

Antibiotic Susceptibility Profile of Uropathogens in Pregnant Women with Asymptomatic Bacteriuria

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Date of Submission: 01-12-2023

Date of Acceptance: 10-12-2023

I. INTRODUCTION

Urinary tract infections [UTI] in pregnancy are more common among antenatal women because of the physiological and morphological changes that occur during pregnancy. Asymptomatic bacteriuria if not diagnosed earlier and treated properly may lead to maternal complications like pyelonephritis, post partum UTI, hypertension disorders and neonatal complications like prematurity, low birth weight, intrauterine growth retardation and prenatal death etc.¹ In pregnancy physiological changes like increase in plasma volume, decrease urine concentration leading to decreased ability of the lower urinary tract to resist invading microorganisms.² Significant bacteriuria is defined as the presence of $>10^5$ colony forming units (CFU)/ml of bacteria in the urine sample.

More than 85% of cases of urinary tract infection are caused by the gram negative bacteria that are normal inhabitants of the intestinal tract. For most urinary tract infections, the infecting organisms from the patients own fecal flora. By far the most common is Escherichia coli, followed by Proteus, Klebsiella, and Enterobacter. Streptococcus faecialis, also of enteric origin , Staphylococci, and other organisms cause Urinary tract infections.¹¹

Approximately 2%-11% of pregnant are found asymptomatic women with 20%-40% bacteriuria.Without treatment of pregnant women with asymptomatic bacteriuria will develop symptomatic UTI like pyelonephritis.³ Bacteriuria is a significant cause of morbidity in pregnant women affecting both women and fetus.^{4,5} Hence screening and treatment of asymptomatic bacteriuria should be incorporated as a routine in antenatal care along with health education, personal hygiene and nutrition.

Majority of the UTI are symptomatic and the patients will become aware of it and take the necessary treatment. As mentioned above 2%-11% of pregnant women are found with asymptomatic bacteriuria. These women will be unaware of this infections and if the condition is not diagnosed, it will lead to many complications.

Therefore, to determine the prevalence of asymptomatic bacteriuria in pregnant women, to

find out the major causative agents of UTI and the antibiotic susceptibility of the causative agents in order to find out which antibiotics is more effective against the causative agent, in order to overcome the complications caused by UTI in pregnancy, this study is being conducted.

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This study was done by collecting a total of 100 mid-stream clean catch urine samples from the sample collection center of those asymptomatic antenatal patients (women whose age ranges between 18 to 45 years) attending Outpatient Department of Obstetrics & Gynecology at District Hospital Of Chamarajanagar, Chamarajanagar. The study was done for 2 months (August and September). The samples were collected only after taking the patient's consent. Relevant case history was taken from the antenatal patients.

II. REVIEW OF LITERATURE

Shalima S, Kalaivani R, Seethesh G, See tha KS et al ¹, did a study to determine the prevalence, bacterial profile and asymptomatic bacteriuria among pregnant women visiting a tertiary hospital in Pondicherry. 637 mid stream urine samples from asymptomatic pregnant women were collected and were immediately processed onto appropriate culture media(onto blood agar and Cysteine Lactose Electrolyte Deficient(CLED) agar). Antimicrobial susceptibility testing was done by Kirby bauer's disc diffusion method. The culturing methods & antibiotic susceptibility testing method done is similar to my study work. Out of 637 samples, 315 (49.5%) were found to have bacteriuria. The most predominant pathogens isolated were Gram negative bacilli, of which 56% were E.coli followed by others. The isolates showed very significant amount of resistance towards all available group of drugs.

B.Prasanna, M Naimisha, K Swathi and Mahaboob V Shaik et al², did a study to determine the prevalence of Asymptomatic bacteriuria in pregnant women, isolates & their culture sensitivity pattern. 500 urine sample were collected from the antenatal OPD in Narayana Medical College Hospital, Nellore for a period of 2 years. Pregnant women with UTI symptoms, history of past UTI , patients with diabetes , hypertension and other preexisting medical disorders urina samples were not



collected. All sample were cultured on blood agar, Nutrient agar plates. Antibiotic susceptibility test was done using Kirby-Baeur's disc diffusion method. The culturing methods & antibiotic susceptibility testing method done is similar to my study work. The exclusion criteria followed in this study is similar to my study. Out of 500 samples, 85 samples (17%) were positive for significant bacteriuria. E.coli was the commonest organism(62%)followed by Klebsiella species(18%) & Staphylococcus species(12%). Most of the isolates were sensitive ton drugs like Meropenam(90%). Amikacin(80%), Nitrofurantoin(76%), & Norfloxacin(72%).

