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Asymptomatic Bacteriuria among Pregnant Women Attending Antenatal Care at Sinza District Hospital, Dar es Salaam, Tanzania

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Abstract

Background

Asymptomatic bacteriuria (ASB) in pregnancy is associated with poor fetal and maternal outcomes. Treatment of ASB in pregnant women is of great concern due to the emergence of antimicrobial resistance. In this study, we determined the prevalence of ASB, bacterial profile, and antimicrobial susceptibility pattern among pregnant women attending an antenatal clinic (ANC) at a secondary-level hospital.

Methods

A cross-sectional study was conducted among pregnant women attending ANC at Sinza District Hospital. A total of 329 pregnant women with no symptoms of urinary tract infection were recruited from November 2019 to January 2020. A structured questionnaire was used to collect sociodemographic and obstetric information of pregnant women following health education session where a minimum of ten pregnant women were enrolled into the study daily. Clean catch mid-stream urine samples were collected from all study participants using sterile containers and transported in an ice-packed cool box to Microbiology laboratory at Muhimbili University of Heath and Allied Sciences for processing. Urinalysis using urine dipstick, urine culture, and antimicrobial susceptibility testing were performed. Bacterial identification was done based on colonial morphological and biochemical tests. Data was analyzed for frequency distribution, proportions and proportional differences using the Chi-square test.

Results

Out of 320 pregnant women included in the final analysis, 21 (6.6%) were diagnosed with ASB. Nine samples were excluded from the analysis due to fungal or mixed growth. *E. coli* was the predominant isolate 9/21(42.8%), followed by *Staphylococci* spp 4/21 (19.0%). Among the gram-negative isolates, *E. coli* demonstrated a high resistance rate to amoxicillin (89%), amoxicillin-clavulanic acid (89.0%), and trimethoprim/sulphamethoxazole (78.0%) but susceptible (100.0%) to amikacin, gentamycin, nitrofurantoin, and meropenem. All *enterococci spp* were resistant to trimethoprim/sulphamethoxazole and 66.6% to nitrofurantoin while *Staphylococci* spp showed 50% resistance. The urine dipstick test showed a sensitivity of 38.1 %, specificity (93.3%), positive predictive value (28.6%) and negative predictive value (95.6%) respectively.

Conclusion

The prevalence of ASB among pregnant women attending ANC at secondary-level hospital was lower than previous findings in Tanzania. *Escherichia coli* was the most isolated organism causing ASB. High rate of resistance was observed to commonly prescribed antibiotics. Urinalysis revealed high false positive results. We recommend large study to be conducted involving secondary level facilities in order to gather enough data on AST pattern as well as to guide patient's management.

Keywords: *Asymptomatic bacteriuria, Pregnancy, Antimicrobial susceptibility testing, Lower-level facility.*

Introduction

Asymptomatic bacteriuria (ASB) is the presence of significant bacteriuria in absence of symptoms suggestive of urinary tract infection (UTI) (1, 2). Pregnant women are more susceptible to symptomatic and asymptomatic UTI due to their anatomical and hormonal changes during pregnancy and the short nature of the female urethra (3). Globally, the reported prevalence rates of ASB in pregnancy range from 2.0% to 10.0% (2, 4). However, studies in Africa showed a wide regional variation. In western Africa, a very high prevalence of 86.6% was reported in Benin City, Nigeria (5). In East Africa, a prevalence of 21.0% had been reported in Kenya (6) and a low prevalence of 3.8% in Uganda (7). Previous studies conducted in Tanzania on ASB, reported the prevalence ranging from 7.0 -13.0% (8, 9, 10).

It has been reported that *Escherichia coli* causes more than three-quarters of ASB; other bacteria commonly associated with ASB include *Klebsiella* spp, *Proteus* spp, *Staphylococcal* spp, enterococci, and group B streptococci, accounting for the remaining 25% (1, 9, 10). The susceptibility to antibiotics of these organisms varies geographically, and antibiotic therapy in the absence of culture and susceptibility tests should be based on established antimicrobial susceptibility patterns in the specific institution (11).

