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Bacterial Profile, Antimicrobial Susceptibility Patterns of Asymptomatic Bacteriuria and Associated Factors among Pregnant Women Attending Antenatal Clinic of Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

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Abstract

Asymptomatic bacteriuria is a common condition among pregnant women and if untreated could lead to maternal and fetal complications. In addition to this, rapid emergence of antimicrobial resistance is became a major health problem and need continuous monitoring of the susceptibility patterns of pathogens. A hospital-based cross-sectional study was conducted among 290 asymptomatic pregnant women attending Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. Data were collected using systematic random sampling technique with interviewer-administered pretested questionnaire. Clean-catch midstream urine specimens were collected and microbiological investigations were performed using standard procedures. Data were analyzed by SPSS version 23. Binary and multivariable logistic regressions were used to identify predictors of asymptomatic bacteriuria. Variables having *p*-value ≤ 0.05 were considered as statistically significant. Out of the 290 study participants, 49(16.9%) were positive for asymptomatic bacteriuria. *Escherichia coli* 21(43%) and *Staphylococcus aureus* 10(20%) were common bacterial isolates. Most bacterial isolates were susceptible to nitrofurantoin. Most *E. coli* isolates were resistant to amoxicillin (86.4%). The prevalence of multi-drug resistance in all study isolates was 57.1%. Pregnant women with a history of urinary tract infection (AOR=3.11, P=0.004), history of catheterization (AOR=2.31, P=0.040), hemoglobin level less <11g/dl (AOR=10.49, P<0.0001) and natural abortion (AOR=2.36, P=0.023) were found to be statistically significant associated with asymptomatic bacteriuria. Asymptomatic bacteriuria was prevalent among pregnant women in our study area setting. Therefore, pregnant women should be screened by urine culture and treatment be guided by antimicrobial susceptibility data. Continuous local monitoring of resistance patterns is important for empirical therapy as well as for epidemiological reasons

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Keywords

Asymptomatic bacteriuria; Antibiotic susceptibility; Pregnant woman; Addis Ababa; Ethiopia

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections that affect both in the community and hospital settings (1, 2). It is defined as microbial contamination of the urine as well as tissue invasion of any part of the urinary tract (3). UTI affects all age groups, but women particularly pregnant women are more susceptible than men, due to their short and wide urethra, absence of prostatic secretion and easily contamination of urinary tract with fecal flora (4, 5). Such infections can be either symptomatic or asymptomatic in pregnant women (6).

Asymptomatic bacteriuria (ASB) is presence of a significant quantity of bacteria in a properly collected urine specimen from a person without signs and a symptom of UTI (7). ASB may precede to symptomatic, and pregnancy itself is one of factor, which increase the risk of UTIs partly due to combination of alternation of hormonal, anatomical, physiological and maternal immunity during the periods of pregnancy (8). In addition, other factors predisposing pregnant women to bacteriuria are increasing age, lower socioeconomic status, multiparty, past history of UTI, history of catheterization, sickle cell trait/anaemia and diabetes mellitus (9, 10). Glycosuria and aminoaciduria during pregnancy are additional risk factors for the development of ASB (11). *Escherichia coli* is the most common infecting organism, which is responsible for 75 to 90% of bacteriuria in pregnancy (12). Other Gram-negative bacteria, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Proteus* species *and* Gram-positive bacteria, such as *Streptococcus agalactiae, Staphylococcus saprophyticus*, and *Enterococcus* species, are the most implicated bacterial species for ASB in pregnancy (13).

Asymptomatic bacteriuria in pregnancy if left untreated, about 30% of the affected mothers will develop acute pyelonephritis compared to 1.8% of non-bacteriuria controls (14). Pyelonephritis can leads to poor maternal and perinatal outcomes including prematurity, low birth weight, increased perinatal mortality, preeclampsia, anemia, hypertension, postpartum UTI, renal insufficiency, preterm labour and delivery (15, 16).

Routine screening and treatment bacteriuria in pregnancy has been associated with improved maternal and perinatal outcomes (17). A study estimated that screening and treatment of bacteriuria in pregnancy has the potential to reduce the incidence of prematurity and low birth weight by 20-55% and neonatal mortality due to prematurity by 15-40% (18). Current management of UTIs are usually empirical, without the use of a urine culture or susceptibility testing to guide therapy (19). However, antimicrobial resistance among pathogens that cause UTIs is increasing and is a major health problem in the treatment of UTI, especially in pregnant women who are restricted to only a few safe first line antibiotics drug like ampicillin, nitrofurantoin and cephalexin (20).

