Asymptomatic Bacteriuria Among Pregnant Women in Debre Markos, Ethiopia: Prevalence, Risk Factors, and Antimicrobial Susceptibility of Isolates

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Abstract

In low-income countries, asymptomatic bacteriuria (ASB) during pregnancy is a major cause for both maternal and foetal health risks. Rapid emergence of antimicrobial resistance also needs continuous monitoring of susceptibility profiles of uropathogens. We conducted a hospital-based cross-sectional study to deterimne prevalence, antimicrobial susceptibility, and associated risk factors of ASB among pregnant women. Socio-demographic and clinical data were collected by interview and extracted from women's medical records. Identification of bacteria from urine, and their susceptibility tests were done by using recommended methods. Logistic regression analyses were done by SPSS versions 20. The *p-value <0.05* at 95% CI was considered as statistically significant. Of the 172 study participants, 24 (14%) had ASB. Among 24 isolates, 13 (54.2%) were gram-negative, and of these, *E. coli* (8; 61.5%) was predominant followed by *K. pneumoniae* (4; 30.8%). Previous UTI and antibiotic use were significantly associated risk factors for ASB. *E .coli*, *S. areus* and CoNS were resistant to tetracycline (87.5%), cotrimoxazole (83.3%), and gentamycin (80%), respectively. Prevalence of ASB was lower than many previous reports in the country. Commonly used antibiotic susceptibility profiles of isolates were known. Hence, hard work ought to assume to decline the consequence of ASB and antibiotics resistance.

Background

Urinary tract infection (UTI) is a complicated public health problem and is caused by a range of uropathogens, but most commonly by *E. coli*, *K. pneumoniae*, *P. mirabilis, E. faecalis* and *S. saprophyticus*[1]. It is among the most common reasons for antibiotic overuse or misuse which induces the emergence of antibiotic resistance[2]. UTI is also the commonest illness during pregnancy following anemia; with clinical presentations of asymptomatic bacteriuria (ASB), acute cystitis and/ or pyelonephritis[3]. Asymptomatic bacteriuria is the identification of a specified quantitative count of bacteria ($\geq 10^5$cfu/ml) in the midstream urine without clinical signs or symptoms indicative of UTI, or it is also referred to as asymptomatic UTI, and occasionally called as bladder colonization[4,5].

Pregnancy can be a risk for ascending of uropathogens to the kidneys because of dilations of renal pelvis and ureters[2], which attributes to a 20 to 30 fold increased risk of pyelonephritis[6]. Little attention is given for ASB even if it has numerous health impacts, such as pyelonephritis in advanced pregnancy. Late diagnosis and improper treatment of pyelonephritis and ASB can result in chronic kidney disease which may move to chronic renal insufficiency[7]. Early screening and treatment of ASB decreases the risk of pyelonephritis by 90% which could attribute to decrease preterm labor with improving fetal survival[8]. Despite its significant prevalence and increased morbidity and mortality in women and their neonates, ASB is poorly screened and treated in resource-limited countries[9]. Bacteriological identification and antibiotics susceptibility tests[10]are impractical in developing settings, rather clinical diagnosis and empirical use of antibiotics that in turn favors for emergence of resistant strains[2,11].

A review conducted in Iran demonstrated 8.7% overall estimated prevalence of ASB, ranging from 2–29.1%. Such a study also described that *E. coli* (63.2%) was the most common isolates of ASB[11]; 11.6–13.1% in India with the predominant isolates of *K.pneumoniae* (50%), *E. coli* (35.7%), *S.aureus* (28.57%) and *Citrobacter* spp (21.4%) [12,13]; Egypt, 10% with common isolates of *E. coli* (71%) and *Klebsiella* spp (29%)[14]; Nigeria, 25% – 59.2% with *S. aureus* (21% – 45.9%), *S. saprophyticus* (10.4%), *E. coli* (15.1%-28.4%), *K. pneumoniae* (23.9–37.8%), *P. aeruginosa* (7.5–30.2%) and *P. mirabilis* (10.8%) [15–17]; Ghana, 33.3%[18]; Tanzania, 17.7% with isolates of *E. coli* (50.8%), *Klebsiella* spp. (17%) and *S. aureus* (8.7%) [19]; Kenya, 21% with common isolates of *E. coli* (38.8%), *S. aureus* (29.7%), CoNS (13.2%), *Klebsiella* spp. (7.8%), *pseudomonas* spp. (2.7%), *Proteus* spp. (2.7%), *Citrobacter* spp. (3%), *Enterococcus* spp. (1.9%), and *Enterobacter* spp. (0.9%) [20]; Adigrat / Ethiopia, 21.2% with the isolates of *E. coli* (34.6%), *Klebsiella* spp (18.2%), *P. mirabilis* (9.1%), *P. aeruginosa* (5.5%), *Enterobacter* spp. (1.8%), *S. aureus* (18.2%) and *S. saprophyticus* (12.7%) [21]; Hawassa / Ethiopia, 18.8% with isolates of CoNS (32.6%), *E. coli* (26.1%) and *S. aureus* (13%) [22]; Ambo / Ethiopia, 17.8% with isolates of *E. coli* (48.5%), *S. aureus* (20 %), CoNS(11.4%) and *Proteus* spp. (5.7%) [23]; and Amhara National Regional State/Ethiopia, 11.5–15.6% with the isolates of

S. aureus (22.2%-31%), *S. saprophyticus* (48.2%), *E. coli* (11.1–31%), *K. pneumoniae* (3.4%), *K. ozanae* (3.7%), *S. agalactiae* (3.4%-3.7%), *Enterobacter* spp. (1.7% – 3.7%) and *Serratia* spp. (3.7%)[24,25].

