

Bacterial etiologic agents, prevalence and associated risk factors of asymptomatic bacteriuria among pregnant and non-pregnant women in primary health care centers in South-South Nigeria

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Abstract

Background: Asymptomatic Bacteriuria (ASB) has been demonstrated to have the adverse maternal and pregnancy outcomes like low birth weight, pyelonephritis, preterm labour and preterm premature rupture of membranes. Pregnant women are known to be more susceptible to ABS leading to UTIs if not treated properly. This study aimed to determine the prevalence, bacteriological agents, risk factors of ASB and antimicrobial sensitivity pattern among pregnant and non-pregnant women attending antenatal clinic in two primary health centers.

Methodology: We conducted cross sectional study involving 246 pregnant women and 100 aged-matched non-pregnant women attending antenatal clinic at two primary health centers in Uyo and Osuk Ediene, South-south Nigeria. All women who met the inclusion criteria and gave their informed consent were invited to participate. Interviews using a questionnaire were conducted to collect socio-demographic data while urine samples were collected for laboratory processing. Clean catch mid-stream urine sample was collected and microbial analysis carried out immediately. Significant ASB was identified and antimicrobial sensitivity test conducted.

Results: The prevalence of ASB among pregnant women in this study was 29.7%. Regarding their parity in the two PHC among pregnant women, 77(31.3%), 89(36.2%) and 80(32.5%) were nulliparous, monoparous and multiparous respectively. Also, 23(9.3%), 83(33.7%) and 140(56.9%) of the pregnant women were in the first, second and third trimester. One hundred and four (42.3%), 87(35.4%), 36(14.6%) and 19(7.7%) pregnant subjects were housewife, self-employed, employed and unemployed in their occupational status. Trimester was a factor for ASB in the second and third trimester. There was association between age, parity, trimester, education, occupation, numbers of partners and ASB; pointing them as risk factors. The microorganisms isolated from the urine sample according to the frequency of occurrence was *Escherichia coli* (28.7%), *Staphylococcus aureus* (20.5%), *Klebsiella pneumoniae* (19.2%), *S. saprophyticus* (11%), *Pseudomonas aeruginosa* (9.6%), etc. The rate of antibiotic sensitivity among gram negative bacteria ranged from 52% and above for all antibiotic tested with respect to *Escherichia coli*; except Azithromycine that was 14.3%. The *Escherichia coli* and other uropathogens isolates were multiple drug sensitive between 50-100%. Previous bacteriuria treatment seeking pattern in the pregnant women was 103(41.9%), 73(29.7%), 42(17.1%) and 8(3.3%) for individuals who had seek treatments in hospital, chemists, multi-centres and traditional herbs dealers respectively.

Conclusion: The prevalence of ASB was 29.7% among pregnant women and the wide array of organisms isolated in this population using PHC warrant the development of protocols for routine ASB screening and proper treatment.

Keywords: asymptomatic bacteriuria, bacterial agents, prevalence, risk factors, pregnant women

1. Introduction

Urinary tract infections (UTIs), are among the most common known public health problems affecting women in both under developed and developing countries; in their reproductive ages [1-4]. These infections account for approximately 25% of all infections in geriatric patients [5] and unnecessary antimicrobial treatment of ASB in older individuals is very common [6-7]. Urinary tract infection can be symptomatic or asymptomatic in pregnant women [8-9]. Asymptomatic bacteriuria (ASB) refers to actively and persistently growth of microorganisms in the urinary tract of human begins without any observed presentations of

symptoms [9-10]. Asymptomatic bacteriuria during pregnancy may lead to acute pyelonephritis, low birth weight of foetus and preterm labour (maternal and obstetric outcome) if not well treated [11-12] medically.

Pregnant and non-pregnant women are known to be more susceptible to UTIs due to the short length of urethra along with proximity to warm, moist and canal [13]. Sexual intercourse facilitates movement of bacterial into bladder of human [14]. Other risk factors include multi-parity, low educational level, social economic status, diabetes mellitus, age, delayed medical care bad hygiene and nephrolithiasis [15]. Pregnancy in females predisposes such individual to

UTIs and they are two times more commonly affected than age-matched non-pregnant females. This is because of urinary stasis due to the effect of progesterone during pregnancy; in addition to various anatomic changes occurring during gestation [16]. All clinical forms of UTIs (ABS, acute cystitis and acute pyelonephritis) may lead to serious complications either to mother or fetus [16]. These complications seem to result from the effect of bacterial/microbial exotoxins and endotoxins that damage the body's tissues including endothelium, although not all causes associated with them are fully proven [15, 17].

