# BACTERIAL ISOLATES AND THEIR CURRENT DRUG SUSCEPTIBILITY PROFILE FROM URINE AMONG ASYMPTOMATIC PREGNANT WOMEN ATTENDING AT A REFERRAL HOSPITAL, NORTHWEST ETHIOPIA; CROSS-SECTIONAL STUDY

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## ABSTRACT

### BACKGROUND:

Asymptomatic bacteriurias (ASB) are common during pregnancies thatcould have potentially serious consequences for maternal and fetal health. The rapid emergence of antimicrobial resistance necessitates continuous monitoring of the susceptibility patterns of pathogens.

### OBJECTIVE:

The purpose of this study was to identify bacterial pathogens from asymptomatic pregnant women attending antenatal clinic and by extension to determine the antimicrobial susceptibility profile of these isolates.

### METHOD:

A cross-sectional study was conducted at Felege Hiwot Referral Hospital (FHRH) from February 1 to May 30, 2016. Freshly voided clean catch midstream urine samples were collected and processed using conventional culture and biochemical tests as per the standard protocol. A concentration of >105 cfu/ml in urine sample was considered culture positive for ASB. Isolates were tested against the commonly used antibiotics by Kirby-Bauer disc diffusion methods. The degree of susceptibility pattern was determined based on the Clinical Laboratory Standards Institute. Descriptive and Chi-square test was done using SPSS version 22, $p < 0.05$ was considered to be significant.

### RESULTS:

A total of 234 study participants were involved in the study. The mean age of participants was 26.8 years (ranged 18–41 years). The majority, 139 (59.4%) of them were multigravida. Most of the participants at 134 (57.3%) were in the 3rd trimester. Among the study subjects, 20 (8.5%) were HIV sero-positive. Out of the 234 pregnant women 11.5 % (27/234) were positive for ASB. History of diabetes was significantly associated with ASB (p=0.019). A total of 27 bacterial uropathogens were identified. Out of these, Gram positives consisted at 20 (74.1%). The predominant isolates were S. saprophyticus at 48.2% (13/27) followed by S. aureus at 22.2% (6/27) and E. coli at 11.1% (3/27). Eleven (84.6%), 10 (76.9%) and nine (69.2%) of 13 isolates of S. saprophyticus were found resistant for co-trimoxazole, oxacilin and tetracycline, respectively.

### CONCLUSIONS:

In the studied area, the prevalence of ASB was at 11.5 %. Considerable drug susceptibility profile of the isolates was documented. Thus, efforts should be given to decrease the effect of ASB and antimicrobial resistance.

**KEY WORDS/PHRASES:** Asymptomatic bacteruria, antimicrobial resistance, Bahir Dar

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# INTRODUCTION

## BACKGROUND

Urinary tract infection is more prevalent in women [1]. Women's lifetime risk of having the infection in is >50%[1-3]. This may be due to the short urethra and its anatomical proximity to the anal orifice, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora[4-9]. Different research findings showed that UTI is the most common medical complication during pregnancy[10-14]. A significant growth of uropathogens $>10^5$ bacterial colony forming units (cfu) per ml of urine without the client showing symptoms of UTI is termed as asymptomatic bacteriuria (ASB) [13, 17-18]. It is a major risk factor for the development of UTIs during pregnancy[5, 10]. Pregnancy enhances the progression from asymptomatic into symptomatic bacteriuria [6,7,10]. This is due to the apparent reduction in immunity of pregnant women [10, 13]. In addition, the physiological increase in plasma volume and glucosuria, that encourages bacterial growth in the urine plays an important role in the conversion of asymptomatic into symptomatic[8, 10].

