



Bacterial Profile, Antimicrobial Susceptibility and Associated Factors of Asymptomatic Bacteriuria and Symptomatic Urinary Tract Infections Among Antenatal Women Attending Tertiary Care Hospital – A Prospective Study.

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Abstract

Introduction: Urinary tract infections (UTIs) are prevalent among pregnant women, with asymptomatic bacteriuria (ASB) being a primary concern. If untreated, approximately 25% of cases progress to clinical infection. The unique physiological and anatomical changes during pregnancy predispose antenatal women to these infections, which can adversely impact maternal and foetal outcomes. Routine screening and timely treatment can significantly reduce morbidity. **Methodology:** This prospective cross-sectional study was conducted at the Department of Microbiology, NRI Institute of Medical Sciences, Sangivalasa, Visakhapatnam, from November 2022 to December 2023. A total of 226 antenatal women attending the OPD of Obstetrics and Gynaecology at Anil Neerukonda Hospital were enrolled. Mid-stream clean-catch urine samples were collected and processed by semi-quantitative culture on Blood agar and CLED agar. Antibiotic susceptibility testing was performed by disc diffusion (Kirby-Bauer) as per CLSI guidelines. Drug resistance mechanisms (ESBL, MBL) were detected by confirmatory phenotypic tests. Obstetric outcomes were followed until delivery. Statistical analysis was performed using SPSS v20 and Chi-square test ($p < 0.05$ considered significant). **Results:** Out of 226 urine samples, 56 (24.7%) were culture positive. Asymptomatic bacteriuria accounted for 16.3% and symptomatic UTI for 8.4% of the study population. The majority of culture positive subjects were aged 20–30 years (80.3%), of lower socio-economic status (64.2%), and multigravida (60.7%). The second trimester had the highest culture positivity (58.92%). *Escherichia coli* was the predominant isolate (55.35%), followed by *Klebsiella pneumoniae* (14.28%) and *Staphylococcus aureus* (10.71%). Gram-negative bacilli constituted 76.7% of isolates. ESBL producers were found in 19.6% and MBL producers in 1.7% of organisms. Nitrofurantoin (94.33%) and Imipenem (97.6%) showed the highest sensitivity. Anaemia ($p=0.04$) and preeclampsia ($p=0.02$) showed significant association with bacteriuria. Low birth weight was significantly associated with bacteriuria ($p=0.05$). **Discussion:** The bacteriuria prevalence of 24.7% is consistent with other Indian and global studies. *E. coli* dominance and high ESBL rates underscore the need for local antibiogram-guided therapy. Nitrofurantoin remains the drug of choice for uncomplicated UTI in pregnancy. Significant associations with anaemia and preeclampsia highlight the importance of early detection and management of bacteriuria in antenatal women.

Keywords: Asymptomatic bacteriuria, Antenatal women, Antimicrobial susceptibility, *Escherichia coli*, ESBL, Pregnancy outcomes, Urinary tract infections.



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INTRODUCTION

Urinary tract infections (UTIs) represent a prevalent medical concern, particularly among pregnant women. The unique physiological and anatomical changes that occur during pregnancy predispose expectant mothers to these infections [1,2]. Urinary tract infections pose a significant risk to pregnant women, with asymptomatic bacteriuria (ASB) being a primary concern. If left untreated during pregnancy, approximately 25% of ASB cases progress to clinical infection [1].

To mitigate this risk, screening for ASB is recommended for all pregnant women at their initial prenatal visit, typically through a clean-catch mid-stream urine culture, as timely treatment can reduce the rate of clinical infection to 3% to 4% [1]. In non-pregnant women, the prevalence of ASB ranges from 5% to 6%, which closely aligns with estimates for pregnant

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women, ranging from 2% to 7%. ASB tends to be more frequent in parous women, individuals of low socioeconomic status, and carriers of sickle cell trait [1].

The pathogenesis of UTI in pregnancy is driven by physiological changes including hydroureter, reduced bladder tone, vesicoureteral reflux, and glucosuria, all of which facilitate bacterial ascension and growth [9,10,11]. The spectrum of organisms and their antibiotic susceptibility patterns are known to vary regionally and temporally, emphasising the need for institution-specific surveillance [12,13]. Emergence of ESBL and MBL producing organisms further complicates empirical therapy [14].

