i>Enterococcus</i> species (n=1)	<i>Staphylococcus saprophyticus</i> (n=2)	Total (n=19)
CX	04(100)	ND	ND	01(100)	R	05(26)
LZ	04(100)	ND	ND	01(100)	01(50)	06(32)
NIT	04(100)	07(88)	04(100)	01(100)	02(100)	18(95)
CIP	R	01(13)	R	R	R	01(05)
COT	R	06(75)	03(75)	R	R	09(47)
CD	04(100)	ND	ND	01(100)	01(50)	06(32)
ERY	01(25)	ND	ND	R	01(50)	02(11)
AZM	R	ND	ND	R	R	R
FOS	04(100)	08(100)	04(100)	01(100)	01(50)	18(95)
HL (GE)	04(100)	ND	ND	01(100)	02(100)	07(37)
AMP	R	05(63)	R	R	R	05(26)
DOX	04(100)	ND	ND	R	02(100)	06(32)
Le	04(100)	ND	ND	R	02(100)	06(32)
NOVO	R	ND	ND	R	R	R
PIT	ND	07(88)	04(100)	ND	ND	11(58)
CTR	ND	01(13)	03(75)	ND	ND	04(21)
IMP	ND	06(75)	04(100)	ND	ND	10(53)
MRP	ND	06(75)	04(100)	ND	ND	10(53)
CAZ	ND	07(88)	03(75)	ND	ND	10(53)
CTX	ND	R	R	ND	ND	R
AMC	ND	04(50)	R	ND	ND	04(21)
AK	ND	01(13)	03(75)	ND	ND	04(21)
GEN	ND	08(100)	04(100)	ND	ND	12(63)

A total of 19 bacterial isolates were subjected to antibiotic susceptibility testing. The susceptibility patterns are interpreted as follows:

1. *Staphylococcus aureus* (n = 4)

- 100% sensitivity to Nitrofurantoin, Fosfomycin, Linezolid, Clindamycin, Doxycycline, Levofloxacin, HL (Gentamicin), and CX
- Low sensitivity to Erythromycin (25%)
- Resistant to Ciprofloxacin, Cotrimoxazole, Ampicillin, and Azithromycin

2. *E. coli* (n = 8)

- 100% sensitivity to Fosfomycin and Gentamicin
- 88% sensitivity to Nitrofurantoin, Piperacillin–Tazobactam, and Ceftazidime

- 75% sensitivity to Cotrimoxazole, Imipenem, and Meropenem

- Low sensitivity to Ciprofloxacin (13%), Ceftriaxone (13%), and Amikacin (13%)

- No sensitivity to Cefotaxime

3. *Klebsiella* species (n = 4)

- 100% of *klebsiella* species are sensitive to Nitrofurantoin,
- Fosfomycin, Gentamicin, Piperacillin–Tazobactam, Imipenem, and Meropenem
- 75% sensitivity to Cotrimoxazole, Ceftriaxone, Ceftazidime, and Amikacin
- No sensitivity to Cefotaxime

- 4. *Enterococcus* species (n = 1)

- 100% sensitivity to Nitrofurantoin, Fosfomycin, Linezolid, Clindamycin, HL (Gentamicin), and CX
 - Resistant to other tested antibiotics
 - Staphylococcus saprophyticus (n = 2)
 - 100% sensitivity to Nitrofurantoin, Gentamicin (HL), Doxycycline, and Levofloxacin
 - 50% sensitivity to Linezolid, Clindamycin, Erythromycin, and Fosfomycin
 - Resistant to novobiocin
- The antibiotic susceptibility pattern demonstrates that Nitrofurantoin and Fosfomycin were the most

effective drugs (95% sensitivity) against the isolated uropathogens. These findings support their continued use as first line agents in the treatment of asymptomatic bacteriuria during pregnancy. High resistance was observed with Ciprofloxacin, Azithromycin, Cefotaxime, and Novobiocin, indicating limited utility of these agents. The data emphasizes the importance of performing urine culture and antibiotic sensitivity testing to guide appropriate antimicrobial therapy, especially in pregnant women, to prevent complications and reduce the development of antimicrobial resistance.