Globally, the prevalence of UTIs in pregnant women is between 13% - 33% with symptomatic bacteriuria occurring in 1% - 18% [18, 19] while asymptomatic cases are observed in 2% - 45% in different populations [20-21] among females. The rate of prevalence has varied including most of the recent epidemiological and cross-sectional researches in developing and under-developed nations [1-4, 18, 20-21]. Epidemiological surveillance of outpatient urine culture offers relevant insights into the changing prevalence and even antimicrobial drugs that are susceptible to specific uropathogens [22], particularly in patients presenting nonspecific symptoms. The frequent microbial uropathogens causing both symptomatic and asymptomatic urinary tract infection is *E. coli* in the range of 70% - 80% as reported by Masinde *et al.* [23] and others are *Klebsiella pneumoniae*, *S. aureus*, *protens spp*, etc [3-4, 18 - 24]. Colonization with multidrug resistant organisms is high in clinics and hospitals, because of unnecessary antibiotic treatment of ASB in older adults [6], resulting in wide spread of these micro-organisms to other settings along with the colonized patients [25]. The multi-drug resistance bacteria implicated in UTIs are extended-spectrum beta-lactamase (ESBL) producing organisms [26], Carbapenem-resistant enterobacteriaceae (CRE) and recently Colistin resistant gram-negative bacilli [27-28] detected in cases among residents of United States. Also researches stipulated that scientists have increasingly discovered that some commonly prescribed antimicrobial drugs are linked to severe and sometimes life-threatening adverse events [29]. These re-occurring trends highlighted the urgent need for renewed emphasis on antimicrobial stewardship in the treatment of UTIs, not excluding increased recognition and a good understanding of ASB.

In numerous hospitals in developing countries like Nigeria, routine urine culture may not be done promptly, even for pregnant mothers. Many patients are treated empirically without routine culture and antimicrobial susceptibility testing (AST) in villages and treatment is therefore administered based on empiric guidelines that are rarely updated [30]. In some situations where UTIs tests are carried out in villages only dipstick analysis and direct wet microscopy of urine are utilized. These tests have poor positive and negative predictive values to detect ASB in individuals [8]. We are suggesting that over-reliance on such method and absence of culture and susceptibility testing have led to under-diagnosis of UTIs partially and this may be triggering the rising cases of treatment failures/antimicrobial multiple drug resistance. In developed nations, UTIs in pregnant women affect approximately 4 - 7% of them [31], but in Nigeria it is as much as 18.2% to 72.5% in south eastern Nigeria and south west Nigeria [32 -

33]. There is paucity of documented research on ASB profile using primary health centers. Thus, this study seeks to investigate on the prevalence, bacterial profiling and associated risk factors of ASB among pregnant and non-pregnant women visiting primary health centers in Ikono and Uyo, South-south, Nigeria.

2. Methodology

2.1 Research Design and Area

This research was designed as a cross-sectional survey, made up of registered pregnant women at all stages of pregnancy attending antenatal clinics in two primary health centers in Ikono and Uyo, Akwa Ibom State, Nigeria. Also, non-pregnant women (control group) were also recruited from the same primary health centers. Ikono is bounded on the north by Ini Local Government Area, south by Abak and Uyo Local Government Areas, east by Itu and west by Ikot Ekpene Local Government Area. Ikono have a landmass of 407.16 square kilometers. The total population of Ikono is 131,904; comprising 69,501 males and 62,403 females. Uyo serves as a state capital and a Local Government Headquarter and it have a total population of 309,573; comprising 153,113 males and 156,460 females, according to 2006 national census. The two health centers used for this study are: primary health center Barracks road, Uyo and primary health center Osuk Ediene, Ikono. These primary health centers provide medical services to the villages and cities within their environs.

2.2 Sample Size Calculation

The sample size for the study was calculated using a single proportion formula, $N = Z^2 p (1-p) / d^2$. Where N = sample size, Z = Z-score for 95% confidence interval (1.96), p = prevalence rate, d = error (5%). The prevalence of 20% was utilized according to the study conducted in Uyo, Akwa-Ibom state in a secondary health facility [34]. Then $N = (1.96)^2 \times 0.2(1-0.2) / (0.005)^2 = 246$. Therefore, the sample size was 246.

2.3 Ethical Considerations

Ethical approval was obtained from the Akwa Ibom State Ministry of Health Research Ethical Review Board, Uyo, Akwa Ibom State, Nigeria. The study adhered strictly to the tenets of the Ethical Review Board. Informed written consent was obtained from all the study participants.