Our study therefore aims to assess the prevalence, clinical characteristics, microbiological profiles, patterns of antibiotic susceptibility, and impact on pregnancy outcomes of ASB and symptomatic UTIs in antenatal women attending a tertiary care hospital in Visakhapatnam.

Objectives of the study:

- To determine the prevalence of asymptomatic bacteriuria (ASB) and symptomatic UTI among antenatal women.
- To isolate and characterize bacterial pathogens responsible for ASB and symptomatic UTI.
- To determine antibiotic susceptibility patterns and detect drug resistance mechanisms (ESBL, MBL) among isolates.
- To analyze the impact of bacteriuria on maternal and foetal outcomes.

METHODOLOGY

This prospective cross-sectional study was conducted in the Department of Microbiology, NRI Institute of Medical Sciences (NRIIMS), Sangivalasa, Visakhapatnam, from November 2022 to December 2023. About 226 antenatal women attending the outpatient department (OPD) of Obstetrics and Gynaecology of Anil Neerukonda Hospital (ANH), Sangivalasa, Visakhapatnam, belonging to any gestational age and parity, were enrolled after obtaining informed consent. The sample size of 226 was calculated using the formula $n = z^2 pq / d^2$.

Inclusion Criteria:

- All antenatal women belonging to any gestational age and parity attending the Obstetrics and Gynaecology OPD.
- Subjects who provided written informed consent for enrolment in the study.

Exclusion Criteria:

- Antenatal women with prior antibiotic usage in the preceding month.
- Antenatal women who could not give consent.

Sample Collection and Transportation:

All antenatal women were instructed to collect 30 ml of mid-stream clean-catch urine sample in a wide-mouth sterile universal container after cleaning the periurethral area with soap and water and spreading the labia apart. Samples were transported immediately to the laboratory and processed within 1 hour of collection.

Examination of Urine Specimen:

Macroscopic examination:

Each urine sample was macroscopically inspected for colour, turbidity, and volume.

Microscopic examination:

Uncentrifuged urine wet mount was examined under the microscope for pus cells per high-power field (HPF); a count of more than 5 cells/HPF indicates UTI. **Urine dipstick test:** SIEMENS MULTISTIX® 10 SG Urinalysis strip was used to detect glucose, ketone bodies, specific gravity, blood, pH, protein, urobilinogen, nitrate, and leucocyte esterase.

Urine Culture:

Semi-quantitative urine cultures were performed on Blood agar and CLED agar (HIMEDIA Laboratories) using the standard loop method with a 4 mm (10 µL) nichrome wire loop holding 0.01 ml of urine. Cultures were incubated at 37°C for 18–24 hours. Colonies were counted using a colony counter and multiplied by 100 to obtain the number of colony forming units (CFU) per millilitre. Interpretation was done as per general guidelines for urine cultures (Table 1) [30].

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Table 1: General Interpretive Guidelines for Urine Cultures [30]

Result	Specimen / Clinical Condition	Work Up
$\geq 10^4$ CFU/ml of a single potential pathogen or for each of two potential pathogens	Clean catch mid-stream / pyelonephritis, acute cystitis, asymptomatic bacteriuria or catheterized urines	Identification of organism and appropriate antibiotic susceptibility testing
2 or 3 organism types with predominant growth of 1 organism and $<10^4$ of other	Clean catch mid-stream urine sample	Identification and susceptibility testing for predominating organisms
$\geq 10^3$ CFU/ml of a single potential pathogen	Clean catch mid-stream urine / Catheterized / Acute cystitis	Identification of organism and appropriate antibiotic susceptibility testing

Identification of Organisms:

Significant isolates were identified by standard microbiological techniques including Gram staining, colony morphology on CLED agar with Andrade indicator, and biochemical tests (catalase, coagulase, oxidase, indole, urease, citrate, triple sugar iron agar, MIU). *Staphylococcus aureus* was identified by tube and slide coagulase tests. *Enterococcus* species were identified by growth on Bile Esculin Agar (BEA).

