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Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae Associated with Asymptomatic Bacteriuria among Pregnant Women Attending Antenatal Clinic at Tertiary Referral Hospital, Tanzania

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Abstract

Background

Asymptomatic bacteriuria occurs in 2-15% of pregnancies resulting in acute pyelonephritis, preterm labor, pre-eclampsia, anemia, amnionitis, low birth weight, stillbirths, bacteremia and toxic septicemia. Asymptomatic bacteriuria in pregnancy (ASBP) caused by extended-spectrum beta-lactamases producing Enterobacteriaceae (ESBL-PE) further complicates the health of a pregnant woman, affecting treatment and spread of resistant bacteria strains to newborns and the community. However, in Tanzania, screening for resistant bacteria such as ESBL-PE in ASBP is not routinely done.

Broad objective

To determine the prevalence of ASBP associated with ESBL-PE and the antimicrobial susceptibility pattern of ESBL-PE isolated from pregnant women at Muhimbili National Hospital (MNH) in Dar es salaam, Tanzania.

Methodology

A hospital-based cross-sectional study was conducted at MNH. A total of 182 pregnant women with the gestational age of 37 weeks and above were enrolled. A semi-structured questionnaire and antenatal cards were used to collect socio-demographic and pregnancy information. Clean catch mid-stream urine was collected for screening of asymptomatic bacteriuria. Bacteria were identified using conventional biochemical methods and antimicrobial susceptibility testing (AST) was performed by Kirby-Bauer method following Clinical Laboratory Standard Institute (CLSI) guidelines. The isolates resistant to ceftazidime and cefotaxime were confirmed for ESBL production using a double-disc synergy test (DDST).

Results

Asymptomatic bacteriuria was observed in 13% (24/182) of the pregnant women attending antenatal clinic at MNH. We report that, 61.9% (13/21) of women with asymptomatic bacteriuria associated with gram negative bacteria were infected with ESBL-PE. Among the ESBL-PE species mostly isolated include *E. coli* (69.2%), followed by *K. pneumoniae* (23.1%), and *K. oxytoca* (7.7%); and non ESBL-PE species isolated were *E. coli* (87.5.6%), and *K. pneumoniae* (12.5%). In addition, three *S. aureus* isolates were detected in women with ASBP. ESBL-PE isolates showed high resistance to aztreonam, sulphamethoxazole-trimethoprim, amikacin and nalidixic acid; while for the few detected non ESBL-PE high resistance was seen to sulphamethoxazole-trimethoprim, aztreonam, meropenem and nalidixic acid.

Conclusion and recommendation

The present study revealed that a high proportion of bacteriuria in pregnancy is associated with ESBL-PE. These findings suggest a need for screening of resistant bacteria such as ESBL in cases of ASBP.

Keywords: *Pregnancy, Asymptomatic bacteriuria, Extended Spectrum Beta-Lactamase, Enterobacteriaceae, Resistance.*

Introduction

Pregnant women are prone to infections, including urinary tract infection (UTI) (1-3). The immunosuppression state and the physiological changes during pregnancy, such as increased plasma volume, urethral dilation, increased bladder volume, and decreased bladder tone, cause the stagnancy of urine flow; that supports bacterial growth in the urine (4, 5). In pregnant women, UTI can be symptomatic (symptomatic bacteriuria) or asymptomatic (asymptomatic bacteriuria) (3). Both gram-positive and gram-negative bacteria cause asymptomatic bacteriuria (ASB) (3) . The most common reported causative agents are *Escherichia coli, Staphylococcus aureus, Staphylococcus saprophyticus, Klebsiella pneumoniae, Klebsiella oxytoca, Pseudomonas aeruginosa, Streptococcus agalactiae, Enterococcus faecalis,* and *Proteus spp* (3, 6-9). Unlike immunocompetent groups, treatment of ASB and the right choice of drugs with minimum risk to the pregnancy are necessary for management in pregnant women (10).

Bacteriuria in pregnancy, even without symptoms, may result in several complications: preterm labor, pre-eclampsia, hypertension, pyelonephritis, anemia, amnionitis, low birth weight, stillbirths, neonatal deaths, bacteremia, and toxic septicemia (11). In addition, infection with multi-drug resistant bacteria like extended spectrum beta lactamase (ESBL) producers further complicate the treatment of ASBP and increase the risk of transmission to newborn babies and the community (11, 12).