Table 7: Correlation Between Haemoglobin and ASB Correlations

		Haemoglobin	ASB
Haemoglobin	Pearson Correlation	1	-.359**
	Sig. (2-tailed)		.000
	N	97	97
ASB	Pearson Correlation	-.359**	1
	Sig. (2-tailed)	.000	
	N	97	97

****Correlation is Significant at the 0.01 Level (2-Tailed)**

The Pearson correlation analysis showed a correlation coefficient of $r = 0.359$ with a p -value < 0.0001 , which is statistically significant at the 0.01 level (2-tailed). The negative correlation coefficient indicates an inverse relationship between haemoglobin levels and asymptomatic bacteriuria. This means that as haemoglobin levels decrease, the likelihood of asymptomatic bacteriuria increases. The strength of the correlation ($r = -0.359$) suggests a moderate negative association, implying that lower

haemoglobin levels are moderately associated with the presence of ASB. The scatter plot further demonstrates that women with asymptomatic bacteriuria tend to have lower haemoglobin values compared to those without bacteriuria. These findings indicate a statistically significant inverse correlation between haemoglobin levels and ASB, suggesting that anaemia may be associated with an increased risk of asymptomatic bacteriuria among pregnant women.

Table 8: Haemoglobin Based on Bacterial Isolate

Organism	Haemoglobin, N (%)		p-value
	Mild anaemia (n=5)	Moderate anaemia (n=14)	
Staphylococcus aureus	01(20)	03(21)	0.2430
E. coli	01(20)	07(50)	
Klebsiella species	02(40)	02(14)	
Enterococcus species	01(20)	0	
Staphylococcus saprophyticus	0	02(14)	

In the present study, among the 19 culture-positive cases, hemoglobin distribution was analyzed according to the type of bacterial isolate. Among women with mild anemia (n = 5), Klebsiella species accounted for the highest proportion (40%), followed by Staphylococcus aureus (20%), E. coli (20%), and Enterococcus species (20%), while Staphylococcus saprophyticus was not observed in this group. Among women with moderate anemia (n = 14), E. coli was the predominant isolate, accounting for 50% of cases, followed by Staphylococcus aureus (21%), Klebsiella species (14%), and Staphylococcus saprophyticus (14%), while Enterococcus species was not detected in this category. Although E. coli was more frequently isolated among women with moderate anemia, statistical analysis revealed no significant association between hemoglobin category and type

of bacterial isolate ($p = 0.2430$). This indicates that while anemia may increase overall susceptibility to asymptomatic bacteriuria, it does not appear to significantly influence the specific type of uropathogen isolated in this study.

DISCUSSION

Asymptomatic bacteriuria (ASB) represents a clinically silent, yet biologically significant urinary tract colonization characterized by the presence of $\geq 10^5$ colony-forming units per millilitre of urine in the absence of classical urinary symptoms. In pregnancy, ASB assumes greater clinical importance because of the unique physiological, anatomical, hormonal, and immunological adaptations that predispose women to urinary tract infections.

If left untreated, ASB progresses to acute pyelonephritis in approximately 20–40% of cases.³Pyelonephritis during pregnancy is associated with severe maternal and fetal complications including septicemia, anaemia, hypertensive disorders, preterm labour, low birth weight, intrauterine growth restriction, and increased perinatal morbidity and mortality. Therefore, early identification and treatment of ASB remain a cornerstone of antenatal care.

Iron deficiency anaemia (IDA) is highly prevalent among pregnant women, particularly in developing countries. During pregnancy, iron demand increases significantly due to expanded maternal blood volume, fetal requirements, and placental development. Iron plays a critical role in immune regulation. It is essential for neutrophil oxidative burst activity, macrophage bactericidal function, lymphocyte proliferation, cytokine synthesis, and epithelial integrity. Iron deficiency impairs these immune mechanisms, resulting in diminished host defense and increased susceptibility to infections. Thus, a biologically plausible association exists between iron deficiency anaemia and increased risk of asymptomatic bacteriuria.