K. pneumoniae, *P.aeruginosa*, *E. coli*, and *Staphylococcus* species showed high resistant to mostly used antibiotics in a Nigerian study. Their resistance ability to ceftazidime, gentamicin, and ciprofloxacin was found to be (28–67%) [15]. In such a study, *Staphylococcus* species showed (90%) and (85%) resistant to cefoxitin and vancomycin, respectively. In Brazil, *E. coli*(24.4%), *Klebsiella* spp.(24.4%), *Proteus* spp. (30.2%), and *Staphylococcus* spp.(9.4%) were resistant to ciprofloxacin. Moreover, *E. coli* (50.6%), *Klebsiella* spp. (34.4%), *Proteus* spp.(53.8%), and *Staphylococcus* spp. (18.6%) were resistant to sulfamethoxazole-trimethoprim. Moreover, *E. coli* (57.2%), *Klebsiella* spp. (27.7%), and *Proteus* spp. (44.4%) were resistant to cephalothin. But, *E. coli* (92.2%), *Klebsiella* spp.(86.1%), and *Proteus* spp.(80%) were susceptible to Ceftriaxone. *E. coli* (93.4%) was again susceptible to nitrofurantoin. The *Staphylococcus* spp. (43.7%) was resistant to oxacillin [26]; in India, most gram negatives were susceptible to Amikacin (90%) and nitrofurantoin (80%) [13]; in Nigeria, most isolates were susceptible to amoxicillin-clavulanic acid (58–92%), ciprofloxacin (53% – 86%), cefotaxime (50% – 86%), gentamicin (50% – 92%), imipenem (50% – 71%) and ceftazidime (50% – 100%) [17]; in Ambo/Ethiopia, *Proteus* spp, *Klebsiella* spp, *Citrobacter* spp and *E. coli* showed high resistance to ceftriaxone (66%), gentamicin (68%), and nitrofurantoin (64%), gentamicin (62%), respectively [23]; in Bale / Ethiopia, isolates showed 90.9%, 88.6%, and 86.3% sensitivity to amoxicillin/clavulanic acid, gentamycin and norfloxacin, respectively [27].

In Nigeria, 89% – 100% of gram-negative isolates showed multidrug resistance (MDR) [15]; in Uganda, 82.4% (14/17) of gram negative isolates demonstrated MDR with the highest MDR from *Pseudomonas* spp. (100%, 2/2), *Klebsiella* spp. (100%, 2/2) and *E. coli* (92.3%, 12/13). Multidrug resistance in gram positive isolates was (72.7%, 8/11) with the highest MDR in *Enteroccocus* spp.(100%, 2/2) followed by *S. aureus* (66.7%, 6/9) [28]. In addition, 57% and 82% of the *E. coli* and *K. pneumoniae*, respectively were resistant to at least three classes of the antimicrobials tested in Uganda [29]. Another study in Uganda noted that 33% of the isolates were MDR [30]. In Nepal, 92 (96.84 %) of enterobacteriaceae displayed MDR [31]; and in Egypt, 95% (124/130) Gram negative isolates were MDR [32].

Regarding risk factors, studies conducted in Tanzania and Ethiopia showed that: a single marital status, $CD4^+$counts of < 200/µl, lack of formal occupation, hospital admission, preeclampsia, and presence of co-morbidities (HIV/ADIS); and age ranging from (18–25 yrs old), family income (< 1000ETB), and gestational period at the 1st - and 2nd -trimesters, history of catheterization and previous UTI were risk factors associated with the prevalence of ASB [21, 25, 33], respectively. As tried to illustrate above, in Ethiopia, though some studies were conducted in pregnant women regarding the ASB, the emphasis is still not given for early screening and treatment of ASB. Owing to this, drug-resistant strains are emerging continuously, making the treatment of UTI difficult. Moreover, at the study setting, little is known about the current uropathogenic ASB and their antibiotic susceptibility patterns. Therefore, this study was aimed to determine prevalence of ASB, antibiotic susceptibility profiles and possible associated risk factors among pregnant women at the Debre Mrkos Comprehensive Specialized Hospital, Northwest Ethiopia.

Materials And Methods

Ethical consideration

The study was conducted after obtaining ethical clearance from the ethical review committee of the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences University of Gondar as per the declaration and regulation of Helsinki as a statement of ethical principles. Permission was obtained from the Debre Markos Comprehensive Specialized Hospital administrators. Informed consent (and assent from parents or legal guardian for those who were below 18 years) has been obtained from each participant. In addition, for those who are illiterate, informed consent has been obtained from parents or legal guardians. Confirmed positive cases were linked to the attending physicians for better management of the participants. Information obtained at any course of the study was kept confidential which was maintained by numeric coding of specimens and questionnaires.

Study area

The study was conducted at the Debre Mrkos Comprehensive Specialized Hospital, Debre Markos city, Northwest Ethiopia. The city is located 299 km away from the Addis Ababa, the capital city of Ethiopia, and 265 km from Bahir Dar, the capital city of the Amhara National Regional State. The reports of the Central Statistical Agency of Ethiopia (CSA) population projection and the Amhara National Regional State Health Bureau showed that the region has about 20,018,988 people and nearly half of it is the females. Debre Markos city has one governmental hospital and four health centers, more than seven medium, and eight specialty private clinics as well as two non-governmental organization clinics. Debre Markos Comprehensive Specialized Hospital gives health services for diagnosis and management of various infectious diseases, antenatal and postnatal cares, laboratory, pharmacy, integrated maternal and neonatal care and other services for more than 5 million people living in urban and rural Kebeles, East- and West- Gojjam Administrative zones of the Amhara National Regional State and from the other neighboring zones and regional states. The Hospital provides antenatal care services for more than 330 pregnant women per month.