Several factors have been reported to associate with a high risk of ASB, including prior history of UTI, pre-existing diabetes mellitus, increased parity, low socioeconomic status, sexual activity, anatomical urinary tract abnormalities, sickle cell disease and elderly (1, 12, 13, 14). Treatment of ASB in pregnant women involves antibiotic therapy tailored to culture and susceptibility results. World Health Organization (WHO) recommends a seven-day antibiotic regimen for all pregnant women with ASB (1). Untreated ASB predisposes pregnant women to severe outcomes like pyelonephritis, maternal septicemia, renal dysfunction, and anemia, as well as adverse fetal outcomes, such as low birth weight, preterm birth, and perinatal mortality (1).

Pregnant women with ASB are at an increased risk for severe outcomes; thus, approximately 30% will end up with severe fetal complications without appropriate treatment. Unfortunately, many health facilities do not offer appropriate ASB or UTI investigations (15). In most health facilities in Tanzania, urine analysis is done at the first prenatal visits using urine dipstick tests aiming at detecting nitrites and leucocytes in urine for UTI diagnosis. The test may, however, give false positive or negative results. Several studies reported low sensitivity ranging 43.8% - 50.0%, and specificity of up to 89% for dipstick urine test (16, 17). Based on this, urine culture remained the gold standard for diagnosing UTI/ASB. Urine culture is usually done on a few pregnant women presenting with symptomatic UTI and in a few centers, especially tertiary hospitals, where skilled personnel and laboratory facilities are available. The test is expensive and usually takes two or more days to get the results. However, WHO still recommends screening for ASB using urine culture and treating ASB during pregnancy to prevent persistent bacteriuria, and poor maternal and fetal outcomes (18).

The emergence of bacterial resistance to antibiotics and the wide geographical variations in the susceptibility pattern of antimicrobials are of great concern, hence the need for continuous monitoring of the susceptibility patterns of bacterial isolates (1). However, data regarding the magnitude of ASB and antimicrobial resistance on the lower-level facilities in Tanzania are limited. Most of the studies were conducted in tertiary hospitals (8, 9, 10, 19, 20), and only few involved the lower-level health facilities (8, 20). This study was therefore conducted to determine the prevalence of ASB, bacterial profile, and antimicrobial sensitivity pattern among pregnant women attending the antenatal clinic at Sinza District Hospital.

Material and Methods

Study Design, Setting and Population

We conducted a cross-sectional facility-based study between November 2019 and January 2020 at Sinza District Hospital in Dar es Salaam, Tanzania, a public lower-level health facility in Ubungo district. The study involved pregnant women who attended antenatal care during the study period. The sample size of 329 pregnant women was estimated using the Kish–Leslie formula and reported a prevalence of 8.9 by Mwei et al. from Northern Tanzania (9). Pregnant women with symptoms suggestive of UTI or a history of antibiotic use at the time of recruitment or within two weeks before enrollment were excluded from the study.

Data Collection

A systematic sampling technique was used to obtain participants. All pregnant women attending ANC are usually registered daily. The first participant was randomly selected from the register then every 5th client was included until the required sample size was reached. The immediate next client was picked if the selected client did not meet the inclusion criteria. A minimum of ten pregnant women were recruited in the study daily. A structured questionnaire was used to collect social-demographic characteristics and obstetric information following a daily health education session. After the interview, each participant was provided with a sterile container for urine collection. Instruction on how to collect midstream urine was given to each participant. A mid-stream urine sample was collected into a pre-labeled wide-mouthed sterile container and kept at 2-8°C in an ice-packed cool box before being transported to the Microbiology teaching laboratory at Muhimbili University of Health and Allied Sciences (MUHAS) for processing.

Laboratory Procedures

At the laboratory, the urine sample was aliquoted into two portions; one for urinalysis using a urine dipstick and the other for culture and antibiotic susceptibility test (AST).

Urinalysis

Dipstick urine analysis was done using urine reagent strips (Cybow-England) according to manufacturer's instructions. Dipstick urine test was regarded as positive when the infection-related markers, nitrites, leucocyte esterase or both were detected.