Previous meta-analysis studies estimated that the prevalence of ASBP by extended spectrum beta lactamase producing enterobacteriaceae (ESBL-PE) among pregnant/postpartum women worldwide is 28% and 3.9% to 45% in Africa (13, 14). In Tanzania, data on the role of multi-drug resistant bacteria such as ESBL-PE in ASBP is limited.

Therefore, this study aimed to investigate the prevalence of ASBP associated with ESBL-PE and antimicrobial susceptibility pattern of ESBL-PE isolated from pregnant women attending antenatal clinic at Muhimbili National Hospital (MNH) in Dar es salaam, Tanzania.

Methods

Study design and study area

A hospital-based cross-sectional study was conducted in 2019 at Muhimbili National Hospital (MNH) in Dar es Salaam, Tanzania. MNH is the largest tertiary health care facility in Tanzania, a research center, university teaching hospital, and referral hospital. The study settings have been previously described in detail (15).

Study population, sample size, and sampling procedure

The study enrolled 182 pregnant women who were at the gestational age of 37 weeks and above attending the antenatal clinic (ANC) at MNH and consented to participate in the study. The sample size was obtained using the Kish Leslie formula, where the expected proportion was estimated at 18.5% (16); and the margin of error was 6%. Part of the data on ESBL-PE carriage in stool samples from these participants has been previously published (15). As previously described briefly, convenient sampling was used to recruit pregnant women in the study. The eligible pregnant women were consecutively enrolled during their ANC visit until the representative sample size was attained and were then screened for ASBP (15).

Data collection

Social demographic data (age, level of education), and history of antibiotic use, hospitalization and hygienic risk behaviours were collected by using a semi-structured questionnaire; and clinical information (gestational age, parity, HIV and syphilis status) was obtained from the antenatal card. The study participants' social demographic and clinical information have been described in detail previously (15).

Study variables

We defined asymptomatic bacteriuria in pregnancy (ASB) as the presence of at least one species of bacteria growing in the urine at specified quantitative counts ($\geq 10^5$ colony-forming units [CFU]/mL), irrespective of the presence of pyuria, in the absence of signs or symptoms attributable to urinary tract infection (UTI) (17).

Sample collection and laboratory procedures

Clean catch mid-stream urine specimens were collected from the pregnant women using sterile urine containers as recommended by WHO (18). Specimens were sent to the Central pathology laboratory (CPL) at MNH and were analyzed within an hour of collection. Standard loops of one µl were used to inoculate urine specimens on Cystine Lactose Electrolyte Deficient, CLED (OXOID, England) medium. The plates were incubated for 24 hours at 37^{o}C; the diagnosis of ASBP was made when there was a pure growth of at least one species at $\geq 10^5$ colony-forming units (CFU) of bacteria/ml of urine. For (CFU)/ml of below 10^5 and colony counts with two or more species of bacteria were considered as contaminations, and no further tests were performed on the samples (19). Preliminary identification of pure isolates was based on colonial morphology and gram staining

properties. All isolates were sub-cultured on Nutrient agar (NA) and incubated aerobically at 37ºC overnight. Isolates from NA were identified using conventional biochemical tests. The identification tests included the Oxidase test, Kligler Iron Agar, Sulphur-Indole-Motility test, Simmons' citrate test, and Urease test for gram-negative bacteria, while catalase and coagulase tests were used for gram-positive bacteria, as previously described (20). Antimicrobial susceptibility test (AST) was done by disc diffusion method, whereby individual colonies were suspended in normal saline to obtain 0.5 McFarland turbidity, and using sterile swabs; the suspensions were inoculated on Muller Hinton agar then incubated overnight at 37ºC. All procedures were done as recommended by Clinical Laboratory Standard Institute (CLSI) (21). With the omission of aztreonam (30 µg) for *S. aureus* antibiotic disks such as gentamicin (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), amoxicillin-clavulanic acid (20/10 µg), sulphamethoxazole-trimethoprim (1.25/23.75), ciprofloxacin (5 µg), meropenem (10 µg), nitrofurantoin (300 µg), nalidixic acid (30 µg), amikacin (30 µg) and cefoxitin (30 µg) were tested for ESBL-PE, non ESBL-PE and *S. aureus* isolates. By using erythromycin (15 µg) and clindamycin (2 µg) discs, D test was performed for *S. aureus*. Briefly, a 15-µg erythromycin disk was placed in proximity to a 2-µg clindamycin disk on an agar plate that has been inoculated with a staphylococcal isolate; the plate was then incubated overnight. A flattening of the zone of inhibition around the clindamycin disk proximal to the erythromycin disk (producing a zone of inhibition shaped like the letter D) was considered a positive result and indicated that the erythromycin has induced clindamycin resistance (a positive “D-zone test”).