Kheya Mukherjee, Saroj Golia, Vasudha CL, Babita, Debojyoti Bhattacharjee, Goutam Chakroborti et al 3^{3} , did a study on prevalence, etiology of asymptomatic bacteriuria in pregnancy and comparison of screening methods. 250 samples of urine was collected from asymptomatic pregnant women visiting a tertiary care hospital in Bangalore, for a period of 1 year(November 2008-November 2009). Urine samples were inoculated on CLED agar by a semi quantitative method .The isolates were further identified on the basis of colonial morphology ,cultural characteristics, gram's staining & biochemical reactions. The antibiotic susceptibility test of isolates was performed on Mueller Hilton agar plates by Kirby Bauer disc diffusion method. The culturing methods & antibiotic susceptibility testing method done is similar to my study work. The inclusion & exclusion criteria followed in this study is similar to my study. Out of the 250 pregnant women, 21(8.4%) had significant bacteriuria. E.coli (57.14%) was the most commonest isolates. gram staining Among screening of tests uncentrifuged urine had a sensitivity of (85.71%).

Jyoti Jojan, Milind Davane, Chandrakala Dawle (Patil), Basavraj Nagoba et al⁴, did a study to find out the prevalence of UTI in asymptomatic pregnant women from rural area of Latur district of Maharashtra, India and to study the antimicrobial susceptibility pattern of the isolated bacteria. A total of 100urine samples from asymptomatic antenatal care cases were screened for significant bacteriuria by using standard procedures. The bacterial isolates wrer subjected to antimicrobial susceptibility studies. The culturing methods & antibiotic susceptibility testing method done is similar to my study work. Significant bacteriuria wass found positive in 23% of the cases. S.aureus was found to be the most common uropathogen. Imipenam and meropenam(82.60% each) were found to be the most effective antimicrobial agents.

Alemu A, Moges F, Shiferaw Y, Tafess K, Kassu A, Anagaw B, Agegn A et al⁷, did a study to assess bacterial profile that causes urinary tract infection and their antimicrobial susceptibility pattern among pregnant women visiting antenatal clinic at University of Gondar teaching Hospital ,Northwest Ethopia from march 22 to april 30,2011. Midstream urine samples were collected and inoculated into Cysteine Lactose Electrolyte Deficient medium(CLED), mac conkey and blood agar plates.Identification of isolates was done by a series of biochemical tests and morphology of isolates. The bacterial isolates wrer subjected to antimicrobial susceptibility studies. The culturing methods & antibiotic susceptibility testing method done is similar to my study work. 10.4% of samples was positive with significant Bacteriuria. The predominant bacterila pathogens were Staphylococcus E.coli(47.5%) followed by aureus(32.5%) and Klebsiella pneumonia(10%).gram negative isolates were resulted low susceptibility to cotrimoxazole(51.9%) and teratcycline(40.7%) where as Gram positive showed susceptibility to ceftriaxon(84.6%) & amoxicillin-clavulinic acid(92.3%). Multiple drug resistance was observed in 95% of the isolates.

In all these study works, the methodology followed were similar, but they differed in the sample sizes . Most of the study works found out E.coli to be predominant uropathogen.

Aims and Objectives

To study the prevalence of asymptomatic bacteriuria and it's antibiotic susceptibility among pregnant women.

III. MATERIALS AND METHODS

This study is of research type.

A total of 100 Mid-stream catch urine samples was collected from the sample collection center of those asymptomatic antenatal patients (whose age ranges between 18 to 45 years) attending Outpatient Department (OPD) of Obstetrics & Gynecology at District Hospital Of Chamarajanagar, Chamarajanagar; after taking their consent for a duration of 2 months (August to September).Relevant case history was taken from the antenatal patients.

The patient was told about the purpose of collecting the urine sample and then only their consent was taken by making them sign the consent form.