Antimicrobial Susceptibility Testing (AST):

Antibiotic susceptibility testing was performed by disc diffusion method (Kirby-Bauer) on Mueller Hinton Agar (MHA) as per CLSI guidelines. The following antibiotics were tested: Nitrofurantoin (NIT), Cefotaxime (CTX), Ceftazidime (CAZ), Cefazolin (CZ), Piperacillin-Tazobactam (PIT), Ampicillin (AMP), Ciprofloxacin (CIP), Cotrimoxazole (COT), Gentamicin (GEN), Amoxicillin-Clavulanic acid (AMC), and Imipenem (IPM). Quality control was maintained using ATCC strains.

Detection of ESBL and MBL:

ESBL detection: Double Disc Synergy Test (DDST) was used. An increase of ≥ 5 mm in zone of inhibition around clavulanic acid combination discs confirms ESBL production as per CLSI guidelines. MBL detection: Disc diffusion test against Imipenem (10 µg) was used for screening. Imipenem-EDTA Combined Disc Test (CDT) was used as confirmatory test; augmentation of zone of inhibition ≥ 7 mm around the Imipenem-EDTA disc confirms MBL production.

Follow-up:

Women with bacteriuria were treated with appropriate antibiotics as per drug sensitivity patterns. Repeat cultures were obtained 2 weeks after completion of therapy. All antenatal women in both groups were followed until delivery for complications such as preeclampsia, IUGR, anaemia, and premature labour. Mode of birth, gestational age at delivery, and birth weight were recorded.

Statistical Analysis:

Data were entered in MS Excel and analysed using SPSS v20. Frequencies and percentages were used for categorical variables. Group comparisons were performed using Chi-square test. A p-value < 0.05 was considered statistically significant. All tests were two-tailed.

RESULTS

Maternal Age Distribution

In the distribution of maternal age, 7.1% of culture positive subjects were under 20 years, compared to 5.8% in culture negative subjects. The majority of both groups were aged 20–30 years (80.3% culture positive vs 80.5% culture negative). For those over 30 years, 12.5% were culture positive and 13.5% were culture negative (Table 2).

Table 2: Maternal Age Distribution among Study Population

Age Group (years)	Culture Positive Subjects	Culture Negative Subjects
<20	4 (7.1%)	10 (5.8%)
20–30	45 (80.3%)	137 (80.5%)
>30	7 (12.5%)	23 (13.5%)
Total	56 (100%)	170 (100%)

Socio-economic Status

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The study population was categorised by socio-economic status. Among culture positive subjects, 64.2% belonged to the lower socioeconomic class, 33.9% to the middle class, and 1.7% to the upper class. Corresponding figures for culture negative subjects were 62.9%, 34.7%, and 2.3% respectively (Table 3).

Table 3: Distribution of Study Population Based on Socio-economic Status

Socio-economic Status	Culture Positive Subjects	Culture Negative Subjects
Lower class	36 (64.2%)	107 (62.9%)
Middle class	19 (33.9%)	59 (34.7%)
Upper class	1 (1.7%)	4 (2.3%)
Total	56 (100%)	170 (100%)

Culture Positivity and Prevalence

Out of 226 urine samples, 56 (24.7%) were culture positive for bacteriuria. Among the study population, 16.3% had asymptomatic bacteriuria (ASB) while symptomatic UTI was present in 8.4% of subjects, accounting for 24.77% in total. The remaining 75.2% were culture negative (Table 4).

Table 4: Distribution of Study Population Based on Culture Positivity (n=226)

Study Population	Total	Percentage
Culture positive subjects	56	24.7%
Culture negative subjects	170	75.2%
Total	226	100%

Distribution Based on ASB and Symptomatic UTI

Among the 56 culture positive subjects, 37 (66.07%) had asymptomatic bacteriuria and 19 (33.9%) had symptomatic UTI. The highest culture positivity was observed in the second trimester (58.92%), followed by the third trimester (21.42%) and the first trimester (19.64%). Among culture positive subjects, 39.2% were primigravida and 60.7% were multigravida.

Table 5: Distribution of Culture Positive Subjects Based on ASB and Symptomatic UTI (n=56)

Culture Positive Subjects	Total	Percentage
Asymptomatic Bacteriuria	37	66.07%
Symptomatic UTI	19	33.9%
Total	56	100%

Screening Tests for Bacteriuria

Among culture positive subjects, 54 individuals had more than 5 pus cells per high-power field (HPF), whereas among culture negative subjects only 6 showed more than 5 pus cells/HPF. Nitrate reduction tests were positive in 34 culture positive cases and 3 culture negative cases. Urine albumin was positive in 18 culture positive and 22 culture negative subjects (Table 6).