Ultimately symptomatic bacteriuria could leads to pyelonephritis and adverse obstetric outcomes such as prematurity, low-birth weight and higher fetal mortality rates [6, 7, 10]. Pregnant women with ASB are more likely to deliver pre-mature or low-birth-weight infants[1, 15-16]. Furthermore, a 20 to 30-fold increased risk of developing pyelonephritis was reported among women with bacteriuria and untreated bacteriruia during pregnancy is associated with low birth weight and premature delivery [6-7, 9, 17, 19-20].Researchers reported that Escherichia coli, Klebsiella spp., P. mirabilis, P. aeruginosa, Staphylococcus spp. and Enterococcus spp. are the most causative agents of UTI 1, 4. Data on local

bacterial etiology and their susceptibility profile is worthy to trace any change in time. Thus, timely updated reference for empirical therapy of ASB can be made [21-22]. Antimicrobial resistance rates among common uropathogens have been increasing[23], and their susceptibility varies from place and time[21-22]. This call continuous monitoring of the susceptibility profile of uropathogens[16]. With this background information, this study was conducted aimed at determining the types and prevalence of local isolates from asymptomatic pregnant women and by extension to determine their antimicrobial susceptibility profile to the most commonly used antimicrobials.

# MATERIALS AND METHOD

## Study design and population

A prospective cross-sectional study was conducted from February 30- May1, 2016. The study was conducted at FHRH in Bahir Dar, which is the capital city of Amhara National Regional State, 565 km away from Addis Ababa. The hospital is a tertiary health care level hospital serving the population of Bahir Dar town and surrounding areas of Northwest Ethiopia. A total of 234 asymptomatic pregnant women, for UTI attending FHRH for antenatal service who did not take antibiotic therapy two weeks before the data collection period were included in the study. All clients who came to the hospital in the study period were included conveniently regardless of their period of pregnancy.

# VARIABLES

## Independent Variables:

Age, residence, educational background, history of catheterization, pregnancy, gestation period
Dependent variables: Asymptomatic bacteriuria, type of isolates, drug resistance profile of the isolates.

# DATA COLLECTION PROCEDURES

A structured and pretested questionnaire was used to collect demographic characteristics of the study participants and related clinical data. Clients were screened for UTI clinically by health practitioners in charge of attending them. In addition, the types of isolated bacterial uropathogens from urine culture with their respective antimicrobial susceptibility profiles were determined using microbiological laboratory procedures as per the standard protocol.

# LAB PROCEDURE:

## Urine sample collection

All the study participants were requested to bring 5 ml freshly voided, clean catch midstream urine

samples. The urine samples were collected using lick proof wide mouth plastic containers. All of the study subjects have no history of taking antimicrobials in the last two weeks. All of the specimens were analyzed 15-30 minutes after collection24.

Urine culture: Bacterial isolation and identification

Isolation of bacterial uropathogens were performed by a surface streak procedure of well mixed un-centrifuged urine on blood and Mac Conkey agar (Oxoid Ltd. Bashingstore Hampaire, UK) using calibrated loops (0.001 ml) for semi quantitative method and incubated aerobically at 370C overnight for 24 hours. Colonies were counted as colony forming units (CFU) per milliliter (ml) to check significant growth at >105. Identification of isolates was performed using colony characteristics, gram reaction of the organisms and panels of biochemical test following the standard procedures. Biochemical tests used in this study includes indole, citrate, oxidase, H2S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, gas production, catalase, coagulase, manitol fermentation and novobiocin susceptibility testing 24-25

## ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing was performed on Mueller Hinton agar (MHA) plate using Kirby-Bauer disk diffusion method. Pure culture bacterial suspensions were prepared in nutrient broth by picking similar colonies of the test organisms with a sterile wire loop. The turbidity of the suspension was equilibrated to match with 0.5McFarland standards. A sterile swab dipped into the suspension of the isolate in broth, and then speeded over the entire surface of Muller-Hinton agar plate (Oxoid, LTD). The antibiotic disks were placed on the surface of inoculated agar and incubated at 370C for 24-48 hours. After 24-48 hours the diameters of the discs growth inhibition were measured and interpreted as per CLSI24-26. The antimicrobials tested were obtained from Oxoid Ltd., England with the following concentrations: Clindamycin (CL,2mg), Ampicillin (AMP, 10mg), Tetracycline (TE, 30mg), Ciprofloxacin (CIP, 5mg), Trimethoprim+Sulphamethazole (SXT, 25mg), Gentamicin (CN, 10mg), Norfloxacin, amoxicilin + clavulinic acid (20/10mg), Nitrofurantion (300mg), Oxacilin and Cephalotin.