Study design and period

A hospital-based cross-sectional study was conducted from January to May 2020.

Population

Source population

All women visited the Debre Markos Comprehensive Specialized Hospital for their medical problems during the study period.

Study population

All pregnant women visited the Debre Markos Comprehensive Specialized Hospital for their antenatal care follow up during the study period.

Inclusion and Exclusion criteria

Inclusion criteria

Pregnant women who were without any signs and symptoms of UTI and welling to participate in the study were included.

Exclusion criteria

Pregnant women who were on antibiotics or had antibiotics during two weeks prior data collection, women with urogenital fistula and those with active bleeding were excluded.

Variables

Dependent variables

Asymptomatic bacteriuria, bacterial isolates, and antibiotic susceptibility profile

Independent variables

Age, residence, religion, educational status, occupation, monthly income, follow up, gestational periods, catheterization history, hospitalization, previous history of UTIs, previous use of antibiotics, and comorbidities (preeclampsia, diabetes Mellitus, HIV/AIDS).

Sample size and sampling technique

The sample size was calculated based on the single population proportion statistical formula, considering a 95% confidence interval, 5% margin of error, and 11.5% prevalence[24]. The sample size n = $(z\alpha/2)^2(p)(1-p)/d^{2;}$ where, n = sample size, α = level of significance, z = at 95% confidence interval z value (α = 0.05) = > z α/2 = 1.96, p = prevalence of occurrence of the event to be studied, d = margin of error at 5%. So, n = $(1.96)^2 \times (0.115) \times (1-0.115)/(0.05)^2$, i.e n = 156.39 ≈ n = 156. Total sample size was 172 including 10% none–respondent rate.

Sampling method and procedure

After obtaining the monthly estimated average number of pregnant women visiting the Debre Markos Comprehensive Specialized Hospital for the antenatal care from the registration book, the sampling interval "k" was determined by dividing the monthly estimated average number of pregnant women by the number of study participants. Then, the first unit was selected by lottery method and the other units were selected systematically by taking every k^{th} unit interval after the first unit until the specified sample size was achieved.

Operational definition

Asymptomatic bacteriuria

The occurrence of significant bacteriuria yielding positive cultures (10^5 colony-forming units per milliliter or cfu/ml) without signs and symptoms referable to urinary tract infections.

Significant Bacteriuria

refers to the presence of $\geq 10^5$ cfu/ml in the urine sample

Multidrug resistance

A resistance to one or more antibiotics among three or more classes of antibiotics.

Data collection and laboratory methods

Socio-demographic and clinical data

A structured and pre-tested questionnaire was used to collect socio-demographic and clinical characteristics, and pregnancy-related issues. Data were collected by trained data collectors from each study participant. Diabetic, Hypertensive, and HIV/AIDS statuses were extracted from women's medical records.

Specimen collection and processing

One hundred seventy two freshly voided midstream urine specimens were collected by laboratory personnel using a sterile screw-capped test tube and inoculated on to Cysteine Lactose Electrolyte Deficient agar (CLED) using calibrated loop (measures 1.3mm diameter, delivering 1µL) and incubated overnight at 37˚C at the Debre Mrkos Comprehensive Specialized Hospital Microbiology laboratory. The specimens with significant bacteriuria (= 10^5CFU/mL) were further sub-cultured onto 5% sheep blood agar, MacConkey and Chocolate agar. The colonies of isolates were characterized using colony characteristics, gram-staining technique, and biochemical reaction patterns following standard procedures. Most gram-negative bacteria were identified using indole test, lysine decarboxylase, H_2S, and gas production in triple sugar agar, citrate utilization, urease production & motility tests. Gram-positive bacteria were identified using catalase and coagulase tests [35,36].

Antibiotic susceptibility tests

Antimicrobial susceptibility tests were carried out by the disk diffusion technique on the Muller-Hinton agar medium. About 3 to 5 selected colonies were taken from pure culture and transferred to 5mL sterile nutrient broth containing tube and mixed smoothly until a homogenous mixture was formed and incubated at 37^{o}C until the turbidity of the mixture became comparable to 0.5 McFarland standards. A sterile cotton applicator stick was used to distribute the bacteria consistently over the whole surface of Mueller-Hinton agar. The inoculated culture plates were left at room temperature until dried for up to 5minutes. Ciprofloxacin (300µg), gentamicin (10µg), ceftriaxone (30µg), cefoxitin (30µg), meropenem (30µg), norfloxacin (10µg), nitrofurantoin (300µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), and penicillin (30µg) discs were used (all from Abtekbio.Ltd UK). After overnight incubation at 37^{o}C, the diameters of inhibition zones around the discs were measured by a calibrated ruler, and the isolates were classified as susceptible, intermediate, and resistant based on the CLSI criteria[37].

Quality control

Data quality was insured by using pretested (5%) questionnaire, training of data collectors, following standard operating procedures, and use of control strains such *S. aureus* (ATCC-25923), and *E. coli* (ATCC-25922), and daily checking and cleaning of data collected.

Data analysis and interpretation

Data were cleaned and coded using Epi-Data and were entered and analyzed using statistical packaged for social sciences (SPSS) version 20. Characteristics of the study participants were summarized using frequencies, percentages, mean and standard deviation. Bivariate logistic regression was used to determine the presence of an association between several variables. Those variables with *p-value* < 0.2 were subjected to multivariable analysis to control the effect of confounding variables. A *p-value* of < 0.05 at 95% CI was considered statistically significant. Assumption of goodness of the model was checked by Hosmer-lemeshow test (p = 0.828).