About 20ml of each urine sample were collected in a sterile universal container. All the samples were properly labelled, transported to microbiology laboratory within 1 hour. In case of



delay, the samples were refrigerated at 4° C for as long as 24 hours. The urine samples were observed macroscopically for its color, turbidity & deposits and the findings was recorded & the samples were subjected to urine culture at microbiology laboratory.

Screening procedures

A drop of well-mixed urine was smeared on slide, allowed to air-dry, fixed, stained & examination under oil immersion. Presence of atleast one organism per oil immersion field (examining 20 fields) correlates with significant bacteriuria. The specimens were inoculated by standard loop technique on Cysteine Lactose Electrolyte Deficient medium(CLED), blood agar & Mac Conkey agar. A caliberated loop (with internal diameter of three mm delivering 0.001ml of urine) was used. The plates were inoculated at 37[°]C for 18-24 hours in an incubator. After incubation, the colonies were counted on each plate & the number of bacteria present in urine were calculated by multiplying the number of colonies by 1000. Specimens of urine showing $>10^5$ CFU/ml was considered and each colony was further identified on the basis of colony morphology, cultural characteristics, Gram's staining and a battery of biochemical tests.

The antimicrobial susceptibility pattern of each isolate was studied by Kirby-Bauer's disc diffusion method. The antibiotics was employed as per CLSI (Clinical and Laboratory Standards Institute) guidelines⁶.

The following antibiotics were used in the antibiotic susceptibility testing-

- 1. Amikacin (AMK) (30mcg)
- 2. Ampicillin (AMP) (30mcg)
- 3. Ceftazidime (CAZ) (30 mcg)
- 4. Ceftazidime/Clavulanic acid (CAZ/CLA) (30mcg)
- 5. Cephotaxime (CTX) (30mcg)
- 6. Cotrimoxazole (SXT) (30 mcg)
- 7. Ciprofloxacin (CIP) (30 mcg)
- 8. Imipenam (IPM) (10 mcg)
- 9. Piperacillin/Tazobactam (TZP) (30 mcg)
- 10. Tetracycline (TCY) (30 mcg)
- 11. Levofloxacin (LE) (30 mcg)
- 12. Cefixime (CFM) (30 mcg)
- 13. Clindamycin (CLI) (30 mcg)
- 14. Erythromycin (ERY) (30 mcg)
- 15. Nitrofurantoin (NF) (30 mcg)
- 16. Norfloxacin (NX) (30 mcg)

- 17. Teicoplanin (TEC) (15 mcg)
- 18. Vancomycin (VAN) (30 mcg)
- 19. Linezolid (LNZ) (30 mcg)
- 20. Amoxyclav(AMC) (30 mcg)
- Samples were collected on the basis of certain criterias-

Inclusion criteria

- 1. Pregnant women with varying gestational periods attending the antenatal clinic without a history of dysuria , loin pain, or increased frequency of micturition.
- 2. Patients not treated earlier with antibiotics, parenteral or local application for at least preceding one month before her attendance to the hospital.

Exclusion criteria

- 1. Pregnant women <18 years and >45 years of age.
- 2. Patients with vaginal discharge or bleeding per vagina.
- 3. Pregnancy included diabetes mellitus/hypertension or any other pre-existing medical comditions or congeniatl anamolies.

MS-Word and MS-Excel is used for data analysis.

Observations and Results

Out of 100 urine samples collected from asymptomatic pregnant women, 14 of the urine samples were positive for significant bacteriuria i.e out of 100, 14 pregnant women were having asymptomatic bacteriuria & remaining 86 samples were sterile (Table 1, figure 1)

Among the 14 uropathogens (100%) isolated, the predominant uropathogen was 6 Staphylococcus aureus (42.8%), followed by 4 (28.6%) E.coli & 4(28.6%) Klebsiella species. (table 2, figure 2)

Out of 14 (100%) pregnant women with asymptomatic bacteriuria, 9 (64.2%) of them were in 1^{st} trimester, 2 (14.2%) of them were in 2^{nd} trimester, 3 (21.6%) of them were in 3^{rd} trimester.(Table 3, figure 3)

Out of 14 (100%) pregnant women with asymptomatic bacteriuria, 6 (42.8%) of them are primigravida, 8 (57.2%) of them are multigravida. (Table 4)

Out of 14 (100%) pregnant women with asymptomatic bacteriuria, 5 (35.7%) of them are aged between 18 to 20years. 9 (64.3%) of them are aged between 21 to 30 years. (Table 5, figure 4).