The antibiotics were selected based on the CLSI recommendations and local frequent prescriptions. All antibiotics used were from Oxoid, Ltd, UK.

Standard methods were used for screening of ESBL using ceftazidime (30 µg) and cefotaxime (30µg) antibiotics; and the results were interpreted as per CLSI guideline. The isolates that were resistant and intermediate to ceftazidime and cefotaxime were phenotypically confirmed for the production of extended spectrum beta lactamases (ESBL) enzymes using double disk synergy test (DDST) as previously described (20). Briefly, the isolates were incubated at 37ºC for 24 hours in a Muller Hinton agar with amoxicillin/clavulanic acid (20/10µg) disc placed in the center of the plate and ceftazidime (30µg) and cefotaxime (30µg) discs were placed 15 mm apart center to center to amoxicillin/clavulanic acid. After overnight incubation any increase in the inhibition zone towards the disc of amoxicillin/clavulanic acid, the isolate was interpreted as ESBL producer (20).

Quality control

Stains and reagents were clearly and uniquely labelled, dated, and correctly stored. In addition, we monitored the operating temperature of the refrigerator and incubator. Culture media were prepared according to the manufacturer's instructions-tested for performance and sterility. To standardize the turbidity of the bacterial suspension for susceptibility and ESBL production tests, a 0.5 McFarland standard was used. As per CLSI 2019 guideline, *Escherichia coli* (ATCC-25922) was used as a quality control strain for susceptibility testing, while *Klebsiella pneumoniae* (ATCC -700603) and *Escherichia coli* (ATCC-25922) were used as positive and negative control strains for ESBL production tests, respectively (21). For the D-test, *Staphylococcus aureus* (ATCC -700699) and *Staphylococcus aureus* (ATCC -25923) were used as positive and negative control strains, respectively.

Statistical analysis

STATA version 15.1(StataCorp LLC, United States of America) was used for analysis. Data were entered and cross-checked to ensure completeness, accuracy, and reliability. Mean and standard deviation was used to analyze continuous variables, whereas proportions were used to describe categorical variables. Fisher's exact test was used to determine difference among the categorical variables. A p-value of ≤ 0.05 was considered statistically significant for all the statistical tests.

Ethics approval and consent to participate

Ethical clearance was obtained from the Senate Research and Publications Committee of the Muhimbili University of Health and Allied Sciences (MUHAS). Permission to conduct the study was obtained from the MNH administration. Written informed consent was obtained from each participant prior to enrolment in the study. Confidentiality of the study participants was ensured using codes instead of participant's names.

Results

The proportion of ESBL-PE isolated among women with ASBP

In this study, we observed that 13.2% (24/182) of the pregnant women had asymptomatic bacteriuria (with one species of bacteria growing in the urine (≥10^5 Colon forming unit (CFU)). Among the 24 pregnant women with ASB, we detected both gram negative (n=21) and gram positive (n=3) bacteria. ESBL-PE were isolated from 61.9% (13/21) of the women with ASBP associated with gram negative bacteria (Figure 1). The distribution of risk factors

across pregnant women with ESBL-PE associated ASBP and those with non-ESBL-PE associated ASBP and gram positive bacteria are summarized in Table 1. However, these factors; participant's age, gestational age, parity, level of education, previous history of antibiotic use and/or self-prescription of antibiotic medication in pregnancy, handwashing with soap before eating, and hands washing using soap after defecation were not statistically significantly associated with ASBP (Table S1-S3), and were also not associated with ESBL-PE associated ASBP and non-ESBL-PE associated ASBP ($p > 0.05$) (Table 1).