In Ethiopia concerning the prevalence and antimicrobial susceptibility pattern of ASB among pregnant women varies from area to area (5, 8). Even those studies that have been done in Addis Ababa among pregnant women with signs and symptoms of urinary tract infection, it lacks associated risk factors and too old. Hence, knowing current knowledge of the organism that causes UTIs and their antibiotic susceptibility pattern is essential for appropriate empirical therapy as well as for epidemiological reason. Therefore, this study was aimed to isolate and identify the bacterial profile of ASB, antimicrobial susceptibility pattern of isolates and associated risk factors among pregnant women attending at antenatal clinic in Saint Paul's Hospital Millennium Medical College (SPHMMC), Addis Ababa, Ethiopia.

Materials and Methods

Study design and period

A hospital-based cross-sectional study was conducted from July to September 2019.

Study area

The study was conducted at Saint Paul's the hospital Millennium Medical College (SPHMMC), Addis Ababa, Ethiopia. The hospital is providing outpatient and inpatient services with 512 beds and gives the health service for more than 2 million patients per year. Every month approximately 830 pregnant women are visiting antenatal care unit.

Study population

Asymptomatic pregnant women in all gestational periods without any signs and symptoms of UTI and willing to participate in the study were included. Symptomatic pregnant women with suggestive of urinary tract infection such as dysuria, the urgency of urination, haematuria, fever, suprapubic pain, flank pain, cramping in the lower abdomen, and received antibiotics treatment within the last two weeks before the data collection and known congenital anomalies of the urinary tract were excluded from the study.

Sample size determination and sampling technique

The sample size was determined by using a single population proportion formula,

$$n = Z^2 P (1-P) / D^2$$

Where n= required sample size, Z is the reliability coefficient (confidence level) which is (95%) = 1.96, P is the anticipated population proportion of 21.2% was obtained from a previous study conducted in the Tigray region, Ethiopia (4), D= margin of error (5%). We have also considered risk factors during sample size calculation. The total sample size after considering risk factors and 10% contingency was equal to 290. A systematic random sampling technique was used to recruit 290 study participants. The first study participant was selected by lottery method.

Data collection and laboratory processing

Data related to socio-demographic characteristics, associated factors, and past delivery data were collected using a pretested and structured interviewer administered questionnaire.

Collection of urine specimens

After proper instruction, study participates were informed to clean their peri-urethral area with water and soap then cleanse with sterile gauze to collect 10-15ml of freshly voided midstream urine using sterile and wide-mouthed plastic bottles with screw cap top. Urine specimens were transported to Ethiopian Public Health Institute (EPHI) Microbiology Laboratory with cold box and processed within 1-2 hours of collection and specimens that were not processed within 2hours were kept refrigerated at 4°C until it was processed.

Culture and identification of isolates

Using calibrated wire loops, 0.001 ml of uncentrifuged, uniformly mixed, midstream urine samples were aseptically inoculated onto 5% sheep blood agar (Biomark, India laboratories) and MacConkey agar (Oxoid, England BD, USA). After overnight incubation at aerobically at 37°C for 24h colonies were counted to check significant growth. Colony counts yielding bacteria growth of 10^5 CFU/ml of urine considered as significant for bacteriuria (21). All positive urine culture with significant bacteriuria was further identified by their colony characteristics, Gram stain and different biochemical tests using standard procedures. *Enterobacteriacea*e were identified by H_2S production and carbohydrate utilization tests in TSI agar, Motility test, indole test, citrate test, oxidase test and urease tests. The Gram-positive bacteria were identified using catalase, coagulase, and bile esculin agar (22).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using disk diffusion on Mueller-Hinton agar (MHA) (Oxoid Ltd, Hampshire, UK) based on Clinical and Laboratory Standards Institute (CLSI) guidelines, (23). The antibiotics discs used and their concentrations were: ampicillin (10µg), amoxicillin (20µg), amoxicillin-clavulanic acid (20/10µg), ciprofloxacin (5µg), ceftriaxone (30µg), cefuroxime (30µg), nitrofurantoin (300µg), cotrimoxazole (25µg), tetracycline (30µg), clindamycin (2µg), erythromycin (15µg), gentamicin (10µg), penicillin (30µg), azithromycin (15µg) and meropenem (10µg). Isolates were classified as susceptible, intermediate and resistant according to standardized table supplied by CLSI 2019 (23).

Data management and quality control

Strict measures were taken from the pre-analytical to the post-analytical phase. Moreover, the questionnaire was pretested among 5% of sample size at Tikur Anbessa Specialized Hospital. To assure data quality, three BSc nurses and one Gynaecologist were recruited as data collectors and supervisor, respectively. In addition, training regarding the objectives and data collection was given for data collectors and supervisor for two day. Furthermore, intensive supervision was done by supervisor and principal investigator though-out data the collection period. Laboratory analyses were carried out using standard operating procedures. Culture media were tested for sterility and performance by incubating 5% of the batch. Standard reference strains of *E. coli* (ATCC-25922), *S. aureus* (ATCC-25923) and *P. aeruginosa* (ATCC-27853) were used during culture and antimicrobial susceptibility testing.