International Journal Dental and Medical Sciences Research Volume 5, Issue 6, Nov-Dec 2023 pp 128-138 www.ijdmsrjournal.com ISSN: 2582-6018

Table 1				
Total	No.of sterile samples	No.of samples with ASB		
No.of				
samples				
100	86	14		
(100%)	(86%)	(14%)		



Table 2				
Uropathogens causing ASB	S.aureus	E.coli	Klebsiella species	
Prevalence	42.8%	28.6%	28.6%	
(in %)				







Table 3 shows distribution of ASB based on various gestational weeks				
Gestational age	1 st trimester	2 nd trimester	3 rd trimester	
Distribution (in%)	64.2	14.2	21.6	

Figure3



Table 4 shows prevalence of Asymptomatic bacteriuria among Primigravida & Multigravida women

Gravida of	Primigravida	Multigravida
women		
Prevalence	42.85%	57.15%
(in %)		

Table 5 : Age Group-wise Distribution of Cases of Significant Bacteriuria among Asymptomatic Groups

Age Group in Years	Number of cases positive for significant Bacteriuria	Percentage (in %)
18-20 years	5	35.71
21-30 years	9	64.29
>30 years	0	0





Name of the	S.aurues	0	E.coli	• •	Klebseilla	
antibiotics	No.of isola	ates(6)	No.of isolates(4)		Species	
				No.of isolates(4)		
	s	R	S	R	S	R
AMK	6	0	4	0	4	0
AMP	-	-	2	2	0	4
CAZ	-	-	1	3	4	0
CAZ/	-	-	3	1	4	0
CLA						
CTX	-	-	2	2	3	1
SXT	5	1	3	1	4	0
CIP	4	2	2	2	4	0
IPM	-	-	4	0	4	0
TZP	-	-	4	0	2	2
TCY	-	-	2	2	1	3
LE	4	2	3	1	4	0
CFM	3	3	2	2	2	2
CLI	4	2	-	-	-	-
ERY	4	2	-	-	-	-
NF	5	1	3	1	3	1
NX	4	2	2	2	3	1
TEC	5	1	-	-	-	-
VAN	6	0	-	-	-	-
LNZ	6	0	-	-	-	-
AMC	-	-	1	3	2	2

Table 6 showing the Antibiotic susceptibility pattern of the isolates

S-Sensitive

R-Resistant





Figure 5 shows Antibiotic susceptibility pattern of Staphylococcus aureus (y axis= no.of isolates; x axis = antibiotics)

Figure 6 shows Antibiotic susceptibility pattern of E.coli (y axis= no.of isolates; x axis = antibiotics)







Figure 7 shows Antibiotic susceptibility pattern of Klebsiella species(y axis= no.of isolates; x axis = antibiotics)

Name of	S.aureus	<u>E.coli</u>	Klebsiella
antibiotics	Resisitance(%)	Resistance(%)	species
antibiotics			Resistance(%)
AMK	0	0	0
AMP	-	50	100
CAZ	-	75	0
CAZ/	-	25	0
CLA			
CTX	-	50	0
SXT	16.6	25	0
CIP	33.3	50	0
IPM	-	0	0
TZP	-	0	0
TCY	-	50	0
LE	33.3	25	0
CFM	50	50	0
CLI	33.3	-	-
ERY	33.3	-	-
NF	16.6	0	25
NX	33.3	50	0
TEC	16.6	-	-
VAN	0	-	-
LNZ	0	-	-
AMC	-	75	50

Table 7 below shows pattern of Antibiotic Resistance (in %) by the isolates



IV. DISCUSSIONS

Asymptomatic bacteriuria causing Urinary tract infections in pregnant women is a emerging problem in today's society. It leads to many complications such as pyelonephritis, cystitis ,still births, low birth babies, etc. It is harmful for both mother and fetus. Chances of UTI increases with advancing age & increase in bacterial load. It is one of the main reasons for mother and neonate's morbidity.