Culture and Bacterial Identification

A standard sterile wire loop (1 µL) was used to inoculate urine sample into cysteine lactose electrolyte deficient agar (CLED-Himedia-India) and blood agar media (OXOID-England). The plates were incubated at 37°C and read after 24 hours for significant bacteriuria, defined as the presence of equal or more than 10^5 colony-forming units (CFU) of the same bacteria per milliliter of urine. Culture plates with mixed growth of bacteria were considered as contaminations. Bacterial pathogens were identified based on colonial morphology, Gram staining and standard biochemical tests including Indole, citrate, urease, hydrogen sulfide production, motility and oxidase test was used. For gram-positive bacteria, catalase, coagulate, DNase and pyrrolidonyl arylamidase (PYR) test were performed.

Antibiotic susceptibility testing

AST was performed using the Kirby Bauer disc diffusion method according to Clinical Laboratory Standards Institute (CLSI) guideline recommendations (21). Briefly, colonial suspension from pure culture was prepared in normal saline to match with 0.5 McFarland standard turbidity and inoculated on Muller Hinton agar plates (OXOID-England). Plates were incubated at 37^0C for 18-24 hours, and the zone of inhibition was interpreted according to CLSI guidelines (21). For Gram-negative bacteria, the following disks (OXOID-England) were tested; Ampicillin (10µg), Gentamycin (10µg), Ciprofloxacin (5 µg), Ceftriaxone (30µg), Nalidixic acid (30µg), trimethoprim/sulphamethoxazole (1.25/23.75 µg), amikacin (30µg), nitrofurantoin (300µg), Amoxicillin/clavulanic acid (20/10µg) and meropenem (10µg). For gram-positive isolates, the following disks were tested; Azithromycin (15µg), Ciprofloxacin (5 µg), Amoxicillin/clavulanic acid (20/10µg), Trimethoprim/sulphamethoxazole 1.25/23.75 µg, and nitrofurantoin (300µg).

Quality control

Media were prepared according to the manufacturer's instructions, performance and sterility checks were done*.* Standard *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 35218) were used as reference strains for quality control of culture media, biochemical test and AST.

Data Analysis

Data analysis was done using Statistical Packages for Social Sciences (SPSS) software version 23. Descriptive statistics using frequency distribution tables and proportions were used to summarize categorical data. The comparison within variables was performed using the Chi-square test to observe the proportion differences. ASB. *P-value* < 0.05 was considered significant.

Ethical Consideration

The ethical clearance for the study was obtained from the Senate Research and Publication Committee of MUHAS. Permission to conduct the study was obtained from the Municipal Director of Ubungo District, the District Medical Officer, and the medical officer in charge of Sinza District Hospital. Written informed consent was obtained from the participants before recruitment. Information concerning individual participants was kept confidential. Results were communicated to the attending doctor and those identified to have significant bacteriuria were given appropriate treatment.

Results

A total of 329 pregnant women were recruited in this study; however, nine participants were excluded (five samples had candida species growth, and four had mixed bacteria growth), making 320 in the final analysis. The mean age of participants was 26 years ± 5.9 SD, and the majority, 232 (72.5%), were aged less than thirty years old. More than half of the participants, 169 (52.8%), had attained the secondary level of education. The majority, 232 (72.5%), were married, 189 (59.1%) reported being unemployed, and 193 (60.3%) were in their second trimester of pregnancy (Table 1).

Table 1: Sociodemographic and obstetric characteristics of pregnant women enrolled in the study (n=320)

Characteristics	Frequency (n)	Percentage (%)
Age group (Mean 26 ± 5.9 SD)		
<30	232	72.5
≥30	88	27.5
Level of education		
Informal/ Primary	89	27.8
Secondary	169	52.8
University or college	62	19.4
Occupation		
Unemployed	189	59.1
Informal employment	114	35.6
Formal employment	17	5.3
Marital status		
Single/divorced/widow	88	27.5
Married/cohabiting	232	72.5
Gravidity		
Prime gravida	149	46.6
Multigravida	171	51.4
Gestational age		
First trimester	52	16.3
Second trimester	193	60.3
Third trimester	75	23.4

Prevalence of Asymptomatic Bacteriuria

Of the 320 participants, 21 had significant bacteriuria giving a prevalence of 6.6%. In addition, participants aged ≥ 30 years had a high proportion (9, 10.5%) of ASB positive. A high frequency, 14 (8.2 %) of ASB, was tested among multigravida. Pregnant women with secondary education had a high frequency, 13 (7.6%) of ASB positive. A high proportion (7, 9.3%) of significant bacteriuria was observed among pregnant women in their third trimester. No significant difference was observed for all the variables analyzed in this study (Table 2).