Operational definitions

Asymptomatic bacteriuria (ASB)

Asymptomatic bacteriuria is defined as urine culture with significant growth of bacteria ($\geq 10^5$ cfu/ml) of urine collected from a freshly voided mid-stream (clean catch) urine specimen where the source of the urine specimen is a person without signs and symptoms of UTIs (24).

Multidrug-resistance (MDR)

Bacterial isolates resistant to two or more antimicrobial agents (25).

Data processing and analysis

All relevant data were cleaned, coded and entered using Epi Info Version 7.2 and analyzed by statistical package for social science (SPSS) version 23 software. Data were organized, summarized, and presented in descriptive statistical methods. The presence of association was assessed by bivariate analysis and variable with a *p*-value <0.25 were candidate for multivariable model. Finally, adjusted odd ratio with their 95% confidence intervals were estimated to assess the strength of the association, and variables having *p*-values ≤ 0.05 were considered as statistically significant factors.

Results and Discussions

Socio-demographic characteristics of study participants

A total of 290 study participates were included in the study, resulting in a response rate of 100%. The mean age of the participants was 27± 4.5 years within range of 18-43. Majority, two hundred and eighty (96.6%) were married and ten (3.4%) were single. With regards to education status, 58(20.0%) of the participants completed higher education and above. More than half of the study participants were urban residence and housewife (Table 1).

Prevalence of asymptomatic bacteriuria

Out of the 290 pregnant women, 49 (16.9%) (95%, CI=13.1-21.5) had significant bacteriuria. Among cases of significant bacteriuria, 47(95.9%) showed a single bacterial growth while two (4.1%) showed mixed growth. In this study, eight different bacterial were isolated. The majority of the isolates 29(59.2%) were Gram-negative bacteria. The most frequent isolated bacteria were *E. coli* 21(43%) and *S.aureus* 10(20.4%), followed by *S. saprophyticus* 7(14.3%), *K. pneumoniae*, *Enterobacter cloacae, Enterococcus faecalis, Klebsiella oxytoca and Enterobacter aerogens* were 3(6.1%), 2(4.1%) 2(4.1%) 1(2.1%) and 1(2.1%) respectively. The mixed culture contained *E. coli* with *Candida albicans* and *S. saprophyticus* with *C. albicans*.

Factors associated with asymptomatic bacteriuria

The current study showed that, the higher prevalence of ASB was found in the age of 26-34 years, housewives, urban residence, married and completed primary school were 30(61.2%), 35(71.4%), and 44(90%), 48(98%) and 23(46.9%) respectively. However, there is no significant difference between prevalence of ASB and socio-demographic data (Table 2).

In multivariate analysis, the prevalence of ASB was significantly associated with those participants with the history of UTI (AOR=3.11, 95% Cl: 1.42, 6.79), a history catheterization (AOR=2.31, 95% Cl: 1.04, 5.73), hemoglobin level (AOR=10.49, 95% Cl : 4.54, 24.04) and natural abortion (AOR=2.36, 95% Cl: 1.13, 4.94) were found to be independent predictors of asymptomatic bacteriuria during pregnancy ($p<0.05$) (Table 3).

Antimicrobial susceptibility pattern bacterial uropathogens

Gram negative bacteria

The rate of susceptibility for gram-negative isolates ranged from 13.8%-93.1%. The majority of the gram-negative isolates were susceptible to nitrofurantoin (n=27; 93.1%), gentamycin (n=25; 85.2%), ceftriaxone (n=24; 82. 2%), cefuroxime (n=23; 79. 3%), and meropenem (n=22; 75.2%) the most effective antibiotics for the bacterial isolated. However, most Gram-negative isolates were resistant to amoxicillin (n=23; 79.3%), cotrimoxazole (n=19; 65.5%), and amoxicillin-clavulanic acid (n=11; 37.9%). Among Gram-negative bacteria, the most frequent isolated was *E. coli*, and (n=19; 86.4%) of isolate were resistance to amoxicillin and while least resistance to gentamicin and meropenem (for each n=1; 4.5%) and nitrofurantoin (n=2; 9.0%). The antimicrobial testing of K. *pneumoniae*, *E. cloacae*, *K. oxytoca* and *E. aerogens* isolates were showed that all (100%) susceptible to nitrofurantoin. 66.7% of *K. pneumoniae* were resistant to cefuroxime and ampicillin. All isolates of *E. aerogenes* and *K. oxytoca* were susceptible to most of the tested antimicrobial agents whereas fully resistant to ampicillin and amoxicillin (Table 4).