This study was conducted to find out the asymptomatic bacteriuria prevalence among antenatal women and its antibiotic susceptibility so that the findings can aid the future antenatal care and their treatment and it also signifies the importance of urine analysis in antenatal care. This study also aimed to find out better effective antibiotics against the causative agents of UTI.

study shows that Asymptomatic This bacteriuria is prevalent in 14 % of pregnant women (table 1) . The findings are slightly similar to study done by B.Prasanna et al , where prevalence of Asymptomatic bacteriuria among pregnant women was $17\%^2$, the study done by Kheya Mukherjee et al, showed prevalence of Asymptomatic bacteriuria among pregnant women was 8.4 $\%^{3}$, the study done by Alemu A et al, showed prevalence of Asymptomatic bacteriuria among pregnant women was 10.4 %⁷. So compared to these studies, the prevalence of Asymptomatic bacteriuria has increased. The reasons for the increased prevalence is due to unhygeine habits of the women, low socioeconomic status, due to lack of cleaniness of the urogenital parts , sexual practices & surrounding environmental conditions.

This study shows that the predominant pathogen causing Asymptomatic bacteriuria (UTI) in pregnant women is Staphylococcus aureus (table 2).The other isolates were E.coli & Klebsiella species. Similar findings was observed by study done by Jyoti Jojan et al ^{4.} Majority of the studies showed that E.coli is the main causative agent of UTI ^(1,2,3,7). This shows that there are other bacterias which are emerging as major causitive agents of UTI apart from E.coli.

This study shows that Asymptomatic bacteriuria is more prevalent in 1^{st} trimester (64.2%), followed by 3^{rd} trimester (21.6%) and then 2^{nd} trimester (14.2%). The study done by B.Prasanna et al, showed that the Asymptomatic bacteriuria is more prevalent in 3^{rd} trimester, followed by 1^{st} & 2^{nd} trimester². Other study done by Shalima et al ¹ showed that Asymptomatic bacteriuria more prevalent in 3^{rd} trimester, followed by 1^{st} trimester which is fairly similar to

this study's findings. The reason for this finding is because the risk of UTI, in pregnancy increases during beginning of 6 weeks and peaks during 22 to 24 weeks¹. The other reasons is that during pregnancy ,as the gestational week increases there is an increase in size of the uterus and hormonal changes which favours the environment for microbial infection.⁴.

This study shows that Asymptomatic bacteriuria is more prevalent in mutigravida women. The study done by B.Prasanna et al ², showed that the Asymptomatic bacteriuria is more prevalent in mutigravida women. The findings can be due to repeated deliveries, hormonal changes & due to changes taking place in the uterus due to repeated pregnancy.

This study shows that Asymptomatic bacteriuria is more prevalent in women with age between 21 to 30 years old (64.29%), followed by women with ages between 18 to 20 years old (35.71%). The studies made by Shalima S et al ¹ & Jyoti Jojan et al⁴, shows similar findings. This high incidence of Asymptomatic Bacteriuria in the active reproductive age group is due to early pregnancy and multiparity. Many studies show advancing age as a risk factor for acquiring Asymptomatic Bacteriuria in pregnancy because there is decrease in glycogen deposition and reduction in the lactobacillus as a part of ageing process which enhances bacterial adherence and invasion by pathogens and make them more susceptible²

The antibiotic susceptibility test is done for the isolates.

This study shows that Amikacin, Tecoplanin , Linezolid, Cotrimoxazole, Nitrofurantoin & Vancomycin are highly effective Staphylococcus against aureus isolates .. Staphylococcus aureus isolates showed some resistance (in %) against some of the antibiotics such as Cefixime(50%), Clindamycin (33.3%) & Norfloxacin(33.33%) & Erythromycin (33.3%). The increased resistance towards some of the antibiotics can be due to prolonged intake of same antibiotic or it can also be due to some mechanism of the isolates (eg:. Mutated efflux pump system of the bacteria). Study done by Jyoti Jojan et al⁴, showed that imipenam and meropenam are effective against Staphylococcus aureus.

Amikacin , Imipenam, Piperacillin +Tazobactam, are very effective against isolates of E.coli. The isolates of E.coli were resistant (shown in %) to some of the antibiotics such as Cotrimoxazole (25%), Tetracycline (50%), Levofloxacin (25%), Nitrofurantoin (25%) , Ampicillin(50%), Norfloxacin(50%). Maximum



resistance by E.coli was shown against Amoxyclav(75%). The increased resistance towards some of the antibiotics can be due to prolonged intake of same antibiotic or it can also be due to some mechanism of the isolates (eg:. Mutated efflux pump system of the bacteria). Study done by Kheya Mukherjee et al³, showed that Ciprofloxacin is very effective against E.coli.