Table 6: Screening Tests for Bacteriuria among Study Population (n=226)

Study Population	Pus Cells >5/HPF	Pus Cells <5/HPF	Nitrate Reduction Positive	Nitrate Reduction Negative	Albumin Positive	Urine Sugar Positive
Culture positive subjects	54	2	34	22	18	8
Culture negative subjects	6	164	3	167	22	3

Distribution of Culture Isolates

Escherichia coli was the highest isolated organism in our study, accounting for 55.35% of culture positive cases, followed by *Klebsiella pneumoniae* (14.28%), *Staphylococcus aureus* (10.71%), *Enterococcus faecalis* (7.14%), Coagulase Negative *Staphylococcus* – CONS (5.35%), *Pseudomonas aeruginosa* (3.57%), *Enterobacter cloacae* (1.78%), and *Proteus mirabilis* (1.78%). Gram-negative bacilli accounted for 76.7% of isolates, while Gram-positive cocci constituted 23.2% (Table 7).

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Table 7: Distribution of Culture Isolates (n=56)

Organism	Number	Percentage
Gram-negative isolates		
Escherichia coli	31	55.35%
Klebsiella pneumoniae	8	14.28%
Pseudomonas aeruginosa	2	3.57%
Enterobacter cloacae	1	1.78%
Proteus mirabilis	1	1.78%
Gram-positive isolates		
Staphylococcus aureus	6	10.71%
Enterococcus faecalis	4	7.14%
CONS	3	5.35%
Total	56	100%

Drug Resistance Mechanisms

Among the total organisms (n=56), 19.6% were Extended-Spectrum Beta Lactamase (ESBL) producers and 1.7% were Metallo Beta Lactamase (MBL) producers.

Antibiotic Susceptibility Pattern

The antibiotic susceptibility pattern of all isolates is summarised in Table 8. Nitrofurantoin showed the highest sensitivity (94.33%), followed by Imipenem (97.6%) and Piperacillin-Tazobactam (79.06%). High resistance was observed to Ampicillin (57.7%) and Cefazolin (56.09%). Ciprofloxacin sensitivity was 66.07% and Cotrimoxazole sensitivity was 74%.

Table 8: Antibiotic Susceptibility Pattern of Isolates

Antibiotic	Sensitive n (%)	Resistant n (%)	Total Tested
Nitrofurantoin (NIT)	50 (94.33%)	3 (5.66%)	53
Cefotaxime (CTX)	22 (53.6%)	19 (46.3%)	41
Ceftazidime (CAZ)	1 (50%)	1 (50%)	2
Cefazolin (CZ)	18 (43.9%)	23 (56.09%)	41
Piperacillin-Tazobactam (PIT)	34 (79.06%)	9 (20.93%)	43
Ampicillin (AMP)	19 (42.2%)	26 (57.7%)	45
Ciprofloxacin (CIP)	37 (66.07%)	19 (33.9%)	56
Cotrimoxazole (COT)	37 (74%)	13 (26%)	50
Gentamicin (GEN)	43 (76.78%)	13 (23.21%)	56
Amoxicillin-Clavulanic acid (AMC)	37 (69.8%)	16 (30.1%)	53
Imipenem (IPM)	42 (97.6%)	1 (2.3%)	43

Maternal Outcome – Anaemia

Among culture positive subjects, 64.2% (36/56) were anaemic compared to 75.2% (128/170) of culture negative subjects. A Chi-square test of 37.6 with a p-value of 0.04 indicated a statistically significant association between bacteriuria and anaemia (Table 9).