Table.1 Socio-demographic characteristics of sampled pregnant women attending the antenatal clinic of Saint Paul's the hospital Millennium Medical College, Addis Ababa, Ethiopia, 2019, (N= 290)

Variables	Category	Frequency	Percent
Age in years	18-25	101	34.8
	26-34	169	58.3
	35-43	20	6.9
Occupation	Housewife	193	66.6
	Merchant	55	19.0
	Government employed	38	13.0
	Student	4	1.4
Marital status	Married	280	96.6
	Single	10	3.4
Education	No formal education	26	3.4
	Primary school	125	43.1
	Secondary school	81	27.9
	Higher education	58	20.0
Residence	Urban	249	85.9
	Rural	41	14.1

Table.2 Socio-demographic characteristics and magnitude of ASB among sampled pregnant women attending antenatal clinic of Saint Paul's the hospital Millennium Medical College, Addis Ababa, Ethiopia, 2019, (N=290)

Variables	Tested n(%)	ASB Positive n(%)	ASB Negative n(%)	COR (95% CI)	P-value
Age (in years)					
18-25	101(34.8)	17(16.8)	84(83.2)	1	
26-34	169(58.3)	30(17.8)	139(82.2)	1.06 (0.55, 2.05)	0.847
35-43	20(6.9)	2(10.0)	18(90.0)	0.54 (0.12, 2.58)	0.449
Occupation					
Housewife	193(66.6)	35(18.1)	158(81.9)	0.66 (0.07, 6.58)	0.727
Merchant	55(19.0)	8(14.5)	47(85.5)	0.51 (0.05, 5.54)	0.581
Government Employed	38(13.0)	5(13.2)	33(86.8)	0.45 (0.04, 5.27)	0.528
Student	4(1.4)	1(25.0)	3(75.0)	1	
Marital status					
Married	280(96.6)	48(17.1)	232(82.9)	1.86 (0.23, 15.04)	0.560
Single	10(3.4)	1(10.0)	9(90.0)	1	
Educational status					
No formal education	26(9.0)	6(23.1)	20(76.9)	3.18(0.87, 11.59)	0.08*
Primary school	125(43.1)	23(18.4)	102(81.6)	2.39(0.86, 6.64)	0.95*
Secondary school	81(27.9)	15(18.5)	66(81.5)	2.41(0.82, 7.05)	0.11*
Higher education	37(12.8)	4(10.8)	33(89.2)	1	
Place of residence					
Urban	249(85.9)	44(17.7)	205(82.4)	1	
Rural	41(14.1)	5(12.2)	36(87.8)	0.65(0.240-1.742)	0.389

Key: *Candidate variable for multivariate analysis at P <0.25, COR**:** crude odds ratio, CI**:** confidence interval, n: number, 1**:** reference group

Table.3 Bivariate and multivariable analysis of factors associated with ASB among pregnant women attending the antenatal clinic of Saint Paul's the hospital Millennium Medical College, Addis Ababa, Ethiopia, 2019 (N=290)