Results

Socio-demographiccharacteristics

A total of 172 asymptomatic pregnant women for UTIs were recruited in this study. The mean age ± SD of the study participants was 27.5 ± 4.9 years (range 19–43 years). Majority, 120 (69.8%) of the study participants were urban residents, and 163(95%) were attending primary school and above educational level. From the study participants, 158(91.9%) were married. Fifty-six (32.6%), and 54(31.4%) pregnant women were housewives and government-employed, respectively (Table 1).

Table 1
Socio-demographic factors and ASB among pregnant women (N = 172) at the Debre Markos Comprehensive Specialized Hospital, January to May 2020

Asymptomatic bacteriuria				
	Positive no (%)	Negative no (%)	Total no (%)	*p-value*
Age in years				
15–24	11(45.8)	38(25.7)	49(28.5)	.841
25–34	10(41.7)	98(66.2)	108(62.8)	.217
35–44	3(12.5)	12(8.1)	15(8.7)	
Religion				
Christian	21(87.5)	127(85.8)	148(86)	.825
Muslim	3(12.5)	21(14.2)	24(14)	
Residence				
Urban	12(73)	108	120(69.8)	
Rural	12(50)	40(27)	52(30.2)	.027
Marital status				
Single	1(4.2)	6(4.1)	7(4.1)	.999
Married	23(95.8)	135(91.2)	158(91.9)	.999
Divorced	0(0)	7(4.7)	7(4.1)	
Educational status				
Illiterate	1(5.2)	8(5.4)	9(5.2)	.506
Primary	5(20.8)	61(41.2)	66(38.4)	.046
Secondary	8(33.3)	41(27.7)	49(28.5)	.569
College or more/	10(41.7)	38(25.5)	38(27.9)	
Occupation				
Farmer	0(0)	4(2.7)	4(2.3)	.680
Employee	9(37.5)	45(30.4)	54(31.4)	.999
Private business	6(25)	46(31.1)	52(30.2)	.330
House wife	7(29.2)	49(33.1)	56(32.6)	.190
Student	2(2.7)	4(2.7)	6(3.5)	
Monthly Income				
<= 2000 ETB	3(12.5)	23(15.5)	26(15.1)	.807
> 2000 ETB	21(87.5)	125(94.5)	146(84.9)	

Type and prevalence of bacteria isolate from pregnant women

In this study, 24 pregnant women were positive for ASB, which makes the total prevalence of 14%, 24/172. Thirteen (54.2%), and 11(45.8%) isolates were gram negatives and gram positives, respectively. *Escherichia coli* (8/24, 33.3%), *K.pneumoniae*

(4/24, 16.7%), and *P.mirabilis* (1/24, 4.2%) were gram-negative; *S. aureus* (6/24, 25%), and CoNS (5/24, 20.8%) were gram-positive isolates (Table 2).

Table 2
Type and prevalence of bacteria identified from pregnant women at the Debre Markos Comprehensive Specialized Hospital, January to May 2020

Bacterial isolates	**Asymptomatic pregnant women**	
	Number	**%**
E. coli	8	33.3
S. aureus	6	25.0
Coagulase negative Staphylococcus (CoNS)	5	20.8
K. pneumoniae	4	16.7
P. mirabilis	1	4.2
Total	24	100

Antibiotics susceptibility profile of Gram-negative bacteria isolates

Antibiotic susceptibility patterns of the gram-negative isolates ranged from 7.6–100%. Majority of gram-negative isolates were sensitive to nitrofurantoin (12; 92.3%), norfloxacin (12; 92.3%), cefoxitin (10; 76.9%), ciprofloxacin (8; 61.5%), ceftriaxone (7; 53.8%), and all of them were (100%) sensitive to meropenem. However, some gram-negative bacteria were resistant to tetracycline (10; 76.9%), Cotrimoxazole (6; 46.2%), and Gentamycin (8; 61.5%). *E. coli* was the most dominant isolate from gram-negative isolates and it was sensitive to nitrofurantoin (8; 100%), norfloxacin (8; 100%) and cefoxitin (6; 75%), ciprofloxacin (5; 62.5%), but it was resistant to tetracycline (7;87.5%), gentamycin (5;37.5%) and cotrimoxazole (4;50%). *Klebsiella pneumoniae* was the second most dominant gram-negative isolate which was sensitive to norfloxacin (3; 75%) and Cefoxitin(3;75%); however, it was resistant to gentamycin (3;75%), tetracycline (3;75%) and ceftriaxone(2;50%) (Table 3).

Table 3
Antibiotic susceptibility profile of Gram-negative bacteria identified from pregnant women at the Debre Markos Comprehensive Specialized Hospital, January to May 2020

Bacterial isolates	Antibiotic discs tested									
		Cip	Cro	Nit	Nor	Mer	Gen	Ttc	Cef	Cot
E.coli (8)	S	5(62.5)	5(62.5)	8(100)	8(100)	8(100)	3(37.5)	0(0)	6(75)	3(37.5)
	R	2(25.0)	2(25.0)	0(0)	0(0)	0(0)	5(62.5)	7(87.5)	1(12.5)	4(50)
	I	1(12.5)	1(12.5)	0(0)	0(0)	0(0)	0(0)	1(12.5)	1(12.5	1(12.5)
K.pneumoniae (4)	S	2(50)	1(25)	3(75.0)	3(75)	4(100)	1(25)	1(25)	3(75)	1(25)
	R	0(0.0)	2(50)	0(0)	0(0)	0(0)	3(75)	3(75)	1(25)	2(50)
	I	2(50.0)	1(25)	1(25.0)	1(25)	0(0)	0(0)	0(0)	0(0)	1(25)
P.mirabilis(1)	S	1(100)	1(1000	1(100)	1(100)	1(100	1(100)	0(0)	1(100)	1(100)
	R	0(0.0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)
	I	0(0.0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Total (13)	S	8(61.5)	7(53.8)	12(92.3)	12(92.3)	13(100)	5(38.4)	1(7.6)	10(76.9)	5(38.4)
	R	2(15.4)	4(30.8)	0(0)	0(0)	0(0)	8(61.5)	10(76.9)	2(15.4)	6(46.2)
	I	3(23.1)	2(15.4)	1(7.6)	1(7.6)	0(0)	0(0)	2(15.4)	1(7.6)	2(15.4)