Table 9: Comparison of Anaemia among Culture Positive and Culture Negative Subjects

Haemoglobin (g/dl)	Culture Positive Subjects	Culture Negative Subjects
With Anaemia	36 (64.2%)	128 (75.2%)
Without Anaemia	20 (35.7%)	42 (24.7%)
Total	56 (100%)	170 (100%)
Statistical test	Chi-square: 37.6	p-value: 0.04

Maternal Outcome – Preeclampsia

Among culture positive subjects, 3.57% (2/56) were diagnosed with preeclampsia compared to 2.35% (4/170) of culture negative subjects. Chi-square test yielded a statistic of 22.8 with p-value 0.02, indicating a statistically significant association between bacteriuria and preeclampsia (Table 10).

Table 10: Comparison of Preeclampsia among Culture Positive and Culture Negative Subjects

Pre-eclampsia	Culture Positive Subjects	Culture Negative Subjects
With Pre-eclampsia	2 (3.57%)	4 (2.35%)
Without Pre-eclampsia	54 (96.4%)	166 (97.6%)
Total	56 (100%)	170 (100%)
Statistical test	Chi-square: 22.8	p-value: 0.02

Foetal Outcomes – IUGR, PROM, Pre-term Birth, and Low Birth Weight

IUGR was found in 14.28% of culture positive subjects compared to 5.59% in culture negative subjects (Chi-square 11.5, $p=0.08$ – not significant). PROM was present in 8.16% of culture positive and 4.34% of culture negative subjects (Chi-square 15.9, $p=0.09$ – not significant). Pre-term births occurred in 10.20% of culture positive and 8.07% of culture negative subjects (Chi-square 13.6, $p=0.21$ – not significant). Low birth weight (<2.5 kg) was noted in 17.8% of culture positive subjects versus 12.9% of culture negative subjects, with a statistically significant association (Chi-square 34.2, $p=0.05$) (Table 11).

Table 11: Comparison of Foetal Outcomes among Culture Positive and Negative Subjects

Outcome	Culture Positive n (%)	Culture Negative n (%)	Chi-square	p-value
IUGR	7 (14.28%)	9 (5.59%)	11.5	0.08
PROM	4 (8.16%)	7 (4.34%)	15.9	0.09
Pre-term birth	5 (10.20%)	13 (8.07%)	13.6	0.21
Low birth weight	10 (17.8%)	22 (12.9%)	34.2	0.05*

*Statistically significant ($p < 0.05$)

DISCUSSION

The present study aimed to determine the prevalence, clinical, and microbial profile of asymptomatic bacteriuria and symptomatic urinary tract infections (UTIs) in antenatal women attending a tertiary care hospital, along with the impact of these infections on pregnancy and foetal outcomes. A total of 226 antenatal women were included in the study conducted from November 2022 to December 2023.

Prevalence of Bacteriuria

Out of 226 urine samples, 56 (24.7%) were culture positive for bacteriuria. Notably, in our study, 16.3% had asymptomatic bacteriuria and 8.4% exhibited symptomatic UTI. This prevalence aligns with previous studies; a study conducted in Dhaka by Parveen K et al [16] also showed a bacteriuria prevalence of 26%, while Ranjan et al [17] reported 35% in India, and Kant et al [18] noted 33.3%. Various studies show ASB among antenatal patients ranging from 11.33% to 19%, with Irum Shehzadi et al [19] reporting 14.2% in Abbottabad and Abha Gupta et al [20] reporting 13.8% (Table 12).

Table 12: Comparison of Prevalence of ASB and Symptomatic UTI among Studies

Studies	Prevalence of ASB	Prevalence of Symptomatic UTI
Our study	16.3%	8.4%
Irum Shehzadi et al [19]	14.2%	7%
Abha Gupta et al [20]	13.8%	9.2%
Paresh Patel et al [21]	13.8%	–
Kerure RD et al [29]	14.8%	8.1%
Parveen K et al [16]	26%	–

Socio-economic Status and Gravida Distribution

A higher proportion of culture positive subjects (64.2%) belonged to the lower socioeconomic status, consistent with several studies in literature that correlate lower socioeconomic status with higher incidence of bacteriuria [15,16,17], potentially due to limited access to healthcare and sanitation facilities. Among culture positive subjects, 60.7% were multigravida, indicating a higher prevalence of bacteriuria among multigravida women, possibly due to anatomical and physiological changes from previous pregnancies. Parveen K et al [16] reported that multiparity is associated with increased urinary tract infection in pregnancy, while Thakur A et al [22] reported higher bacteriuria among primigravida (58.5%).