Variables	Tested n(%)	Asymptomatic bacteriuria Positive n(%)	Negative n(%)	Bivariate analysis COR (95% CI)	P-value	Multivariate analysis AOR (95% CI)	P-value
History of urinary tract infection							
Yes	91(31.4)	28(30.8)	63(69.2)	3.76(1.99, 7.12)	0.001*	3.11(1.42, 6.79)	0.004**
No	199(68.6)	21(10.6)	178(89.4)	1			
Hemoglobin level							
<11g/dl (Anemic)	33(11.4)	18(54.5)	15(45.5)	8.75(4.01,19.11)	0.0001*	10.49(4.58, 24.04)	0.0001**
≥11g/dl (Normal)	257(88.6)	31(12.0)	226(88.0)	1			
History of catheterization							
Yes	89(30.7)	23(25.8)	66(74.2)	2.34(1.25, 4.39)	0.008*	2.31(1.04, 5.13)	0.04**
No	201(69.3)	26(12.9)	175(87.1)	1			
Educational status							
No formal education	26(9)	6(23.1)	20(76.9)	3.18(0.87, 11.59)	0.08*	3.26(0.72-14.83)	0.126
Primary school	125(43.1)	23(18.4)	102(81.6)	2.39(0.86, 6.64)	0.95*	2.04(0.63, 6.62)	0.233
Secondary school	81(27.9)	15(18.5)	66(81.5)	2.14(0.82, 7.05)	0.11*	2.45(0.72, 8.38)	0.152
Higher education	58(20.0)	5(8.6)	53(91.4)	1			
Average monthly income level (in Ethiopian Birr)							
≤500	20(6.9)	3(15.0)	17(85.0)	0.98(0.26, 3.68)	0.986	0.36(0.06, 1.92)	0.232
501-1000	58(20.0)	13(22.4)	45(77.6)	1.62(0.74, 3.52)	0.226*	1.17(0.46, 2.95)	0.735
1001-1500	29(10.0)	3(10.3)	26(89.7)	0.64(0.18, 2.34)	0.506	0.54(0.13, 2.19)	0.390
1501-2000	51(17.6)	10(19.6)	41(80.4)	1.36(0.59, 3.16)	0.466	0.75(0.26, 2.12)	0.590
>2000	132(45.5)	20(15.2)	112(84.8)	1			
Gestation period							
1st Trimester	51(17.6)	8(15.7)	43(84.3%)	1			
2nd Trimester	66(22.8)	10(15.2)	56(84.8)	0.96(0.349, 2.638)	0.937		
3rd Trimester	173(59.6)	31(17.9)	142(82.1)	1.173(0.502, 2.742)	0.712		
Parity							
Nulliparaus	86(29.7)	17(19.8)	69(80.2)	1			
Primiparaus	109(37.6)	16(14.7)	93(85.3)	0.69(0.33, 1.47)	0.348		
Multiparaus	95(32.8)	16(16.8)	79(83.2)	0.82(0.38, 1.74)	0.611		
History of natural abortion							
Yes	91(31.4)	24(26.4)	67(73.6)	2.49(1.33, 4.66)	0.004	2.36(1.13, 4.96)	0.023**
No	199(68.6)	25(12.6)	174(87.4)	1			
History of still birth							
Yes	14(4.5)	3(21.4)	11(78.6)	1.36(0.17, 2.50)	0.545		
No	276(95.5)	46(16.7)	230(83.3)	1			
History of early neonatal death							
Yes	14(4.8)	1 (7.1)	13(92.9)	0.36(0.35, 21.42)	0.338		
No	276(95.2)	48(17.4)	228(82.6)	1			
History of Diabetes							
Yes	21(7.2)	5(23.8)	16(76.2)	1.59(0.22, 1.79)	0.384		
No	269(92.8)	44(16.4)	225(83.6)	1			
HIV sero-status							
Positive	9(3.1)	2(22.2)	7(77.8)	1.42(0.14 ,1.39)	0.666		
Negative	281(96.6)	47(16.7)	234(83.3)	1			

Key: ** statistically significant at *P*<0.05; COR: crude odds ratio, AOR: adjusted odds ratio, CI: confidence interval, 1**:** reference group, n: number

Table.4 Antimicrobial susceptibility pattern of Gram-negative bacteria isolated from pregnant women with ASB at Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2019 (n=29)

Bacterial isolates (n)		Antibiotics										
		GEN	CIP	AMP	AMX	AMC	TET	NIT	CEF	CRX	MER	COT
E. coli (22)	S	20(91.0)	18(81.8)	10(45.5)	1(4.5)	10(45.5)	15(68.2)	20(91.0)	18(81.8)	18(81.8)	18(81.8)	6(27.3)
	I	1(4.5)	1(4.5)	-	2(9.1)	2(9.0)	-	-	-	1(4.5)	3(4.5.7)	-
	R	1(4.5)	3(13.7)	12(54.5)	19(86.4)	10(45.5)	7(31.8)	2(9.0)	4(18.02)	3(13.7)	1(4.5)	16(77.7)
K.pneumoniae (3)	S	1(33.3)	1(33.3)	2(66.7)	1(33.3)	2(66.7)	1(33.3)	3(100)	1(33.3)	2(66.7)	1(33.3)	1(33.3)
	I	1(33.3)	-	-	-	-	1(33.3)	-	1(33.3)	-	-	-
	R	1(33.4)	2(66.7)	1(33.3)	2(66.7)	1(33.3)	1(33.3)	-	1(33.3)	1(33.3)	2(66.7)	2(66.7)
E. cloacae (2)	S	2(100)	-	2(0.0)	2(0.0)	2(100)	1(50.0)	2(100)	2(100)	2(100)	2(100)	-
	I	-	-	-	-	-	1(50.0)	-	-	-	-	2(100)
	R	-	2(100)	-	-	-	-	-	-	-	-	-
K.oxytoca(1)	S	1(0.0)	1(100)	-		1(100)	1(100)	1(100)	1(100)	1(100)	-	-
	R	-	-	1(100)	1(100)		-	-			1(100)	1(100)
E. aerogens (1)	S	1(100)	1(100)	-	-	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
	R	-	-	1(100)	1(100)	-	-	-	-	-	-	-
Total (29)		25(86.2)	21(72.4)	19(65.5)	4(13.8)	16(55.2)	19(65.5)	27(93.1)	23(79.3)	24(82.8)	22(75.9)	8(27.6)