## QUALITY CONTROL

Proper specimen collection was made through explaining for the client. All of the specimens were analyzed within 15-30 minutes of collection to prevent contamination. Culture media and antibiotic discs were checked for their normal shelf life. All culture plates, biochemical test media and MHA were used after checking sterility and performance using ATCC strains. All culture plates and antibiotic discs were stored at the recommended refrigeration temperature (2-80C) after preparation and sterilized by autoclaving at 121 0C for 15 minutes24. The standard reference strains of E. coli (ATCC 25922), P. aeruginosa (ATCC 27853) and S.aureus (ATCC 25923), were used for quality control of culture and antimicrobial susceptibility testing.

## DATA ANALYSIS

Data were entered, cleaned and analysed using Statistical Software Package for Social Sciences (SPSS) version 22 (SPSS Inc., Chicago, IL, USA) for Windows. Generated data were compiled by frequency tables and figure and other statistical summary measures. Statistical association was employed to compare the proportion of bacterial isolates and antimicrobial resistances profile among participants. A P-value less than 0.05 was considered to indicate statistically significant difference.

## OPERATIONAL DEFINITIONS

In accordance with the national kidney and urologic diseases information (1) the following definitions were applied,

**Mid-stream urine sample:** a urine specimen obtained from the middle part of urine flow (the so called clean catch urine sample)

**Asymptomatic bacteriuria:** is the occurrenceof significant bacteruria (yielding positive cultures (≥ 105CFU/ml)) in the urine without symptoms.

## ETHICAL CONSIDERATION

Ethical clearance was obtained from Amhara Regional Health Bureau Ethics Committee located at Bahir Dar Regional Research Lab Center. After the research staff explained about the purpose of the study informed written consent was taken

from each participant. Bacteriological positive results were communicated for health professionals attending women. Individual records were coded and accessed only by research staff. All information from participants was kept confidential by using lab codes.

## RESULTS

### Socio-demographic characteristics

A total of 234 pregnant women were included in this study. Their ages ranged from 18 to 41 years, with a mean age of 26.8 years and SD 4.7. In this study Based on their gravidity, primigravida accounted at 87(37.2%), multigravida at 139 (59.4%) and gravidity >5 at eight (3.4%). When the number of registered pregnant women are stratified by trimester 17 (7.3%), 83 (35.4%) and 134 (57.3%) of them were in the 1st, 2nd and 3rd trimester of pregnancy respectively. Four (1.7%), 16 (6.8%) and 19(8.1%) of the study participants had history of diabetes, catheterization and urinary tract infection respectively (Table 1).

**Table1: Socio-demographic variables and magnitude of ASB among pregnant women (n= 234) attending antenatal clinic in FHRH, 2016.**

| Characteristics | Tested No (%) | Bacteriological culture result | | Chi-square ($X^2$) | P val |
|---|---|---|---|---|---|
| AGE GROUP IN YEARS | | Negative No (%) | Positive No (%) | | |
| 18-25 | 96 (41) | 86 (89.6) | 10 (10.4) | | |
| 26-33 | 114(48.7) | 102 (89.5) | 12 (10.5) | 13.5 | 0.633 |
| 34-41 | 24(10.3) | 19 (79.2) | 5 (20.8) | | |
| MARITAL STATUS | | | | | |
| SINGLE | 2(0.9) | 2 | 0 | 8 | 1.000 |
| MARRIED | 232(99.1) | 205 (88.4) | 27 (11.6) | | |
| RESIDENCE | | | | | |
| URBAN | 211(90.2) | 189 (89.6) | 22 (10.4) | 7.9 | 0.441 |
| RURAL | 23(9.8) | 18 (78.3) | 5 (21.7) | | |
| EDUCATION | | | | | |
| ILLITERATE | 38(16.2) | 32 (84.2) | 6 (15.8) | | |
| PRIMARY/SECONDARY SCHOOL COMPLETED | 122(52.1) | 110 (90.2) | 12 (9.8) | 13.1 | 0.668 |
| COLLEGE/UNIVERSITY GRADUATE | 74(31.7) | 65 (87.8) | 9 (12.2) | | |
| OCCUPATION | | | | | |
| HOUSE WIFE | 115(49.1) | 110 (95.7) | 5 (5.3) | | |
| GOVERNMENT EMPLOY | 71(30.3) | 64 (90.1) | 7 (9.9) | 33.0 | 0.104 |
| PRIVATE BUSINESS | 27(11.5) | 23 (85.2) | 4 (14.8) | | |
| OTHERS | 21(9.0) | 19 (90.5) | 2 | | |
| MONTHLY INCOME, ETB | | | | | |
| <1000 | 37(15.8) | 32 (86.5) | 5 (13.5) | 22.6 | 0.543 |
| 1000-1999 | 64(27.4) | 58 (90.6) | 6 (9.4) | | |
| 2000-2999 | 51(21.8) | 44 (86.3) | 7 (13.7) | | |
| >2999 | 82(35.0) | 73 (89.0) | 9 (11) | | |