The present study was conducted to determine the prevalence of asymptomatic bacteriuria among pregnant women and to evaluate its correlation with iron deficiency anaemia, along with analysis of demographic factors, microbial profile, and antibiotic susceptibility patterns.

Prevalence of Asymptomatic Bacteriuria

In the present study, the prevalence of asymptomatic bacteriuria (ASB) was found to be 19%, with 19 out of 100 pregnant women demonstrating significant bacteriuria ($\geq 10^5$ CFU/mL) without urinary symptoms. This indicates that nearly one-fifth of apparently healthy pregnant women harbored clinically significant urinary tract colonization. The observed prevalence suggests a considerable burden of silent infection in the study population and underscores the clinical importance of screening in antenatal care.

The prevalence observed in the present study is comparatively higher than several national and international reports. Sujatha and Nawani (2014),⁹ in a study conducted among 550 antenatal women in South India, reported an ASB prevalence of 7.3%. Totadhri et al.^[11] (2013), in a study of 200 pregnant women, reported a prevalence of 8.4%. Sonkar et al.^[10] (2012), in North India, evaluated 200 antenatal women and found a prevalence of 16.7%. Physiologically, pregnancy induces ureteral dilatation and reduced peristalsis due to progesterone-mediated smooth muscle relaxation. Mechanical compression by the gravid uterus further contributes to urinary stasis and vesicoureteral reflux. These changes facilitate bacterial ascent and persistence within the urinary tract. Nicolle (2003)⁵ emphasized that untreated ASB in pregnancy can progress to acute pyelonephritis in up to 20–40% of cases. In

populations with concurrent iron deficiency anaemia, impaired neutrophil oxidative killing, reduced macrophage function, and diminished cell-mediated immunity may further increase colonization rates. Studies by Cuttitta et al.¹² have suggested that anaemia may act as a predisposing factor for urinary infections due to compromised host defense mechanisms.

Age Distribution

In the present study, the majority of pregnant women belonged to the 20–30 years age group (73%), followed by >30 years (14%) and <20 years (13%). Among women diagnosed with asymptomatic bacteriuria (ASB), 53% were between 20–30 years, while 42% were above 30 years. Statistical analysis demonstrated a highly significant association between maternal age and ASB ($p = 0.0004$), indicating that age plays an important role in determining susceptibility to asymptomatic bacteriuria during pregnancy.

Although the highest absolute number of ASB cases occurred in the 20–30 years group due to larger representation in the study population, proportionally higher prevalence was observed among women aged >30 years (42% of ASB cases despite constituting only 14% of the total population). This suggests that advancing maternal age may independently increase the risk of bacteriuria.

Similar findings have been reported in several published studies. Webe et al. (2020),^[13] in a cross-sectional study involving 290 pregnant women in Ethiopia, reported an overall ASB prevalence of 16.9% and found that maternal age >30 years was significantly associated with increased risk ($p < 0.05$). Sonkar et al. (2012),^[10] in a study of 200 antenatal women in North India, also observed higher prevalence among women above 30 years. Fatima et al. (2017), in a study of 300 pregnant women, reported that advancing maternal age was associated with increased odds of bacteriuria, attributing this to cumulative exposure and parity-related factors.

Gestational Age Distribution

In the present study, the majority of pregnant women were between 30–34 weeks of gestation (41%), followed by 35–39 weeks (34%) and 25–29 weeks (25%). Among women diagnosed with asymptomatic bacteriuria (ASB), 53% belonged to the 30–34 weeks group, 37% to the 35–39 weeks group, and 11% to the 25–29 weeks group. Although a higher proportion of ASB cases were observed in the 30–34 weeks category, although a higher proportion of ASB cases were observed in the 30–34 weeks category, statistical analysis revealed that the association between gestational age and ASB was not statistically significant ($p = 0.2472$).

The absence of statistical significance indicates that gestational age did not independently influence the occurrence of asymptomatic bacteriuria in the present study population. Similar findings have been

reported in previous literature. Sonkar et al. (2012),^[10] in their study of 200 antenatal women, did not observe a statistically significant association between trimester and ASB prevalence. Likewise, Totadhri et al. (2013),^[11] reported that bacteriuria was detected across all trimesters without significant variation. Nicolle (2003) 5 emphasized that ASB may be identified at any stage of pregnancy and recommended universal screening irrespective of trimester.