Key: GEN: Gentamycin, CIP: Ciprofloxacin, AMP: ampicillin, AMX: Amoxicillin, AMC: Amoxicillin clavulanic acid, TET: Tetracycline, NIT: Nitrofurantoin, CEF: Ceftriaxone, CRX: Cefuroxime, MER: Meropenem, COT: Co-trimoxazole, S: Susceptible; I: Intermediate and R: Resistance

Table.5 Antimicrobial susceptibility pattern of Gram-positive bacteria isolated from pregnant women with ASB Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2019 (n=20)

Type Bacterial isolates (n)		Antibiotics									
		GEN	ERY	CIP	PEN	COT	TET	NIT	CD	AZT	AMP
S. aureus (10)	S	9(90.0)	6(60.0)	9(90.0)	3(30.0)	7(70.0)	7(70.0)	10(100)	8(80.0)	9(90.0)	NA
	I	1(10.0)	1(10.0)	1(10.0)	-	-	-	-	1(10.0)	-	NA
	R	-	3(30.0)	-	7(70.0)	3(30.0)	3(30.0)	-	1(10.0)	1(10.0)	NA
S. saprophyticus (8)	S	6(75.0)	4(50.0)	6(75.0)	2(25.0)	4(50.0)	4(50.0)	8(100)	6(75.0)	7(87.5)	NA
	I	1(25.0)	2(25.0)	1(12.5)	-	1(12.5)	2(25.0)	-	1(12.5)	1(12.5)	NA
	R	1(25.0)	2(25.0)	1(12.5)	6(75.0)	3(37.5)	2(25.0)	-	1(12.5)	-	NA
E. faecalis (2)	S	2(100)	NA	2(100)	1(50.0)	1(50.0)	1(50.0)	2(100)	2(100)	2(100)	1(50.0)
	I	-	NA	-	-	-	1(50.0)	-	-	-	-
	R	-	NA	-	1(50.0)	1(50.0)	-	-	-	-	1(50.0)
Total Total (29)		17(85.0	10(50.0)	17(85.0)	6(30.0)	12(60.0)	12(60.0)	20(100)	16(80.0)	18(90.0	1(50.0)

Key: GEN: Gentamycin, ERY: Erythromycin, CIP: Ciprofloxacin, PEN: Penicillin, COT: Co-trimoxazole, TET: Tetracycline, NIT: Nitrofurantoin, CD: Clindamycin, AZT: Azithromycin, AMP: Ampicillin, S: Susceptible, I: intermediate, R: Resistant, NA: Not applicable

Table.6 Multi-drug resistance patterns of bacterial isolates among pregnant women with ASB attending in Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2019 (n=28)

Bacterial isolates	Combination of antibiotics	N (%)
E. coli (n=12)	AMX,COT	1 (8.3)
	AMX MER	1 (8.3)
	AMX,AMP,AMC,COT	3 (25.0)
	AMX,AMP,AMC,TET,COT	3 (25.0)
	AMX,AMP,AMC,CRT,CRX,COT	1 (8.3)
	AMX,AMP,AMC,NIT,TET,COT	1 (8.3)
	AMX,AMP,AMC, CTR,CRX,CRP,TET,COT	2 (16.7)
K. pneumoniae (n=2)	AMX, CRP,MER,COT	1 (50.0)
	AMX,AMP,AMC,CTR,CRX,GEN,CRP,TET,MER,COT	1 (50.0)
K. oxytoca (n=1)	AMX,AMP,COT	1 (100)
S. aureus (n=7)	ERY,PEN	3(42.9)
	TET,PEN	1(14.2)
	COT ,PEN	1(14.2)
	PEN,TET,CD,COT	1(14.2)
	PEN,TET,AZT,COT	1(14.2)
S. saprophyticus (n=5)	PEN,CD	1(20.0)
	ERY,PEN	1(20.0)
	COT,ERY	1(20.0)
	GEN,PEN,TET,COT	2(40.0)
E. faecalis (n=1)	PEN, COT	1(100)

Key: AMX: Amoxicillin, COT: Co-trimoxazole, CIP: Ciprofloxacin, AMP: Ampicillin, AMC: Amoxicillin-clavulanic acid, TET: Tetracycline, GEN: Gentamycin, NIT: Nitrofurantoin, CTR: Ceftriaxone, CRX: Cefuroxime, MER: Meropenem ERY: Erythromycin, PEN: Penicillin, CD Clindamycin, AZT: Azithromycin