This study shows that antibiotics such as Ceftazidime/Clavulinic Amikacin, acid. Cotrimoxazole, Ciprofloxacin Imipenam . &Levofloxacin are highly effective against Klebsiella species. However, Klebsiella species show resistance (shown in %) towards Ampicillin (100%), Nitrofurantoin (25%), Norfloxacin (25%), Amoxyclav(50%), Tetracycline (75%) . The increased resistance towards some of the antibiotics can be due to prolonged intake of same antibiotic or it can also be due to some mechanism of the isolates (eg:. Mutated efflux pump system of the bacteria).

V. CONCLUSIONS

After conducting the study, it is found out that there is an increase in prevalence of Asymptomatic bacteriuria among pregnant women. Majority of the studies showed E.coli as the predominant causative uropathogen, but this study showed there are other organisms (Staphylococcus aureus) which are emerging as predominant uropathogen potentially equal to E.coli. It is found out that Asymptomatic bacteriuria is more prevalent in women with age group 21-30 years & risk of asymptomatic bacteriuria also increases with advancing age and in multiparous women. This study also found out that there is an increase in resistance of the uropathogens against some antibiotics. Antibiotic susceptibility test was done. Ceftazidime/Clavulinic Overall, acid Ciprofloxacin, Vancomycin, Amikacin. Piperacillin+tazobactam, Imipenam, Tecoplanin & Linezolid are highly effective antibiotics against uropathogens. Therefore the objectives of the study has been fulfilled.So it is advised to have screening for bacteriuria in every trimester so that if there is an infection, it can be diagnosed as early as possible & appropriate treatment can be given. Screening for bacteriuria should be included in routine antenatal care so that we can prevent complications for both mother and foetus. Thus, I conclude by hoping that the above data will be informative to the antibiotic policy and treatment strategy formulators to prevent and to treat the Asymptomatic bacteriuria among pregnant women.

VI. SUMMARY

tract infections [UTI] Urinary in pregnancy are more common among antenatal women because of the physiological and morphological changes that occur during pregnancy. Asymptomatic bacteriuria (ASB) if not diagnosed earlier and treated properly may lead to maternal complications like pyelonephritis, post partum UTI, hypertension disorders and neonatal complications like prematurity, low birth weight, intrauterine growth retardation and prenatal death etc. The study was undertaken and purpose of this to find out the prevalence of study was asymptomatic bacteriuria in pregnant women, to find out the major causative agents of UTI and the antibiotic susceptibility of the causative agents so that it will aid in future Antenatal care and treatment. A total of 100 urine samples were collected from antenatal patients after taking their consent. The samples were further processed & the Isolates were identified by conventional methods to know the prevalence of Asymptomatic bacteriuria. Antibiotic susceptibility test was done for bacteriuria positive urine samples.

Out of 100 urine samples collected from asymptomatic pregnant women, 14 of the urine samples were positive for significant bacteriuria i.e out of 100, 14 pregnant women were having asymptomatic bacteriuria. Among the uropathogens isolated, Staphylococcus aureus (S.aureus) (42.8%) was predominant, followed by Escherichia coli (E.coli) (28.6%) and Klebsiella species (28.6%). Asymptomatic bacteriuria was more prevalent among 1^{st} trimester women (64.2%) , (14.2%) followed 2^{nd} trimester, & (21.6%) 3^{rd} trimester. Asymptomatic bacteriuria was more prevalent among pregnant women with age group 21-30 years old(64.3%). Asymptomatic bacteriuria was more prevalent among Multigravida women (57.2%). Most of the isolates were susceptible to anitibiotics such as Ceftazidime/Clavulinic acid, Amikacin, Ciprofloxacin, Vancomycin, Piperacillin+tazobactam, Imipenam, Tecoplanin & Linezolid.

Therefore if ASB is left undiagnosed, it leads to adverse maternal and foetal complications & even death. So it is advised to have screening for bacteriuria in every trimester so that if there is an infection, it can be diagnosed as early as possible & appropriate treatment can be given. Screening for bacteriuria should be included in routine antenatal care so that we can prevent complications for both mother and foetus.



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