NB:S = sensitive,R = Resistance,I = Intermidate,Cip = Ciprofloxacin,Cro = Ceftriaxone,Nit = Nitrofrunation,Nor = Norfloxacin,Gen = Gentamycin,Ttc = Tetracyclin,Cef = Cefoxitine,Cot = Cotrimoxole

Antibiotics susceptibility patterns of gram-positive bacteria isolates

In this study, *S.aureus* and CoNS were the gram-positive isolates and showed their different susceptibility profile. As shown in Table 4, *S. aureus* was sensitive to nitrofurantoin (5; 83.3%), norfloxacin (6; 100%), penicillin (3; 50%), and (4; 66.7%) for each ciprofloxacin, cefoxitin, tetracycline. But, it was resistant to ceftriaxone and penicillin for each (3; 50%) and Gentamycin and Cotrimoxazole for each (5; 83.3%). CoNS was also (4; 80%) sensitive for each gentamycin, ceftriaxone, nitrofurantoin, cefoxitin, but it was resistant to cotrimoxazole and gentamycin for each (4; 80%) and tetracycline (3; 60%).

Table 4
Antibiotic susceptibility profile of Gram-positive bacteria isolates from pregnant women at the Debre Markos Comprehensive Specialized Hospital, January to May 2020

Bacterial isolates	Antibiotic discs tested									
		Cip	Cro	Nit	Nor	Gen	TTC	Cef	Cot	pen
S.aureus(6)	S	4(66.7)	1(16.7)	5(83.3)	6(100)	1(16.7)	4(66.7)	4(66.7)	1(16.7)	3(50)
	R	1(16.7)	3(50)	0(0)	0(0)	5(83.3	2(33.3)	1(16.7)	5(83.3)	3(50)
	I	1(16.7)	2(33.3	1(16.7)	0(0)	0(0)	0(0)	1(16.7)	0(0)	0(0)
CoNS*(*5)	S	4(80.0)	4(80)	4(80)	2(40)	1(20)	0(0)	4(80)	0(0)	0(0)
	R	1(20.0)	0(0)	1(20)	2(40)	4(80)	3(60)	1(20)	4(80)	2(40)
	I	0(0.0)	1(20)	0(0)	1(20)	0(0)	2(40)	0(0)	1(20)	3(60)
Total(11)	S	8(72.7)	5(45.5)	9(81.8)	8(72.7)	2(18.2)	4(36.4)	8(72.7)	1(9.9)	3(27.2)
	R	2(18.2)	3(27.2)	1(9.9)	2(18.2)	9(81.8)	5(45.5)	2(18.2)	9(81.8)	5(45.5)
	I	1(9.9)	3(27.2)	1(9.9)	1(9.9)	0(0)	2(18.2)	1(9.9)	1(9.9)	3(27.2)

Key:S = sensitive, R = Resistance, I = Intermidate, Cip = Ciprofloxacin, Cro = Ceftriaxone, Nit = Nitrofrunation, Nor = Norfloxacin, Gen = Gentamycin, Ttc = Tetracyclin,Cef = Cefoxitine,Cot = Cotrimoxole,pen = Penicillin

Multidrug resistance patterns of the isolates

As tried to illustrate in Table 5, 17 isolates showed multidrug resistance profile. Among the total of MDR isolates, 10 (59.0%), and 7(41.2%) were gram-positive and gram-negative bacteria, respectively.

Table 5
Multi-drug resistance profiles of bacterial isolates isolated from asymptomatic pregnant women at the DMRH, January – May 2020

MDR Profiles	**Isolates**					
	S. aureus (n = 6)	*E. coli* (n = 8)	*K.pneumoniae(n = 4)*	CoNS (n = 5)	*P.mirablis(n = 1)*	Total (n = 24)
CEF/TTC/CRO	0%	0%	1(25%)	0%	0%	4.2%
CIP/COT/GEN	0%	1(12.5%)	0%	0%	0%	4.2%
COT/GEN/CEF	1(16.7%)	0%	0%	0%	0%	4.2%
COT/GEN/CRO	0%	0%	1(25%)	0%	0%	4.2%
COT/GEN/TTC	0%	1(12.5%)	1(25%)	1(20%)	0%	12.5%
COT/TTC/CRO	0%	1(12.5%)	0%	0%	0%	4.2%
GEN/CEF/TTC	0%	1(12.5%)	0%	0%	0%	4.2%
COT/GEN/TTC/CRO	1(16.7%)	0%	0%	0%	0%	4.2%
COT/GEN/TTC/PEN	2(33.3%)	0%	0%	0%	0%	8.3%
COT/GEN/TTC/CRO/PEN	1(16.7%)	0%	0%	0%	0%	4.2%
COT/GEN/CEF/TTC/PEN	0%	0%	0%	1(20%)	0%	4.2%
CIP/COT/GEN/TTC/CRO	0%	1(12.5%)	0%	0%	0%	4.2%
CIP/NIT/COT/GEN/TTC/PEN	0%	0%	0%	1(20%)	0%	4.2%