Asymptomatic bacteriuria needs special consideration during pregnancy due to the absence of symptoms and associated adverse effects on maternal and fetal outcomes (5). The prevalence of ASB among pregnant women in the current study was 16.9%, which is in agreement with prior studies done in Bangladesh (16.5%) (26), Adama, Ethiopia (16.1%) (27) and Ambo, Ethiopia (17.8%) (16). However, it is low compared to prevalence rate reported from Iraq (42.9%) (28), Nigeria (37.1%) (29), Saudi Arabia (32.1%) (30) and Adigrat, Ethiopia (21.2%) (4). In addition, the finding of the current study is higher than the prevalence reported from Nigeria (11%) (31) and Bahir dar, Ethiopia (11.5%) (32). The wide-ranging reported prevalence of ASB across different studies from one country to other and among region of the same countries might be to due attributed difference in associated factors, geographical variation, sample size, social habits of the community, health education practices and education levels of the patients being studied.

In the present study, Gram-negative bacterial isolates (59.2%) were more prevalent than Gram-positive bacteria isolates (40.8%). This finding was similar to study done in Gondar, Ethiopia (33), Tanzania (34), and Nigeria (35). This could be due to the presence of unique structure in Gram-negative bacteria that help with the attachment to the uroepithelial cells and to prevent bacteria from urinary lavage, allowing for multiplication and tissue invasion resulting in invasive infection and pyelonephritis during pregnancy (36).

E. coli 21(43%) was found to be the most frequent bacterial isolate in this study. Similar finding reported from in Ambo, Ethiopia (46.4%) (16), Adama, Ethiopia (37.3%) (24), Kenya (38.8%) (37) and Tanzania (64.2%) (38) have also showed that *E.coli* was common bacteria isolated. This could be due to a number of virulent factors specific for colonization and invasion of the urinary epithelium and difficultly of maintaining personal hygienic during pregnancy might increase the risk acquiring UTI by *E. coil* (15).

The second predominant isolate was S. *aureus* 10(20.4%) followed by *S. saprophyticus*, 7(14.3%). This finding is comparable with the study conducted in Iraq *S. aureus* (25.0%) (28), Kenya, *S. aureus* (29.7%) (37). The prevalence of *S. saprophyticus* in the current study is comparable with study done in Adigrat, Ethiopia (12.7%) (4). In contrast, high prevalence of *S. saprophyticus* was reported in Bahir dar, Ethiopia (48.2%) (32) and Nigeria (44.5%) (3). This might be due contamination as normal floral of the urogenital area, sexual behavior of patient studied and also unsafe insertion and long-term utilization of catheters.

The present study showed participants with a history of UTI had significantly higher prevalence of asymptomatic bacteriuria compared to those with no previous history of UTI (p=0.004). The pregnant women with a history of UTI is considered as strongest predictor of ASB, as study conducted in Gondar, Ethiopia (29) and Egypt (39). The possible explanation for this association could be due to existence of resistance strains from earlier uropathogens. The history of catheterization was also found significantly associated with the occurrence of ASB (p=0.040). This was similar to a study done in Dessie, Ethiopia (5). This could be due to contamination while catheter insertion, frequent and long-term catheterization which supports adherence of pathogens to the urinary tract.

Similarly, pregnant women with hemoglobin level less than 11g/dL were found more than ten times (AOR=10.49, 95%Cl (4.54, 24.04), likely to positive for ASB (p<0.0001). This finding is in line with the previous reported from Bahir dar, Ethiopia (40). Finally, in this study we reported pregnant women with a history natural abortion were 2.36 times more at risk to develop ASB (p=0.023). The results of this study disagree with a study conducted in Cameron (41).

Concerning with antimicrobial susceptibility patterns of isolates, the majority of Gram-negative isolates were susceptible to nitrofurantoin (93.1%), gentamicin (85.2%), ceftriaxone (82.2%), cefuroxime (79.3%), meropenem (75.2%), and ciprofloxacin (75.2%). These findings are comparable with a study conducted in Dessie, Ethiopia (5), and Bale, Ethiopia (42). However, most Gram-negative bacteria were resistant to amoxicillin (79.3%), amoxicillin-clavulanic acid (37.9%). The easy accessibility, indiscriminately and frequently used drugs such as amoxicillin and amoxicillin-clavulanic acid may result in an increase in resistance.