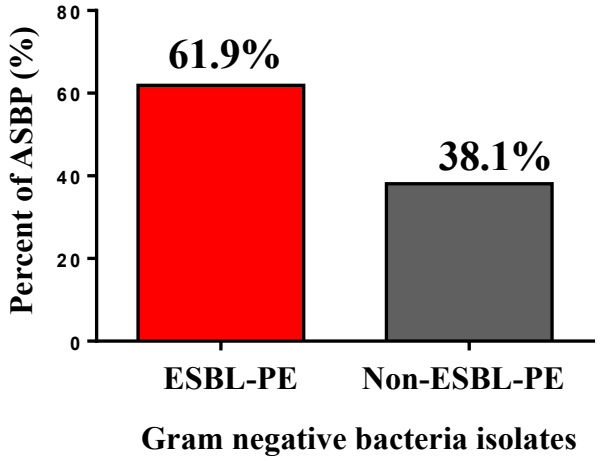

Figure 1: Proportion of ESBL-PE and non-ESBL-PE associated ASBP

The figure illustrates the proportion of ASBP associated with ESBL-PE (n=13) and non-ESBL-PE (n=8) among pregnant women with ASB associated with potential ESBL producer bacteria (n=21).

Table 1: Distribution of ASBP by social-demographic characteristics, clinical factors, and hygienic practices

Variable name	Total number of pregnant with ASBP N=24 (%)	ESBL-PE +ve-ASBP n=13 (%)	non-ESBL-PE and *S. aureus* ASBP n=11 (%)	p-value (Fisher's exact test)
Mean age (Years)	26.4±4.8	25.8±6.0	27.1±3.1	0.5417
Mean GA (weeks)	39.7±1.8	39.2±1.6	40.3±2.0	0.1397
Mean number of parity (Children)	1.8±1.1	1.7±1.1	2.0±1.1	0.5030

Education level				0.697
Low	10 (41.7)	6 (60.0)	4 (40.0)	
High	14 (58.3)	7 (50.0)	7 (50.0)	
Self-prescription of antibiotic in pregnancy				0.386
Yes	17 (70.8)	8 (47.1)	9 (52.9)	
No	7 (29.2)	5 (71.4)	2 (28.6)	
Hands washing with soap before eating				0.630
Yes	19 (79.2)	11 (57.9)	8 (42.1)	
No	5 (20.8)	2 (40.0)	3 (60.0)	
Hands washing using soap after defecation				0.300
Yes	20 (83.3)	12 (60.0)	8 (40.0)	
No	4 (16.7)	1 (25.0)	3(75.0)	

**A p-value of less than 0.05 indicates statistically significant association (Fischer's exact test)*.

Bacterial species isolated among women with ASBP

The bacteria species isolated among the pregnant women with asymptomatic bacteria (ASBP) were *Escherichia coli 16* (66.6%), *Klebsiella pneumoniae* 4 (16.7%), *Klebsiella oxytoca* 1 (4.2%) and *Staphylococcus aureus* 3(12.5%) (Figure 2A). This study identified three different ESBL-PE species among the 13 ESBL producing bacterial isolates whereby, *Escherichia coli* was the most frequently isolated uropathogenic species with a proportion of 69.2% followed by *Klebsiella pneumoniae* (23.1%) and *Klebsiella oxytoca* (7.7%) (Figure 2B).

Out of the 24 participants with ASBP, non-ESBL-PE species (n=8) and gram positive bacteria *Staphylococcus aureus* (n=3) were recovered from 11 participants. The isolated non-ESBL-PE bacteria species included *Escherichia coli* (n=7) and *Klebsiella pneumoniae* (n=1) (Figure 2B).