From a clinical standpoint, the absence of statistical significance reinforces the recommendation that screening for asymptomatic bacteriuria should not be restricted to a particular trimester. International guidelines advocate routine urine culture screening at least once during early pregnancy, with repeat testing in high-risk populations. In settings with high prevalence of iron deficiency anaemia and urinary tract infections, integrating bacteriuria screening into routine antenatal evaluation throughout gestation may improve early detection and prevent complications such as pyelonephritis, preterm labour, and low birth weight.

Overall, the findings of the present study suggest that gestational age alone is not a determining factor for ASB; rather, pregnancy itself creates a sustained physiological predisposition to urinary tract colonization.

Hemoglobin Status (Categorical Analysis)

In the present study, moderate anaemia was more frequently observed among women with asymptomatic bacteriuria (74%) compared to those without bacteriuria (44%). Although the association did not reach statistical significance ($p = 0.0696$), the results demonstrate a trend toward increased prevalence of ASB among moderately anaemic women.

Iron plays an essential role in immune function, including neutrophil bactericidal activity and cytokine-mediated immune responses. Iron deficiency impairs host defense mechanisms and may predispose pregnant women to urinary tract colonization. Beard reported that iron deficiency reduces neutrophil oxidative burst and cellular immunity¹. Similarly, Cuttitta et al,^[12] demonstrated a significant association between iron deficiency anemia and asymptomatic bacteriuria in pregnancy². The borderline p-value in the present study may be attributed to the limited sample size, particularly the low number of severe anemia cases. With a larger sample, statistical significance might have been achieved. Therefore, while categorical analysis did not show statistical significance, the clinical trend and correlation analysis support a potential relationship between iron deficiency anaemia and asymptomatic bacteriuria.

Correlation Between Hemoglobin and ASB (Pearson Analysis)

In the present study, Pearson correlation analysis demonstrated a statistically significant moderate inverse correlation between hemoglobin levels and asymptomatic bacteriuria ($r = -0.359$, $p < 0.0001$).

The highly significant p-value indicates that the observed association is unlikely to be due to chance. Unlike categorical comparison, which did not reach statistical significance, continuous variable analysis revealed a meaningful relationship between declining hemoglobin levels and increasing likelihood of bacteriuria.

The negative correlation coefficient ($r = -0.359$) indicates that as hemoglobin levels decrease, the probability of asymptomatic bacteriuria increases. This inverse relationship suggests that anaemia may act as a risk-enhancing factor rather than merely coexisting with urinary tract colonization. The strength of correlation falls within the moderate range, implying that hemoglobin level has a clinically relevant association with ASB, though other contributing factors may also play a role.

Similar observations have been reported in previous studies. Sonkar et al. (2012),^[10] in their study of 200 antenatal women, found significantly lower mean hemoglobin levels among ASB-positive women compared to ASB-negative controls ($p < 0.05$). Cuttitta et al. identified iron deficiency anaemia as an independent predictor of urinary tract infection risk, highlighting the role of compromised immunity in anaemic individuals. Byna et al. (2015),^[14] also reported that pregnant women with hemoglobin levels below 10 g/dL had significantly higher odds of bacteriuria compared to those with normal levels. The statistically significant moderate inverse correlation observed in the present study strongly supports the primary hypothesis that iron deficiency anaemia is associated with increased risk of asymptomatic bacteriuria among pregnant women. The finding is particularly important because it suggests that correcting anaemia may not only improve maternal hematological status but also potentially reduce susceptibility to urinary tract colonization. Integrating anaemia management with routine bacteriuria screening could therefore provide dual preventive benefits in antenatal care.

Hemoglobin and Type of Bacterial Isolate

In the present study, among the 19 culture-positive cases, moderate anaemia was most commonly associated with *E. coli* isolates (54%), followed by *Staphylococcus aureus*, *Klebsiella* species, and *Staphylococcus saprophyticus*. The predominance of *E. coli* in moderately anaemic women is noteworthy, as *E. coli* is globally recognized as the leading uropathogen in pregnancy.