| | | | | | |
|---|---|---|---|---|---|
| GRAVIDITY | | | | | |
| PRIMIGRAVIDA | 87(37.2) | 79 (90.8) | 8 (9.2) | | |
| MULTIGRAVIDA | 139(59.4) | 121 (87.1) | 18 (12.9) | 13.1 | 0.667 |
| GRAVIDITY >5 | 8(3.4) | 7 (87.5) | 1 | | |
| PERIOD OF GESTATION/ TRIMESTER | | | | | |
| 1ST | 17(7.3) | 16 (94.1) | 1 | | |
| 2ND | 83(35.4) | 73 (88) | 10 (12) | 20.4 | 0.202 |
| 3RD | 134(57.3) | 118 (88.1) | 16 (11.9) | | |
| HISTORY OF DIABETES | | | | | |
| YES | 4 (1.7) | 3 (75) | 1 | 18.4 | 0.019 |
| NO | 230(98.3) | 204 (88.7) | 26 (11.3) | | |
| HISTORY OF CATHETERIZATION | | | | | |
| YES | 16(6.8) | 15 (93.8) | 1 | 1.1 | 0.998 |
| NO | 281(93.2) | 192 (68.3) | 89 (31.7) | | |
| HISTORY OF UTI | | | | | |
| YES | 19(8.1) | 16 (84.2) | 3 (15.8) | 14.8 | 0.063 |
| NO | 215(91.9) | 191(88.3) | 24 (11.7) | | |
| ANAEMIC STATUS | | | | | |
| ANAEMIC | 8(3.4) | 7 (87.5) | 1 | 1.2 | 0.996 |
| NON-ANAEMIC | 226(96.6) | 200 (88.5) | 26 (11.5) | | |
| HIV SERO-STATUS | | | | | |
| POSITIVE | 20(8.5) | 17 (85) | 3 (15) | | |
| NEGATIVE | 209(89.3) | 186 (89) | 23 (11) | 19.3 | 0.254 |
| UNKNOWN | 5(2.1) | 4 (80) | 1 | | |

**Magnitude of asymptomatic bacteriuria (ASB)**

In this study 27 pregnant women harbor bacteria in their urine sample, this makes the overall prevalence of ASB at 11.5 % (27/234). In this study history of diabetes was significantly associated with ASB (X2=18.4, p=0.019) (Table1).

**Identified uropathogens and their current antimicrobial susceptibility profile**

The distribution patterns of bacterial uropathogens recovered from urine sample among pregnant women are found to be 27. Among these Gram-positive cocci constituted of 20 (74.1%) were Gram positives and 7(25.9%) were gram negatives. Out of the gram positives, the predominant isolate was S. saprophyticus at 48.2% (13/27) followed by S. aureus at 22.2% (6/27). Among gram negatives E. coli constituted of 11.1% (3/27) of the isolates. In this study S. agalactae, K. ozanae, K. rihinose, Enterobacter spp and Serratia spp were also identified (Figure 1).