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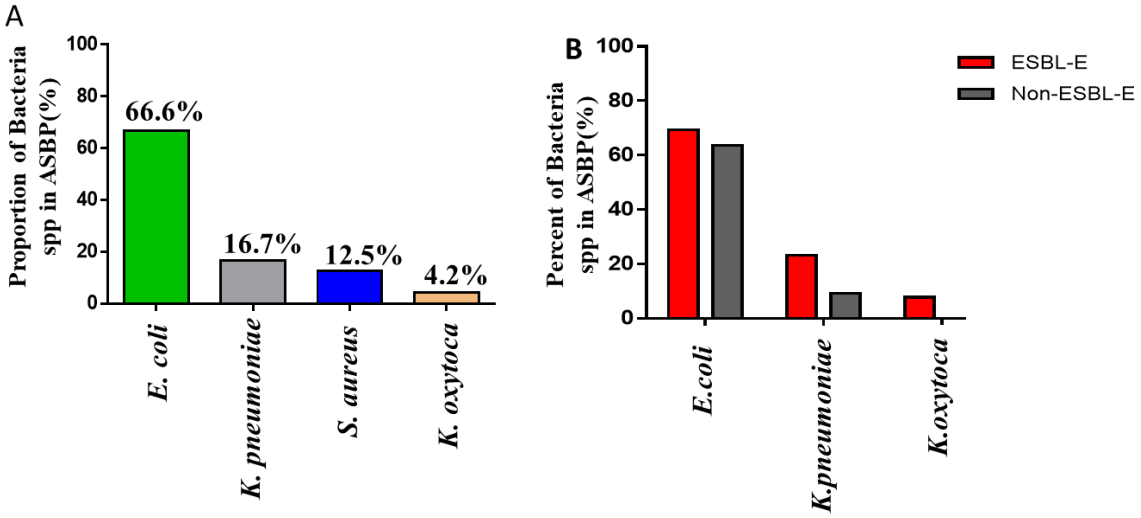

Figure 2A-B: Proportion of isolated bacteria species from pregnant women with ASB

The figure illustrates the distribution of bacteria species isolated from participants with ASBP (n=24) (A). The figure illustrates the proportion of specific ESBL-PE and non ESBL-PE isolates obtained from pregnant women with ASB at Muhimbili National Hospital (n=21).

Antimicrobial resistance pattern of bacterial species isolated from pregnant women with ASB

AST was performed for the all isolated bacterial species including ESBL-PE, non-ESBL-PE and *Staphylococus aureus*. Among the ESBL-PE isolates *E. coli* (88.9%) showed high resistance to sulphamethoxazole-trimethoprim, aztreonam and third generation cephalosporins. In addition, 22.2%, 33.3% and 44.4% of *E. coli* isolates showed resistance to nitrofurantoin, nalidixic acid and amikacin, respectively. *K. pneumoniae* isolates 66.7% were resistant to sulphamethoxazole-trimethoprim and aztreonam. All *K. pneumoniae* isolates were resistant to nalidixic acid and amikacin disks while the only one isolate of *K. pneumoniae* was resistant to gentamicin. In contrast, among non-ESBL-PE isolates, 28.6% of *E.coli* isolates were resistant to meropenem and third generation cephalosporins drugs; and 71.4% were resistant to sulphamethoxazole-trimethoprim and nalidixic acid. The only one isolate of *K. pneumoniae* found was resistant to aztreonam and meropenem. About 66.7% and 33.3% of *S. aureus* isolates were resistant to sulphamethoxazole-trimethoprim and amikacin, respectively (Table 2). In regard to the *S. aureus* species detected, the D-test was negative. In addition, none of the three *S. aureus* species detected were methicillin

resistant *Staphylococcus aureus* (MRSA) as determined by cefoxitin disc (30 µg) disk diffusion testing.

Table 2: Antimicrobial resistance patterns of ESBL-PE and non-ESBL-PE bacteria isolated from women with ASBP

ANTIBIOTIC	ESBL-PE ISOLATES			NON-ESBL-PE ISOLATES	
	E. coli *n*=9 (69.2%)	*K. pneumoniae* *n*=3 (23.1%)	*K. oxytoca* *n*=1 (7.7%)	*E. coli* *n*=7 (87.5%)	*K. pneumoniae* *n*=1 (12.5%)
Gentamicin (10 µg)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)
Ceftazidime(30 µg)	8 (88.9)	3 (100.0)	1 (100.0)	2 (28.6)	0 (0)
Cefotaxime (30 µg)	8 (88.9)	3 (100.0)	1 (100.0)	2 (28.6)	0 (0)
Ceftriaxone (30 µg)	8 (88.9)	3 (100.0)	1 (100.0)	2 (28.6)	0 (0)
Aztreonam (30 µg)	8 (88.9)	2 (66.7)	1 (100.0)	5 (71.4)	1 (100)
Amoxiclav (20/10 µg)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sulphamethoxazole-trimethoprim (1.25/23.75)	8 (88.9)	2 (66.7)	0 (0)	5 (71.4)	1 (100)
Ciprofloxacin(5 µg)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Meropenem(10 µg)	0 (0)	0 (0)	0 (0)	2 (28.6)	1 (100)
Nitrofurantoin (300 µg)	2 (22.2)	0 (0)	0 (0)	3 (42.9)	0 (0)
Nalidixic acid (30 µg)	3 (33.3)	3 (100)	0 (0)	5 (71.4)	0 (0)
Amikacin (30 µg)	4 (44.4)	3 (100)	0 (0)	1 (14.3)	0 (0)
Cefoxitin (30 µg)	9 (100)	3 (100)	1 (100.0)	3 (42.9)	0 (0)
Erythromycin (15 µg)	-	-	-	-	-
Clindamycin (2 µg)	-	-	-	-	-