Key: *CIP =* Ciprofloxacin, *CRO = Ceftraxone, NIT = Nitrofurantoin, NOR = Norfloxacin, MER = Meropenem, GEN = Gentamycin, TTC = Tetracyclin, CEF = Cefoxitine, COT = Cotrimoxazole, PEN = Pencilin*

Risk factors associated with the prevalence of asymptomatic bacteriuria

In this study, bi-variable analysis of rural residency, primary school educational level, previous history of UTI, catheterization exposure history, hospital admission history, diabetic Mellitus, and prior use of antibiotics showed statistically significant association with the prevalence of ASB among pregnant women. However, the multivariable analysis showed that ASB occurrence was associated with previous UTI and antibiotic use histories. Pregnant women who had history of UTI had 6.14 (AOR = 6.14, 95% CI: 2.04–18.45) times the chance of having ASB compared to those who had not previous UTI. In addition, pregnant women who had history of antibiotic use had the odds of 3.85 times ASB developing as compared to their counterparts (AOR = 3.85, 95% CI: 1.15–12.87) (Table 6).

Table 6: Logistic regression analysis of risk factors associated with ASB among pregnant women at the DMRH, January – May 2020

Variable	ASB			COR (95% CI)	AOR (95% CI)	*p-value*
	Yes (n, %)	No (n, %)	Total (n, %)			
Residence						
Rural	12(23.1)	40(76.9)	52(30.2)	2.7(1.12–6.5)		*
Urban	12(10)	108(90)	120(69.8			
History of UTI						.001
Yes	13(46.4)	15(53.6)	28(16.3)	10.5(3.99–27.5)	6.14(2.04–18.45)	
No	11(7.6)	133(92.4)	144(83.7)			
Antibiotic therapy						.029
Yes	10(50.0)	10(50.0)	20(11.6)	9.9(3.5-27.72)	3.85(1.15–12.87	
No	14(9.2)	138(90.8)	152(88.4)			
Catheterization history						
Yes	3(50.0)	3(50.0)	6(3.5)	6.91(1.31–36.45)	.	*
No	21(12.7)	145(87.3)	166(96.5)			
Hospital admission history						
Yes	4(36.4)	7(63.6)	11(6.4)	4.03(1.03-15.0)		*
No	20(12.4)	141(87.6)	161(93.6)			
Diabetic Mellitus						
Yes	4(40.0)	6(60.0)	10(5.8)	4.7(1.23–18.24)		*
No	20(12.3)	142(87.7)	162(94.2)			

* = After multivariable analysis, a statistically significant association was not identified (P > .05). COR = crude odd ratio, AOR = adjusted odd ratio, CI = confidence interval.

Discussion

Asymptomatic pregnant women need special attention concerning ASB due to the absence of signs and symptoms and pending with the negative health impact of both the mother and the fetus [9]. In this study, ASB was (14%) prevalent which is in line with the previous studies conducted in Desie, Ethiopia (15.6%) [25]; and Bahir Dar, Ethiopia (11.5%) [24]; Tanzania (17.7%) [19]; Egypt (10%) [14]; and different regions of India (11.6–13.1%) [12,13]. However, it was less prevalent than the prevalence registered in Adigrat, Ethiopia (21.2%) [21]; in Kenya (21%) [20]; Ghana (33%) [18]; and in different parts of Nigeria (25% – 59.2%) [15 – 17]. The variation of prevalence of ASB in different studies from one country to another and among areas of the same country might be a result of the difference in risk factors, study sites, sample size, geographical location, social habit of the population, and health education system/policies practiced.

Bacterial isolates of this study, namely *E. coli (*33.3%), *K. pneumoniae* (16.7*)*, and *P. mirabilis* (4.2%) were gram negatives and gram positives were *S.aureu* (25%) and CoNS (18.5%,). These isolates were almost similarly identified in Ambo, Ethiopia (*E. coli* 48.5%, *S. aureus*20%, CoNS;11.4%, and *Proteus* spp. 5.7% [23]; Desie, Ethiopia (*E.coli*; 30.4%, *S.aureus*; 30.4% *K .pneumoniae* (*3*.4%) and CoNS (26.8%) [25]; and Bahir Dar, Ethiopia (*S.ures;*22.2%, *E.coli* (11.1%), *K.pneumoniae* (3.4%) [24]; Kenya (*E. coli* (38.8%), *S. aureus* (29.7%), CoNS (13.2%), *Klebsiella* spp. (7.8%) and *Proteus* spp. (2.7%) [20]; and in different regions of Nigeria (*S. aureus*, 21-45.9%, *E. coli*, 15.1–28.4%, *K.pneumoniae*;23.9–37.8%, and *P. mirabilis*;10.8%) [15 – 17]. This in agreement might be due to the fact that most of the uropathogenic bacteria are Gram negatives that usually reside in the intestinal tract and would contaminate women's urethra that in turns aid the ascending of bacteria to the urinary tract in pregnant women[1].

In this study, we noticed that previous antibioitc use and previous UTI history were associated risk factors for ASB among pregnant women. Pregnant women who had previous UTIs history had 6.14 folds more probable to develop ASB than those who hadn't any previous UTI history. This finding was in line with the previous report in Bahir Dar, Ethiopia[33]. In addition, pregnant women who had prior antibiotic exposure had the odds of 3.85 times more risk factor of acquiring ASB than their counterparts which is I line with a study conducted in USA where

P. aeruginosa was significantly more commonly isolated from urine sample in patients with ≥ 2 prior antibiotic exposures (12.6%) compared with no exposure (8.2%; $p = 0.036$) or 1 prior exposure (7.9%; $p = 0.025$)[34].