However, statistical analysis revealed that the association between hemoglobin level and specific bacterial organism was not statistically significant ($p = 0.2430$). This indicates that although moderate anaemia appeared more frequent among *E. coli* cases, the distribution of hemoglobin levels across different organisms did not differ significantly.

Similar findings have been reported in previous literature. Sujatha and Nawani (2014) 9 and Totadhri et al. (2013),^[11] both reported *E. coli* as the predominant isolate irrespective of hemoglobin status. Sonkar et al,^[10] observed that while anaemia

was more common among ASB-positive women, no specific bacterial species showed exclusive association with a particular hemoglobin category. These findings suggest that anaemia increases susceptibility to urinary tract colonization broadly, rather than selectively favoring a particular pathogen.

Overall, the findings suggest that iron deficiency anaemia predisposes pregnant women to urinary infection in general, rather than selectively influencing the type of uropathogen. Clinically, this reinforces the importance of addressing anaemia as a systemic risk factor for infection, while still relying on urine culture and sensitivity testing to guide organism specific treatment.

Microbiological Profile

In the present study, *Escherichia coli* was the predominant isolate, accounting for 8% of total cases and the majority of culture-positive samples, followed by *Staphylococcus aureus* (4%), *Klebsiella* species (4%), *Staphylococcus saprophyticus* (2%), and *Enterococcus* species (1%). Among the 19 culture-positive cases, *E. coli* constituted the largest proportion of isolates, confirming its dominant role in asymptomatic bacteriuria among pregnant women.

The predominance of *E. coli* in the present study is consistent with global epidemiological data. Numerous studies have reported *E. coli* as the leading causative organism in urinary tract infections during pregnancy. Sujatha and Nawani (2014) reported *E. coli* accounting for approximately 45–55% of isolates in antenatal women. Totadhri et al. (2013),^[11] observed *E. coli* in nearly 50% of culture-positive cases. Sonkar et al. (2012) documented *E. coli* in 42% of ASB cases. Internationally, Webe et al. (2020),^[13] reported *E. coli* as the predominant isolate (48%) among Ethiopian pregnant women. This consistent global predominance reinforces the pathogenic importance of *E. coli* in pregnancy-associated bacteriuria.

The presence of Gram-positive organisms such as *Staphylococcus aureus* and *Staphylococcus saprophyticus*, along with *Enterococcus* species, reflects regional microbial variation and possible community acquired colonization patterns. *Staphylococcus saprophyticus* is well known for causing urinary tract infections in young sexually active women. Studies by Fatima et al. and Totadhri et al.,^[11] also reported isolation of Gram-positive organisms, though at lower frequencies compared to *E. coli*. The detection of *Klebsiella* species further indicates the involvement of enteric organisms with potential hospital or community origins.

Understanding the microbial distribution is clinically important for guiding empirical antibiotic therapy, especially in settings where immediate culture results may not be available. Knowledge of prevalent organisms and their resistance trends assists clinicians in selecting appropriate first-line agents and supports antimicrobial stewardship efforts. Additionally, monitoring shifts in microbial

patterns over time helps in early detection of emerging resistant strains.

Overall, the microbiological profile observed in the present study aligns with established global trends, confirming *E. coli* as the principal uropathogen in pregnancy while also highlighting the presence of other Gram-negative and Gram-positive organisms contributing to asymptomatic bacteriuria.

Antibiotic Susceptibility Pattern

In the present study, Nitrofurantoin and Fosfomycin demonstrated the highest sensitivity (95%) among all tested isolates, indicating excellent efficacy against the predominant uropathogens. Out of 19 isolates, 18 were sensitive to Nitrofurantoin and 18 to Fosfomycin. This high sensitivity pattern is consistent with findings from several regional and international studies. Totadhri et al. (2013) reported Nitrofurantoin sensitivity of 85–95% among *E. coli* isolates. Sujatha and Nawani (2014) observed over 90% susceptibility to Nitrofurantoin in pregnant women with ASB. Similarly, Wabe et al. (2014) documented high effectiveness of Nitrofurantoin against Gram-negative uropathogens. Fosfomycin has also been increasingly recognized as an effective single-dose therapy for uncomplicated bacteriuria, with global susceptibility rates exceeding 90% in many surveillance studies. The preserved efficacy of these agents may be attributed to their limited overuse and specific urinary targeting.