Table 2: Asymptomatic bacteriuria among pregnant women enrolled in the study (n=320)

Variable	Culture-positive n(%)	Culture-negative n(%)	*P-value*
Age (years)			0.12
< 30	12 (5.2)	220 (94.8%)	
≥30	9 (10.5)	79 (89.9)	
Level of education			0.68
Informal/ Primary	4 (4.5)	85 (95.5)	
Secondary	13 (7.6)	157 (92.4)	
University or college	4 (6.6)	57 (93.4)	
Occupation			0.96
Unemployed	12 (6.3)	177 (93.7)	
Informal employment	9 (7.9)	105 (92.1)	
Formal employment	1 (5.9)	16 (94.1)	
Marital status			0.45
Single/divorced/widow	4 (4.5)	84 (95.5)	
Married/cohabiting	17 (7.3)	215 (92.7)	
Gravidity			0.26
Prime gravida	7 (4.7)	142 (96.3)	
Multigravida	14 (8.2)	157 (91.8)	
Gestational age			0.44
First trimester	2 (3.8)	50 (96.2)	
Second trimester	12 (6.2)	181 (93.8)	
Third trimester	7 (9.3)	68 (90.7)	

Spectrum of bacteria causing ASB

E. coli 9/21 (42.8%) was the common bacteria isolated from the urine sample, followed by *Enterococci spp,* which accounted for 3/21 (14.3%) (Figure 1).

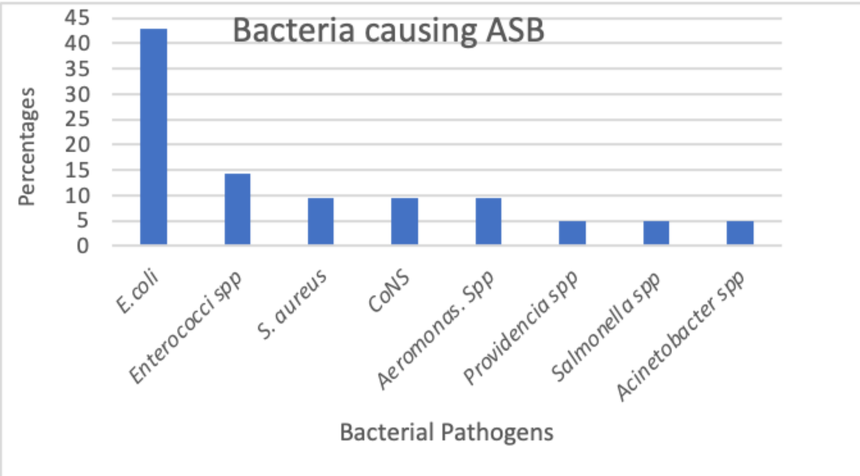

Figure 1. Bacteria pathogens causing ASB among pregnant women

Antimicrobial resistance pattern

E. coli isolates had high resistance rate to ampicillin (89%), Trimethoprim/sulphamethoxazole (78%), and amoxicillin-clavulanic acid (89%) but were all sensitive (100%) to amikacin, gentamicin, nitrofurantoin, and meropenem. Among Gram-positive isolates, *Enterococci* spp demonstrated high resistance to Trimethoprim/sulphamethoxazole (100%), nitrofurantoin, and ciprofloxacin (66.7%). High overall resistance rate was demonstrated among Gram -negative and Gram-positive towards ampicillin (71%), Trimethoprim/sulphamethoxazole (62%) and amoxicillin-clavulanic acid (52%) for gram-negative bacteria. None of the isolate tested showed resistance to meropenem (Table 3).