Discussion

In the present study, the overall prevalence of ASB in pregnancy was 13.2%. This finding is consistent to similar previous study conducted in Tanzania (3). However, it is higher compared to the general populations (22) therefore, further supporting an increased risk of ASBP in pregnancy. We observed 61.9% of bacterial species isolated from pregnant women with ASB were ESBL-PE. The observation is relatively comparable to that reported in Egypt (23) and in a systematic review and meta-analysis in Africa (13). The high prevalence of ESBL-PE associated ASBP suggests an increased risk for ESBL-PE associated complications in pregnancy and transmission of infection to newborn and community.

In this study, *E. coli, K. pneumoniae, S. aureus* and *K. oxytoca* were the reported etiological bacteria of ASB in pregnancy, with *E. coli* being the dominant uropathogenic bacteria. These

findings are similar to those reported in a systematic review and meta-analysis by Mansouri F *et al* (2019), and Belete *et al* (2020) in Ethiopia that reported the most common causative agent of asymptomatic bacteriuria in pregnant women was *E. coli* followed by the *Klebsiella* species (13, 19). This observation suggests that the trends of distribution of uropathogenic bacteria and the uropathogenic virulence factors may yet not be affected by the selective pressures including antibiotics and other biological factors.

We report that ESBL-PE had low resistance to nitrofurantoin, and gentamicin. However, ESBL-PE demonstrated moderate and high resistance to nalidixic acid and amikacin, and sulphamethoxazole-trimethoprim, respectively. The present findings indicate that ESBL-PE associated with ASBP has high rate of multidrug resistance than non-ESBL-PE. These findings agree with those reported by other studies including studies by Mansouri *et al*. (2019) (13), and Belete *et al*. (2020) Ethiopia (19). Therefore, the present study clearly demonstrates the continuous need for periodic surveys to determine the susceptibility patterns of microorganisms that cause ASB in pregnant women. Despite the small number of isolates included in AST in our study, from our results we suggest the following potential antibiotics to be considered for empirical treatment of ESBL-PE associated ASBP: amoxiclav(augmentin), meropenem, gentamicin, and nitrofurantoin. However, further, studies involving a larger sample size and inclusion of minimum inhibitory concentration (MIC) analysis are warranted to determine the antibiotic concentration that will be appropriate for treatment of ESBL-PE associated ASBP.

In addition, in this study, logistic regression analysis was performed where applicable to identify the factors associated with ASBP and ESBL-PE associated with ASBP. However, we did not observe any statistically significant association between ASBP, ESBL-PE associated ASBP, non-ESBL-PE associated ASBP and the participants' socio-demographic characteristics and clinical information, including; maternal age, gestational age, parity, educational level, previous history of antibiotic use and/or self-prescription of antibiotic medication in pregnancy; hygienic behaviors such as handwashing with soap before eating and hands washing using soap after defecation. In contrast, other studies have reported the association between ESBL-PE in ASBP and history of previous antibiotic use three or six months before sample collection (19, 23). This difference could be due to the variation in duration of previous history of antibiotic use and the small sample size in our study.

Our study had some limitations; we recruited pregnant women at term, so we cannot deduce when these participants acquired ESBL-PE infection, either during other antenatal visits at the hospital or from the community. In addition, we had a limited sample size, hence we

could not establish significant association between ASBP, ESBL-PE associated ASBP and the participants' socio-demographic characteristics and clinical data.