Gentamicin (63%) and Piperacillin–Tazobactam (58%) also showed moderate to good sensitivity in the present study. Carbapenems such as Imipenem and Meropenem demonstrated 53% sensitivity. While these agents are effective against resistant organisms, their use in pregnancy is generally reserved for complicated infections due to cost, intravenous administration, and the need to prevent development of multidrug resistance. Studies by Fatima et al. and Sonkar et al. have similarly reported moderate sensitivity of aminoglycosides and beta-lactam combinations against Gram-negative isolates. However, aminoglycosides are used cautiously in pregnancy due to potential fetal ototoxicity with prolonged use.

High resistance was observed with Ciprofloxacin (5%), Azithromycin (0%), and Cefotaxime (0%), indicating limited utility of these agents in the current setting. Fluoroquinolone resistance among *E. coli* has been rising globally due to widespread community use. Sonkar et al. and Totadhri et al. reported increasing resistance to Ciprofloxacin ranging between 40–70% in Indian cohorts. Third-generation cephalosporin resistance, particularly Cefotaxime, may reflect emerging extended-spectrum betalactamase (ESBL) producing strains. Azithromycin and Novobiocin demonstrated negligible sensitivity, suggesting they are unsuitable for empirical treatment of ASB in pregnancy.

Nitrofurantoin remains a preferred first-line agent for asymptomatic bacteriuria in pregnancy due to several advantages: high urinary concentration, minimal systemic distribution, low resistance rates,

and relative safety profile. It is considered safe during most of pregnancy except near term (due to risk of hemolysis in G6PD-deficient neonates). International guidelines including those from ACOG and WHO support Nitrofurantoin as an appropriate agent for uncomplicated lower urinary infections in pregnancy. Fosfomycin is also increasingly recommended due to its single-dose regimen, good compliance, and high sensitivity rates.

The findings of the present study strongly emphasize the importance of culture-guided therapy rather than empirical antibiotic use. Rising antimicrobial resistance is a major global health concern, particularly in developing countries where over-the-counter antibiotic access is common. Routine urine culture and sensitivity testing in antenatal women not only ensures appropriate therapy but also reduces the risk of treatment failure, progression to pyelonephritis, and adverse pregnancy outcomes. Moreover, rational antibiotic selection helps preserve the efficacy of higher-generation drugs and supports antimicrobial stewardship programs.

Overall, the antibiotic susceptibility pattern observed in the present study reinforces the continued effectiveness of Nitrofurantoin and Fosfomycin as first-line agents for asymptomatic bacteriuria in pregnancy, while highlighting emerging resistance trends to fluoroquinolones and certain cephalosporins. Continuous surveillance of resistance patterns is essential to guide evidence-based treatment protocols. The present study provides a comprehensive evaluation of asymptomatic bacteriuria (ASB) and its correlation with iron deficiency anaemia among pregnant women.

Firstly, the study demonstrated a relatively high prevalence of asymptomatic bacteriuria (19%) among pregnant women. This indicates that nearly one-fifth of apparently healthy antenatal women harbored significant bacteriuria without urinary symptoms. This prevalence is clinically significant and reinforces the silent nature of ASB, which may remain undetected without routine screening. Given the known association of untreated ASB with acute pyelonephritis, preterm labor, low birth weight, and perinatal morbidity, the findings strongly support the incorporation of routine urine culture screening into antenatal care protocols.

Secondly, a statistically significant association was observed between maternal age and ASB ($p = 0.0004$). Although the majority of participants were between 20–30 years of age, proportionally higher prevalence was noted among women above 30 years. This suggests that increasing maternal age may act as an independent risk factor for bacteriuria, possibly due to higher parity, repeated exposure to infections, altered vaginal flora, and age-related decline in mucosal immunity. Identification of advanced maternal age as a risk determinant allows clinicians to classify older pregnant women as a

higher-risk subgroup requiring vigilant screening and follow-up.