Table 3: Antimicrobial resistance patterns of bacteria pathogens causing ASB in the study population

Bacteria (n)	Antimicrobial resistance (%)										
	AMK	AMP	GEN	CIP	STX	NIT	CRO	AMC	NAL	MEM	AZK
E. coli n=9	0	89	0	22	78	0	22	89	11	0	NA
Aeromonas spp n=2	0	50	50	0	50	50	0	50	0	0	NA
Acinectobacter spp	0	NA	100	100	100	NA	NA	NA	0	0	NA
*Other GNB n=2	0	100	0	0	50	50	50	50	0	0	NA
S.aureus n=2	50	50	50	50	50	50	NA	NA	-	NA	50
CoNS n=2	0	50	0	50	50	0	NA	NA	NA	NA	0
Enterococci spp n=3	33	67	33	67	100	67	NA	NA	NA	NA	33
Overall	9.5	71	19	29	62	24	9.5	52	5	0	9.5

*Amikacin AMK, Ampicillin AMP, Gentamycin GEN, Trimethoprim/sulphamethoxazole SXT, Nitrofurantoin NIT, ceftriaxone CRO, amoxicillin-clavulanic AMC, nalidixic acid NAL, meropenem MEM, Azithromycin AZR. *Other-GNB, gram negative bacteria includes Salmonella and Providencia species.*

Sensitivity, Specificity and predictive values of urine dipstick test using culture as a gold standard

The prevalence of ASB among pregnant women by urine dipstick test was 28 (8.8%). The sensitivity and specificity of the urine dipstick were 38.1 % and 93.3%, respectively. Positive and negative predictive values were 28.6% and 95.6%, respectively (Table 4).

Table 4: Sensitivity, Specificity and predictive values of urine dipstick test using culture as a gold standard

		Culture results		
		Positive n (%)	**Negative n (%)**	**Total**
Urine Dipstick test	Positive	8 (28.6)	20 (71.4)	28 (100)
	Negative	13 (4.5)	279 (95.5)	292 (100)
	Total	**21 (6.6)**	**299 (93.4)**	**320 (100)**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Urine dipstick	38.1	93.3	28.6	95.6

PPV; Predictive positive value, NPV; Negative predictive value.

Discussion

In this study, we found that the overall prevalence of ASB among pregnant women attending ANC at Sinza District hospital was 6.6 %. *E. coli* (43%) was the predominant bacterial pathogen isolated, followed by *Staphylococcus species* and *Enterococcus spp,* which accounted for 19% and 14%, respectively. In general, gram-negative and gram-positive bacteria demonstrated high resistance to the commonly used antibiotic, including ampicillin and trimethoprim/sulphamethoxazole but highly sensitive to amikacin, gentamicin, ciprofloxacin, nitrofurantoin, and meropenem.

The prevalence of ASB obtained from this study (6.6%) is within the reported global prevalence range of (2% to 10%) in pregnancy (2,4) but higher than the reported prevalence in Uganda (3.8%) and Kenya (4.3%), respectively (7, 22). Similar studies in Tanzania reported a higher prevalence of 8.9% in Kilimanjaro and 13% in Mwanza (9,10). Another study in Kenya by Ayoyi et al. reported a high prevalence of 21.5% (6). The variations observed among these studies, especially in Tanzania, could be attributed to the differences between the studied populations and facility levels. Our study was conducted at a lower-level health facility with low-risk women as compared to higher risk pregnant women most likely with medical and/or obstetric conditions referred to the higher-level health facilities for additional care. A multicenter study in Mwanza reported a similar trend where pregnant women attending lower-level health facilities had lower prevalence of significant bacteriuria (13.9%) compared to tertiary hospital (21.5%) (20).