In the current study, antibiotic susceptibility pattern of Gram-negative bacteria verified that all of the isolates exhibited susceptibility to meropenem (13; 100%) and the majority of them were susceptible to nitrofurantoin (92.3%), norfloxacin (92.3%), cefoxitin (76.9%), ciprofloxacin (61.5%), gentamycin (38.4%), cotrimoxazole (38.4%), and ceftriaxone (53.8%). But the study in Hawasa, Ehiopia showed lower susceptibility to norfloxacin (64.7%)[22]. The increase in the antibiotic resistant profile might be due to antibiotic misuse and self-prescribing. Additionally, it is stated that antibiotics resistance rates among common bacterial isolates continue to change and look to be growing to numerous frequently prescribed antibiotics and their susceptibility varies from place to place and across time[24].

Among the total isolates (n = 24) in the present study, 17 (70.1%) showed multidrug resistance which was in line with the study conducted in Desie, Ethiopia[25]. which showed that multidrug resistance was identified to be very high to the commonly used antibiotics. The possible reasons for this frightening figure of multidrug resistant cases might be due to the misuse of antibiotics without laboratory confirmation and inappropriate and incorrect administration of antimicrobial agents in empirical treatment. Besides, over-the-counter sales of antimicrobials without prescription are probable factors for increased bacterial resistance to antimicrobial agents.

Conclusion And Reommendation

In this study area, 14 % ASB was identified, which is an essential health alarm of pregnant women. Both Gram-positive and Gram-negative bacteria were identified from pregnant women. Asymptomatic bacteriuria might lead to harmful maternal and perinatal consequences, unless recognized and treated early. Therefore, screening and treating of ASB should be incorporated in a routine culture laboratory guidance based for better management of pregnant women. Additionally, pregnant women who reside in the rural catchments require special consideration. Larger sample sizes are necessary in the impending to assess the associated risk factors for ASB among pregnant women. All Gram-negative bacteria isolated demonstrated susceptibility to meropenem. Among the total isolates (n = 24) in the present study, 17 (70.1%) showed multidrug resistance which needs warrants continues surveillance of antimicrobial susceptibility profiles of ASB.

Declarations

Acknowledgment

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Authors' contributions

YA participated in conception and design of the study, acquisition of data, analysis and interpretation of findings. SB participated in conception and design of the study, and interpretation of data; he also participated in revision of the manuscript. MG participated in design of the study, acquisition and interpretation of data; in drafting and revision of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Data Availability

All data generated or analyzed during this study are included in this manuscript and when there is a need of raw data, it would be available through formal request of the corresponding author.

References

1. Flores-Mireles, A.L., Walker, J.N., Caparon, M. & Hultgren, S.J. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature reviews Microbiol* **13**(5),269-84 (2015).
2. Gilbert, N.M. *et al.* Urinary tract infection as a preventable cause of pregnancy complications: opportunities, challenges, and a global call to action. Glob Adv Health Med **2**(5),59-69(2013).
3. Szweda, H. & Jozwik, M. Urinary tract infections during pregnancy, an updated overview. *Dev. period med* **20**(4),263-72(2016).
4. Nicolle, L.E. Asymptomatic Bacteriuria and Bacterial Interference. *Microbiol spectr* **3**(5) (2015).
5. Nicolle, L.E. *et al*. Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. Clin. Infect. Dis: an official publication of the Infectious Diseases Society of America **40**(5),643-54(2005).
6. Delzell, O.E. & Lefevre, M.L. Urinary Tract Infections During Pregnancy. *Am Fam Physician* **61**(3),713-20(2000).
7. Nogayeva, M.G. & Tuleutayeva, S.A. Asymptomatic Bacteriuria in Pregnant Women in Outpatient Facilities. *Cent Asian J Glob Health* **4**(1),53(2015).
8. Schnarr, J. & Smaill, F. Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy. *Eur J Clin Investig* **38**,50-7(2008).
9. Lee, A.C.C. *et al.* Screening and treatment of maternal genitourinary tract infections in early pregnancy to prevent preterm birth in rural Sylhet, Bangladesh: a cluster randomized trial. *BMC Pregnancy Childbirth* **15**,326(2015).
10. Geibel, S., Procko, E., Hultgren, S.J., Baker, D. & Waksman, G. Structural and energetic basis of folded-protein transport by the Fimusher. *Nature* **496**(7444),243(2013).
11. Azami, M. *et al*. The etiology and prevalence of urinary tract infection and asymptomatic bacteriuria in pregnant women in Iran: a systematic review and Meta-analysis. *BMC Urol* **19**(1),43(2019).
12. Rajaratnam, A., Baby, N.M., Kuruvilla, T.S. & Machado, S. Diagnosis of asymptomatic bacteriuria and associated risk factors among pregnant women in mangalore, karnataka, India. *JCDR* **8**(9), (2014)
13. Jayachandran, A.L., Gnanasambandam, U., Balan, K., Sangeetha, A. & Vijayalakshmi, T. Asymptomatic bacteriuria among antenatal women attending a tertiary care hospital in Kanchipuram: evaluation of screening tests and antibiotic susceptibility pattern. *Int J Res Med Sci* **4**(2),540-4 (2016).