Period of Gestation

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In the study, the majority (63.7%) of participants were in their second trimester. Among culture positive subjects, 58.92% were in their second trimester. This distribution suggests the second trimester is the most important time for the development of bacteriuria, presumably as a result of structural changes in the urinary system during this period.

Screening Tests

Screening tests showed that 54 culture positive subjects had more than 5 pus cells/HPF. Nitrate reduction tests were positive in 34 cases. Urine albumin was positive in 18 culture positive cases. Among culture negative subjects, most had fewer than 5 pus cells/HPF and nitrate reduction tests were largely negative. This emphasises the utility of simple screening tests like pus cell count and nitrate reduction in identifying bacteriuria. Studies have shown that the nitrate reduction test exhibits good sensitivity in detecting nitrate-reducing bacteria in urine samples [24,25].

Distribution of Culture Organisms

Gram-negative bacilli accounted for 76.7% of isolates, while Gram-positive cocci made up 23.2% in our study population. *Escherichia coli* was the highest isolated organism (55.35%), consistent with numerous studies identifying *E. coli* as the predominant pathogen in urinary tract infections, primarily due to its ability to adhere to the uroepithelium and form biofilms enhancing its virulence. Paresh Patel et al [21] had contradicting results where Gram-positive isolates were more predominant, while Sabri M et al [26] reported higher incidence of Gram-negative bacilli consistent with our study. Gargi Mudey et al [28] concluded in their study that Gram-negative bacteria in pregnant women with significant bacteriuria showed highest susceptibility to amikacin, gentamicin, imipenem, and meropenem, while resistance was observed against ceftazidime, cotrimoxazole, and ciprofloxacin.

Antibiotic Susceptibility

The antibiotic susceptibility pattern among bacteriuric pregnant women showed *E. coli* as the prevalent pathogen with highest susceptibility rates to Nitrofurantoin and Nalidixic Acid as reported by Ilusanya OA et al [23]. In our study, Nitrofurantoin (94.33%) and Imipenem (97.6%) showed the highest sensitivity. Significant resistance was observed to Ampicillin (57.7%), Cefazolin (56.09%), and Ceftazidime (50%). Another study concluded that Gram-negative bacteria were prevalent with 95% being multidrug-resistant [27]. The high prevalence of ESBL producers (19.6%) underscores the need for antibiotic stewardship and avoidance of empirical third-generation cephalosporins.

Comparison Between Preeclampsia, IUGR, PROM, and Foetal Outcomes

The study further analysed the correlation between maternal infections and adverse pregnancy outcomes. Among the women with culture-positive UTIs, 3.57% developed preeclampsia, 14.28% had IUGR, and 8.16% experienced PROM. Statistical analysis showed that the presence of UTI significantly increased the risk of preeclampsia ($p < 0.05$). Pre-term births were higher among culture positive subjects (10.20%) compared to culture negative subjects (8.07%). Low birth weight (<2.5 kg) was significantly more common in culture positive subjects (17.8%) compared to culture negative subjects (12.9%), with statistical significance ($p=0.05$). Adverse maternal and foetal outcomes including low birth weight, premature membrane rupture, preterm labour, and increased risk of conditions like hypertension and preeclampsia have been associated with the presence of asymptomatic bacteriuria in pregnant women [19-21].

CONCLUSION

This study highlights the significant prevalence of bacteriuria (24.7%) among antenatal women attending a tertiary care hospital in Visakhapatnam, with a higher incidence in those of lower socioeconomic status and multigravida. Asymptomatic bacteriuria was more common than symptomatic UTI, necessitating routine screening of all antenatal women. *Escherichia coli* was the predominant isolate, with high ESBL prevalence (19.6%) underscoring the need for antibiotic stewardship. Nitrofurantoin remains the most effective drug for UTI in pregnancy. Significant associations were found between bacteriuria and anaemia ($p=0.04$), preeclampsia ($p=0.02$), and low birth weight ($p=0.05$). Adverse pregnancy outcomes, including pre-term birth, low birth weight, and preeclampsia, emphasise the importance of early detection and treatment. Regular screening, effective management of anaemia, and appropriate antibiotic use are critical to improving maternal and foetal outcomes in antenatal women with bacteriuria.

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