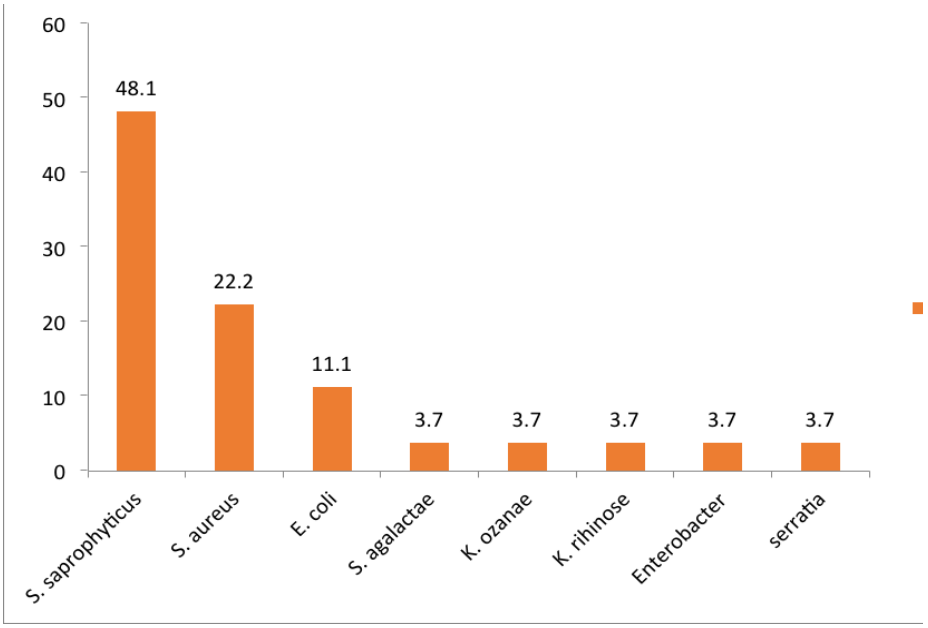


**Figure 1: Type and percentage distribution of uropathogens isolated from pregnant women with ASB in FHRH, 2016.**

With regard to antimicrobial susceptibility pattern of the isolates 11 (84.6%), 10 (76.9%) and nine (69.2%) of the 13 isolates of S. saprophyticus were found to be resistant to co-trimoxazole, oxacilin and tetracycline respectively. Similarly, five and three of 6 isolates of S. aureus were found resistant to tetracycline and co-trimoxazole respectively. No resistance (0%) was documented for amoxicillin-clavulinic acid and ciprofloxacin among gram positives. On top of this, all the three isolates of E. coli were resistant to tetracycline. However, all of them were sensitive to Norfloxacin (Table 2).

Table 2: Antimicrobial resistance profile of uropathogens identified from pregnant woman with ASB in FHRH, 2016.

| | | | RESISTANCE TO ANTIBIOTICS | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TYPE OF ISOLATES | NO | PROFILE | AMP | AMC | CIP | CN | CLN | N | NOR | SXT | TTC | OXA | CEP |
| GRAM POSITIVES (20) | | | | | | | | | | | | | |
| S. SAPROPHYTICUS | 13 | R* | - | 0 | 0 | - | 0 | 1 | 4 | 11 | 9 | 10 | 3 |
| S. AUREUS | 6 | R | - | 0 | 0 | - | 0 | 0 | 0 | 3 | 5 | 0 | 1 |
| S. AGALACTAE | 1 | R | - | 0 | 0 | - | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| GRAM NEGATIVES (7) | | | | | | | | | | | | | |
| E. COLI | 3 | R | 2 | 2 | 1 | 0 | - | 1 | 0 | 1 | 3 | - | 1 |
| K. OZANAE | 1 | R | 1 | 0 | 0 | 0 | - | 0 | 0 | 1 | 1 | - | 0 |
| K. RIHINOSE | 1 | R | 1 | 0 | 0 | 0 | - | 0 | 0 | 0 | 1 | - | 0 |
| ENTEROBACTER | 1 | R | 1 | 1 | 0 | 0 | - | 0 | 1 | 1 | 1 | - | 1 |
| SERRATIA | 1 | R | 1 | 0 | 0 | 1 | - | 0 | 0 | 1 | 1 | - | 0 |
| TOTAL | 27 | R | 6 | 3 | 1 | 1 | 1 | 2 | 5 | 18 | 21 | 12 | 6 |

*R*= resistant, AMP = ampicillin, AMC = amoxicilin + clavulinic acid, CIP = ciprofloxacin, CN = gentamicin, CLN= Clindamycin, N=Nitrofurantion, NOR = norfloxacin, SXT = co-trimoxazole, TTC = tetracycline, OXA= oxacilin and CEP= Cephalotin*

## DISCUSSION

Most Ethiopian women are living in the rural settings where they are unable to get regular antenatal follow up during pregnancy. For those who live in urban and semi- urban areas government owned health centers and hospitals provide follow up for a minimum of four times throughout their pregnancy period for free. There is no routine urine culture test for pregnant women to screen ASB during follow up instead they tested urinalysis using urine chemical strip tests.