Conclusion

The current study has revealed a high burden of ESBL-PE among pregnant women with ASB. With the current global threat of an increase in antimicrobial resistance, our findings further emphasize the need to conduct screening of antibiotic resistant bacteria in pregnant women with ASBP at each trimester. Our study also suggests that nitrofurantoin remains to be the drug of choice for treating ASBP. However, multi-drug resistance screening should be performed to assist in the appropriate selection of treatment choices.

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The authors would like to acknowledge all participants in this study. In addition, we wish to thank the nurses from MNH who assisted with participants' recruitment and specimen collection. We also extend our sincere appreciation to all the laboratory technicians of the departments of Microbiology and Immunology, MUHAS.

Authors' contributions

DK and AMM were involved in the study's conceptualization and data collection. DK and AMM performed the laboratory work and statistical analysis. DK, AMM, UK, SM, MMM, SEM, JM, and MM were involved in manuscript preparation. SEM and MM critically reviewed the manuscript.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Abbreviations

ASBP	Asymptomatic bacteriuria in pregnancy
AMR	Antimicrobial Resistance
ANC	Antenatal Clinic
AST	Antibiotic Susceptibility Test
CLSI	Clinical Laboratory Standard Institute
DDST	Double Disc Synergy Test
ESBL	Extended Spectrum Beta-Lactamase

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ESBL-PE	Extended Spectrum Beta-Lactamase producing Enterobacteriaceae
HIV	Human Immunodeficiency Virus
CLED	Cystine Lactose Electrolyte Deficient
MNH	Muhimbili National Hospital
MUHAS	Muhimbili University of Health and Allied Sciences
NA	Nutrient Agar
UTI	Urinary Tract Infection
WHO	World Health Organization.

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Supplementary Tables

Table S1: ASB among pregnant women by social-demographic, clinical factors, and hygienic factors

Variable name	Total Pregnant women	ASBP+	ASBP-	P-value (Fisher's exact test)
	N = 182 (%)	n=13 (%)	n=169 (%)	
Mean age (Years)	28.8±5.2	25.8±6.0	29.0±5.0	**0.0307**
Mean GA (weeks)	38.9±1.5	39.2±1.6	38.9±1.5	0.5938
Mean number of parity (Children)	2.1±1.3	1.7±1.1	2.1±1.3	0.2409
Education level				1.000
Low	80 (44.0)	6 (7.5)	74 (92.5)	
High	102 (56.0)	7 (6.9)	95 (93.1)	
HIV status				1.000
Positive	9 (5.0)	0 (0.0)	9 (100)	
Negative	173 (95.0)	13 (7.5)	160 (92.5)	
Self-prescription of antibiotic in pregnancy				0.572
Yes	95 (52.2)	8 (8.4)	87 (91.6)	
No	87 (47.8)	5 (5.8)	82 (94.3)	
Hands washing with soap before eating				0.671
Yes	159 (87.4)	11 (6.9)	148 (93.1)	
No	23 (12.6)	2 (8..7)	21 (91.3)	
Hands washing using soap after defecation				0.696
Yes	156 (85.7)	12 (7.7)	144 (92.3)	
No	26 (14.3)	1 (3.9)	25 (96.1)	

In bold p-value of less than 0.05 indicate statistically significant association (Fischer's exact test for categorical data and Student's t-test for continuous)

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Table S2: Socio-demographic, clinical and hygienic factors associated with ASB by bivariate analysis

Variable	Total number	UTI+, n (%)	cOR	95% CI	p-value
Age (p=0.0307)	182	13 (7.1)	2.11	0.77-0.99	**0.035**
Number of parity (p=0.2409)	182	13 (7.1)	1.17	0.44-1.23	0.244

cOR stands for crude odds ratio and Ref stands for reference (Binary logistic regression)

Table S3: Multivariate logistic regression for the factors independently associated with ASB

Variable	UTI+, n (%)	cOR	95% CI	p-value	aOR	95% CI	*p*-value
Age (p=0.0307)	13 (7.1)	2.11	0.77-0.99	0.035	1.81	0.75-1.01	0.071
Number of parity (p=0.2409)	13 (7.1)	1.17	0.44-1.23	0.244	0.02	0.53-1.85	0.985

cOR stands for crude odd ratio, aOR stands for adjusted odd ratio and Ref stands for reference association (Log likelihood)