In this study, *Escherichia coli* was the most organism isolated, this finding is consistent with a study by Moyo et al done at MNH, Dar es Salaam and Seni et al in Mwanza among pregnant women (19, 20). *Escherichia coli* is a normal flora of the bowel, and therefore contamination of the urethral area by fecal matter may contribute to its high prevalence among pregnant women. In addition, Gram-negative bacteria have a distinct structure such as adhesions, pili or fimbriae that enables the organisms to attach, grow and invade the urinary epithelium (23). *Staphylococcus species* were the second most isolates, similar to related studies in Eastern Uganda and Ethiopia in which *Staphylococcus* was the second most common isolate (7, 24). However, this is in contrast to a study by Masinde et al. in Tanzania, among pregnant women, where *Enterococcus spp* was the second most isolated bacterial pathogen (10). Another study in Nigeria reported *Klebsiella species* as a dominant uropathogen which differ to our study findings (25). The differences in the distributions of the isolates might be due to the differences in the geographical settings of the studied populations. *Salmonella spp* as a rare cause of UTI was also isolated among pregnant women in this study (26, 27).

Regarding the antimicrobial pattern, most isolates were resistant to commonly used first-line antibiotics. Gram-negative bacteria were highly resistant to ampicillin, amoxicillin-clavulanic acid, and trimethoprim/sulphamethoxazole, unlikely gram-positive, which were highly resistant to trimethoprim/sulphamethoxazole and nitrofurantoin. A study by Moyo et al at MNH, Dar es Salaam reported a resistance of 38.5% towards trimethoprim/sulphamethoxazole and 57.7% to ampicillin slightly lower than a 62% and 71% obtained from our study, reflecting an increase in resistance to antimicrobial agents over time (19). However, a study done in Mwanza, Tanzania, a resistance rate of 65% was noted towards trimethoprim/sulphamethoxazole, a finding similar to our study (10). Notably, high resistance rate of 52% against urinary pathogens was observed towards amoxicillin-clavulanic acid, which is higher than reported 31% among pregnant women in Kano, Nigeria (25). Such a high resistance rate negatively impacts patient management since these are the most used, accessible, and affordable antibiotics. A possible explanation for the higher resistance might be due to the issuance of prescriptions without antibiotic susceptibility data (28). This highlights the need for routine AST to guide therapeutic decisions, reducing the overuse of antibiotics without susceptibility tests in our settings and curbing the rising antibiotic resistance.

Bacteriuria during pregnancy has been linked with adverse obstetric outcomes, including low birth weight, premature delivery, and perinatal mortality (29). Such outcomes can be avoided with treatment if screening for bacteriuria can be done early. Rapid urine dipsticks are easy to perform and interpret and can be done in low-level health facilities, and results are obtained within a very short time. Despite this benefit, poor sensitivity and positive predictive values in detecting bacteriuria, especially in asymptomatic pregnant women, have been reported elsewhere (16, 30). In the current study, we also assessed the use of a rapid urine dipstick test for screening UTI or bacteriuria, which is the common method used in primary care facilities. In this study, we observed a significant difference in determining bacteriuria among pregnant women between the two tests; urine dipstick vs. culture as the gold standard method. The sensitivity of the urine dipstick test was 38.1%, similar to findings observed from a study done in Mwanza, Tanzania, by Masinde et al. (10) but higher than a sensitivity of 5.6% observed in Nigeria (25). Nevertheless, the urine dipstick test was found to have a high false positive, indicating that it may be unreliable in determining ASB among pregnant women, leading them to unnecessary antibiotic exposure and increasing antimicrobial resistance.

Strengths and limitations of the study

The study findings are reproducible and will add value to prescription practices management of patient with ASB. The study was done in low-level health facility with low-risk group but higher rate of antimicrobial resistance indicating an increase of resistance over time. Unable to follow up the participants after treatment for those who were diagnosed to have ASB to declare clearance of infection. The AST data obtained cannot be generalized due to low number of isolates tested.

Conclusion

This study demonstrated that ASB is common among pregnant women attending ANC at Sinza District Hospital. *Escherichia coli* was the most isolated organism causing ASB, and a high level of resistance was observed to commonly prescribed antibiotics. We recommend large study to be conducted in various secondary-level health facilities in order to gather enough data on AST pattern as well as to guide patient's management.

Disclosure

The authors declared no conflict of interest.

Authors Contributions

NJ, WT and AJ participated in the conception and design of the study. NJ and AJ participated in the Laboratory work, acquisition, and analysis of data. NJ drafted the initial manuscript. AJ and WT critically reviewed the manuscript. All authors read and approved the final manuscript.

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