Asymptomatic bacteriruia (ASB) during pregnancy needs special consideration due to the absence of indication and its adverse consequences. An early detection and treatment of such cases may be of considerable importance not only to prevent acute pyelonephritis and chronic renal failure in the mother, but also to reduce the prematurity and fetal mortality16. In the present study the prevalence of asymptomatic bacteriuria was at 11.5% (27/234). Our findings are in agreement with data from other studies worldwide reported, including in Ethiopia ranges 16.1%-48%16, 27-30.

The design of the studies, including factors such as social habits and socio-economic status, practice of personal hygiene, and educational levels of the study subjects may have contributed for the discrepancies of the results. The study finding also showed that the prevalence of ASB among pregnant women with diabetes was significantly higher compared to those without diabetes (P = 0.019). Similar findings were reported from the studies conducted by Rizk et al (2001) that indicated diabetes mellitus could complicate up to 5% of the pregnancies and has been associated with an increased risk of both fetal and maternal morbidity [31].

In this study, we documented higher isolation rate of Gram positive bacteria at 20 (74.1%) compared to Gram-negatives at seven (25.9%). This proportion is higher than the proportions reported in Goner 11 but lower than in Hawassa27. In the present study, the predominant isolates from gram positive bactria were S. saprophyticus accounted at 13 (48.1%) E. coli was found to be the highest isolate at three (11.1%) among gram negatives from pregnanet women. The prevalence of staphylococci at 19(70.4%) in the present study was higher.

This could be due to the poor genital hygienic practices by pregnant women who may find it difficult to clean their anal or genitalia properly after defecating or passing urine[27]. Senani in 2011 reported predominantly S. agalactia and E. coli. from pregnant women[10]. However, Alemu et al in Gondar, Ethiopia reported overall prevalence of UTI among pregnant women at 10.4% with the predominant isolate of E. coli at 47.5 % followed by coagulase-negative staphylococci at 22.5 %, S. aureus and K. pneumonia at 10% each[11]. Similarly, a study by Imade et al[13]. and Ezechi et al[31] reported E. coli followed by S. aureus and E. coli followed by Proteus mirabilis were the most commonly bacterial isolates from pregnant women. The difference on the pattern of the isolates as compared with the present study might be due to the difference in the sample size, the lab diagnostic procedure and urine sample collection as it needs clean catch urine sample.

Out of 13 isolates of *S. saprophyticus*; 11 (84.6%), 10 (76.9%) and nine (69.2%) were found to be resistant to co-trimoxazole, oxacilin and tetracycline respectively. Similarly, five and three of the six isolates of *S. aureus* were resistant to tetracycline and co-trimoxazole respectively. All isolates of *E. coli* were resistant to tetracycline however, they are found sensitive to Norfloxacin. Comparable antimicrobial resistant pattern was reported from Gonder and Hawassa, Ethiopia[11, 27] although there is elevated resistance level against tetracycline and better sensitivity to Norfloxacin in the present study. The upsurge in the antibiotic resistant pattern could be due to antibiotic abuse and self-medication. Furthermore, it is reported that antimicrobial resistance rates among commonly isolated uropathogens continue to evolve and appear to be increasing to many commonly used agents[23], and their susceptibility varies from place and time[21-22].

Urine sample was collected once from each study participant regardless of their period of gestation, which could influence to reveal the real status of ASB during the entire period of pregnancy. This study lacks data on extended spectrum betalactamase production status of the isolates due to limited resources available in laboratory.

# CONCLUSIONS

In the studied area, 11.5 % ASB was documented, which is an important health concern of pregnant women that needs to be addressed. Furthermore, pregnant women with the history of diabetes merit special attention. Larger studies are warranted in

the future to assess the associations more precisely. Both gram positive and gram-negative bacteria were isolated from pregnant women. If unrecognized and untreated, asymptomatic bacteriuria could lead to adverse maternal and perinatal outcomes. Hence screening and treatment of such cases should be incorporated as a routine procedure in antenatal cares.

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