In contrast, gestational age did not show a statistically significant association with ASB ($p = 0.2472$). Although a larger proportion of ASB cases were observed between 30–34 weeks, this distribution did not reach statistical significance. This suggests that susceptibility to bacteriuria persists throughout pregnancy and is not restricted to a particular trimester. Therefore, screening strategies should not be trimester-specific but rather universally applied during antenatal visits.

With respect to hematological parameters, categorical analysis of hemoglobin levels did not demonstrate a statistically significant association with ASB ($p = 0.1455$). However, descriptive trends showed that a higher proportion of ASB-positive women had moderate anaemia. More importantly, Pearson correlation analysis revealed a statistically significant moderate inverse correlation between hemoglobin levels and ASB ($r = -0.359$, $p < 0.0001$). The negative correlation coefficient indicates that as hemoglobin levels decrease, the likelihood of asymptomatic bacteriuria increases. This finding provides strong quantitative support for the hypothesis that iron deficiency anaemia increases susceptibility to urinary tract colonization. Pathophysiologically, iron plays a critical role in immune competence. Iron deficiency impairs neutrophil oxidative burst, reduces macrophage bactericidal activity, diminishes cytokine response, and compromises mucosal immunity. These immune alterations may predispose anaemic pregnant women to bacterial persistence in the urinary tract.^[19] The statistically significant inverse relationship observed in the present study substantiates this biological plausibility and strengthens the causal inference between iron deficiency anaemia and asymptomatic bacteriuria. Microbiologically, *Escherichia coli* emerged as the predominant pathogen, consistent with global epidemiological patterns of urinary tract infections. The ability of *E. coli* to produce adhesins, hemolysins, biofilms, and siderophores enhances its survival in iron-limited environments, which may further explain its predominance in anaemic populations. The identification of other Gram-negative and Gram-positive organisms reflects regional microbial diversity and underscores the importance of culture-based diagnosis.

Antibiotic susceptibility analysis demonstrated that Nitrofurantoin and Fosfomycin were highly effective first-line agents, showing 95% sensitivity among isolates. These findings align with current obstetric infectious disease guidelines recommending Nitrofurantoin for uncomplicated bacteriuria in pregnancy (except near term). Conversely, high resistance rates were observed with Ciprofloxacin, Azithromycin, and Cefotaxime, indicating limited utility of these agents and highlighting the growing concern of antimicrobial resistance.

Collectively, the study establishes:

- A significant burden of asymptomatic bacteriuria in pregnancy
- Maternal age as an important epidemiological determinant
- Iron deficiency anaemia as a statistically significant risk factor for ASB
- E. coli as the principal uropathogen
- Nitrofurantoin and Fosfomycin as effective empirical treatment options

Most importantly, the statistically significant inverse correlation between hemoglobin levels and ASB provides strong evidence supporting the central hypothesis of this research — that iron deficiency anaemia increases susceptibility to asymptomatic bacteriuria among pregnant women.

From a public health perspective, these findings emphasize the dual importance of:

1. Routine urine culture screening in antenatal care
 2. Early identification
 3. and correction of iron deficiency anaemia
- Addressing both conditions simultaneously may reduce maternal infectious morbidity, prevent obstetric complications, and improve perinatal outcomes.

CONCLUSION

The present study concludes that asymptomatic bacteriuria (ASB) is common among pregnant women, with a prevalence of 19% in the study population. *Escherichia coli* was the most common organism isolated. Nitrofurantoin and Fosfomycin were found to be the most effective antibiotics

The study emphasizes the importance of:

- Routine urine culture screening during pregnancy
- Early detection and treatment of asymptomatic bacteriuria
- Early identification and correction of iron deficiency anaemia
- Use of culture-guided antibiotic therapy
- Improving maternal hemoglobin levels along with routine screening may help reduce the risk

of asymptomatic bacteriuria and associated complications during pregnancy.

Conflict of Interest: None

Funding Support: Nil

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