14. Elzayat, M.A.A., Barnett-Vanes, A., Dabour, M.F.E. & Cheng, F. Prevalence of undiagnosed asymptomatic bacteriuria and associated risk factors during pregnancy: a cross-sectional study at two tertiary centres in Cairo, Egypt. *BMJ Open* **7**(3),e0131980(2017).
15. Onanuga, A., Omeje, M.C. & Eboh, D.D. Carriage of multi-drug resistant urobacteria by asymptomatic pregnant women in Yenagoa, Bayelsa State, Nigeria. *Afr J Infect Dis* **12**(2),14-20(2018).
16. Onu, F.A. *et al.* Profile and microbiological isolates of asymptomatic bacteriuria among pregnant women in Abakaliki, Nigeria. *Infect Drug Resist* **8**,231-5(2015).
17. Akpan, N.G. *et al*. Asymptomatic Uropathogenic Bacteriuria Among Pregnant and Non-pregnant Women at St Luke's Hospital Anua, Offot Ukwa District Uyo: A Reassessment Case-Control Approach. *Am J Lab Med* **4**(1),1-10(2019).
18. Afoakwa, P. & Domfeh, S.A. Asymptomatic Bacteriuria and Anti-Microbial Susceptibility Patterns among Women of Reproductive Age. A Cross-Sectional Study in Primary Care, Ghana. *Med Sci (Basel)* **6**(4),118(2018).
19. Seni, J. *et al*. Multicentre evaluation of significant bacteriuria among pregnant women in the cascade of referral healthcare system in North-western Tanzania: Bacterial pathogens, antimicrobial resistance profiles and predictors. *J Glob Antimicrob Resist* **17**,173-9(2019).
20. Ayoyi, A.O., Kikuvi, G., Bii, C. & Kariuki, S. Prevalence, aetiology and antibiotic sensitivity profile of asymptomatic bacteriuria isolates from pregnant women in selected antenatal clinic from Nairobi, Kenya. *PAMJ* **26**(1),1-12(2017).
21. Tadesse, S. *et al*. Prevalence, antimicrobial susceptibility profile and predictors of asymptomatic bacteriuria among pregnant women in Adigrat General Hospital, Northern Ethiopia. *BMC Res Notes* **11**(1),740(2018).
22. Tadesse, E., Teshome, M., Merid, Y., Kibret, B. & Shimelis, T. Asymptomatic urinary tract infection among pregnant women attending the antenatal clinic of Hawassa Referral Hospital, Southern Ethiopia. *BMC Res Notes* **7**,155(2014).
23. Gessese, Y.A. *et al*. Urinary pathogenic bacterial profile, antibiogram of isolates and associated risk factors among pregnant women in Ambo town, Central Ethiopia: a cross-sectional study. *Antimicrob Resist Infect Control* **6**,132(2017).
24. Habteyohannes, A.D.*et al.* Bacterial isolates and their current drug susceptibility profile from urine among asymptomatic pregnant women attending at a Referral Hospital, Northwest Ethiopia; cross-sectional study. *EJRH* **10**(2),10(2018).
25. Ali, I.E., Gebrecherkos, T., Gizachew, M. & Menberu, M.A. Asymptomatic bacteriuria and antimicrobial susceptibility pattern of the isolates among pregnant women attending Dessie referral hospital, Northeast Ethiopia: A hospital-based cross-sectional study. *Turk J Urol* **44**(3),251-60(2018).
26. Cunha, M.A., Assunção, G.L.M., Medeiros. I.M. & Freitas, M.R. Antibiotic resistance patterns of urinary tract infections in a Northeastern Brazilian Capital. *Rev Inst Med Trop Sao Paulo 58*,2(2016).
27. Taye, S., Getachew, M., Desalegn, Z., Biratu, A. & Mubashir, K. Bacterial profile, antibiotic susceptibility pattern and associated factors among pregnant women with Urinary Tract Infection in Goba and Sinana Woredas, Bale Zone, Southeast Ethiopia. *BMC Res Notes11*(1),799(2018).
28. Nteziyaremye, J. *et al*. Asymptomatic bacteriuria among pregnant women attending antenatal care at Mbale Hospital, Eastern Uganda. Asymptomatic bacteriuria among pregnant women attending antenatal care at Mbale Hospital, Eastern Uganda. *PLoS ONE 15*(3),e0230523(2020).
29. Stanley, I.J. *et al*. Multidrug resistance among Escherichia coli and Klebsiella pneumoniae carried in the gut of out-patients from pastoralist communities of Kasese district, Uganda. *PLoS ONE* **13**(7),e0200093(2018).
30. Najjuka, C.F., Kateete, D.P., Kajumbula, H.M., Joloba, M.L. & Essack, S.Y. Antimicrobial susceptibility profiles of Escherichia coli and Klebsiella pneumoniae isolated from outpatients in urban and rural districts of Uganda. *BMC Res Notes* **9**,235(2016).
31. Yadav, K.K., Adhikari, N., Khadka, R., Pant, A.D. & Shah, B. Multidrug resistant Enterobacteriaceae and extended spectrum β-lactamase producing Escherichia coli: a cross-sectional study in National Kidney Center, Nepal. *Antimicrob Resist Infect Control* **4**,42(2015).

32. Metwally, W.S. & Elnagar, W.M. Multidrug Resistant Uropathogens among Egyptian Pregnant Women. *Am J Infect Dis* **15**(4),115.22(2019).
33. Emiru, T., Beyene, G., Tsegaye, W. & Melaku, S. Associated risk factors of urinary tract infection among pregnant women at Felege Hiwot Referral Hospital, Bahir Dar, North West Ethiopia. *BMC Res Notes* **6**(1),2920(2013).
34. Bidell, M.R., Opraseuth, M.P., Yoon, M., Mohr, J. & Lodise, T.P. Effect of prior receipt of antibiotics on the pathogen distribution and antibiotic resistance profile of key Gram-negative pathogens among patients with hospital-onset urinary tract infections. *BMC Infect Dis*;**17**,176(2017).
35. Wilson, M.L.& Gaido, L. Laboratory diagnosis of urinary tract infections in adult patients. Clini Infect Dis **38**(8),1150-8(2004).
36. Chesbrough, M. District laboratory practice in tropical countries. New York: Cambridge University Press; (2006).
37. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement. 27th ed. CLSI Supplement M100 Wayne, PA: CLSI; (2017).