Most *E. coli* isolated in this study were susceptible to gentamicin (91%), nitrofurantoin (91%), ceftriaxone (81.8%), ciprofloxacin (81.8%) and cefuroxime (81.8%). However, *E. coli* showed the highest resistance to ampicillin, cotrimoxazole, amoxicillin-clavulanic acid. It is higher than a study conducted in North West Ethiopia (30) but it is low compared to a report from Kenya (34). The finding of this study is not in line with a study conducted in India, where 61% and 70% *E. coli* were susceptible to ampicillin and amoxicillin-clavulanic acid respectively (43). These differences could be due to variations in antibiotics prescribing habits between the countries.

This study also showed all *K. pneumoniae* isolates were 100% susceptible to nitrofurantoin and most of them were susceptible to ampicillin, amoxicillin-clavulanic acid and cefuroxime. This finding is not comparable with study a reported from Tanzania (32). *K. oxytoca* and *E. aerogens* were 100% resistant to ampicillin, and amoxicillin. The high resistance to ampicillin and amoxicillin observed in this study may be due to the easily availability of antibiotics in local pharmacies, being commonly prescribed antibiotics by health care workers without susceptibility testing in hospitals.

Our study revealed that, Gram-positive isolates were 100% susceptible to nitrofurantoin. This could be due to drug less commonly used the study area and it is relatively safe in pregnancy but may cause haemolysis with anaemia in the fetus of patients with glucose 6-phosphate dehydrogenase deficiency if used close to term (18). In the current study, most of *S. aureus* was resistant to penicillin, erythromycin, and tetracycline. This finding is lower than a study conducted in Togo (44). The resistance profiles of *S. saprophyticus* isolated in the current to penicillin, cotrimoxazole, and ciprofloxacin are comparable to study done in Iran (45). In addition, all *E. faecalis* were susceptible to gentamicin and ciprofloxacin; however, 50% of them were resistant to ampicillin and penicillin. In contrast, study conducted in Togo, all *E. faecalis* were resistant to ciprofloxacin (44).

Based on our finding 57.1% of the bacterial isolate showed multi-drug resistance (MDR) to those commonly used antimicrobial agents. This is relatively lower than MDR reported from Jimma, Ethiopia (90%) (46), Ambo, Ethiopia (100%) (16), Dire Dawa, Ethiopia (100%) (33), and Nigeria (100%) (47). The emergences of MDR is a global challenge and accelerated antimicrobial resistance could be due to widespread misuse of antimicrobials by the patient, inadequate dosing, poor drug quality, incomplete treatment courses and inappropriate prescription of antimicrobial without susceptibility tests are factors that increased bacterial resistance to antimicrobial agents.

Limitation of the study

The present study had limited to track patients though follow-up urine specimen testing to determine the efficacy of antimicrobial treatment due to lack antibiotic disc and financial constraints and also pregnant women with significant bacteriuria did not know their adverse maternal and perinatal outcomes.

In conclusion, the prevalence of ASB among pregnant women in the present study was at 16.9%. Women's with a past history of UTIs; history of catheterization, hemoglobin level less than 11g/dl and history of natural abortion were significantly associated with ASB. The most frequent identified isolates were *E. coli* (43.0%), followed by *S. aureus* (20.4%) and *S. saprophyticus* (14.3%). The antibiotic susceptibility result raveled that nitrofurantoin and gentamicin were the most effective against bacteria isolates. The study also showed that most of the bacteria were multidrug resistant. Therefore, the empirical antibiotic selection should be based on the knowledge of the local prevalence of bacterial organisms and antibiotic sensitivities rather than on universal guidelines. This study recommends that, there is need for early routine screening of all antenatal care pregnant women with or without clinical symptoms of urinary tract infection, in order to prevent adverse outcome to mother and fetus.

List of Abbreviations

ANC: Antenatal care, ASB: Asymptomatic bacteriuria, ATCC: American Type Culture Collection CFU: Colony Forming Unit, MDR: Multidrug-resistance, HIV: Human Immunodeficiency Virus, SPHMMC: Saint Paul's Hospital Millennium Medical College, UTI: Urinary tract infection

Availability of data and materials

All relevant data generated and analyzed are available within the paper.

Ethical approval and consent to participant

The study was approved by Institutional Review Board of Hawassa University, College of Medicine and Health Sciences (Ref No IRB/205/11) and Saint Paul's Hospital Millennium Medical College (Ref No pm23/368). An official permission letter was obtained from the Saint Paul's Hospital Millennium Medical College administration office. Participants enrolled in study were informed about the study objectives, expected outcomes and benefits and risks associated with it. Written informed consent was taken from the all participants before the interview.

Authors' contributions

YAW Conceived, designed the experiments, laboratory work, data analysis and write up **MMA** supervision, review, analysis and manuscript preparation **DYR** review, supervision, **ET&DBG** support laboratory work. All authors have read and approved the manuscript.

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Competing Interests

The authors declare that they no competing interests.

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