2.4 Recruitment of Study Population

Participants were recruited from two primary health centers in Ikono and Uyo, Akwa Ibom State, South-south Nigeria and they were given the full right to continue or withdraw from the research. Information obtained at each course of the study was kept confidential. Cases identified positive for bacteriuria during the study period were referred to attending medical doctor and treatment administered accordingly with appropriate drugs in line with the national guidelines for treatment of pregnant women and non-pregnant women. Urine samples were collected from a total of 346 participants, comprising 246 pregnant women (100 cases from primary health center Barracks road, Uyo and 146 cases from primary health center in Osuk Ediene, Ikono) between the ages of 14-43 years and 100 age-matched non-pregnant women as control subjects. The one

hundred (100) non-pregnant women (controls) were also recruited within the same primary health centers. The study lasted over a six month period in the primary health center Barracks road, Uyo and ten month at the primary health center Osuk Ediene, Ikono.

2.5 Inclusion and Exclusion Criteria

All pregnant women not on antibiotic(s) therapy were recruited for the study. In both cases (pregnant women) and controls (non-pregnant women) subjects recruited, there must be no clinical signs or symptoms of urinary tract infections (UTIs). All pregnant women currently receiving antimicrobial drugs for any urinary tract infection or any other type health problem in the health centers were excluded from the study. In addition, patients and the control subjects who refused to participate and/or fully cooperate with the guidelines of the study were also excluded. Asymptomatic uropathogens is defined as the presence of significant bacteria ($\geq 10^5$ cfu/ml) in two consecutive clean-voided mid-stream urine specimen in a patient without signs or symptoms according to Gessese *et al.*, [35].

2.6 Sample Collection

Clean catch early morning mid-stream urine samples of 5 ml were collected in sterile universal containers as described by the published protocols [36-37]. Recruited participants were instructed to use the cotton wool swabs impregnated with 0.9% aqueous solution of Sodium Chloride in distilled water, to clean the urethra, vulva and retrovaginal areas anterior-posteriorly (from above-downward), with labia widely held apart and midstream urine samples were collected [38]. The clinic matrons, senior and junior nursing staff assisted in the proper instruction of all the participants (in Pidgin English, Annang, Efiks, Igbo and Ibibio languages for the educationally less-privileged ones) and supervision of those needing assistance. The participants were given well-structured questionnaire to fill. Information such as age (Age at last Birthday), marital status, number of years married where applicable, number of children, spacing of children and gestational age derived from the last menstrual period (LMP) of the participants. The information on gestational ages of pregnant women were used to classify them into 3 trimesters namely: first trimesters between 0-13 weeks, second trimesters between 14-27 weeks and third trimesters between 28-40 weeks. Also, previous mode of treatment, the educational background and occupation of both the patients and their spouse were obtained too.

Based on other information obtained, participants were grouped into social classes 1-5 according to the method documented [39]. The information gathered from the medical records of cases recorded in the questionnaire was subsequently probed into the presence of clinical signs of urinary tract infection such as abdominal/renal pain, back/flank pain, fever painful/irritating urination, frequency and dysuria before urinating. The urine samples collected from patients and controls were immediately taken to the laboratory for microbiological examination and biochemical testing. All urine samples were duly labeled and cultured on arrival (between 1-2 hours of collection). This was carried out to prevent bacterial population growth and proliferation in specimens [40].

2.7 Microbiological Examination of the Samples

2.7.1 Macroscopic Examination

All samples were microscopically examined for colour, turbidity and odour.

2.7.2 Urine Microscopy

The microscopy and cell count were carried out using the improved Neubauer's haemocytometer. The haemocytometer was prepared for use by placing a clean grease free cover glass on the chamber and applying a gentle sliding pressure until a correct symmetrical positioning was achieved as indicated by the appearance of interference pattern (Newton's rings) according to the research reported [41]. Broken cover glasses were discarded, then un-centrifuged sample was properly mixed and using a clean Pasteur pipette, the aspirated urine was filled into the counting chamber at about 45°. Care was taken to avoid rapid filling, air bubbles and overflow, but when this happened, the chamber was washed and the exercise repeated. After filling the chamber, it was placed on the microscope stage for a few minutes before counting, this was done to enable the streaming of the fluid to cease and the cells settle on the bottom of the chamber.

A high dry magnification (objective x40) piece was used in counting. Cells touching the left hand and/or the upper lines of a square were counted, while those touching the lower and/or the right lines were considered outside the square. The leucocytes were distinguished from the non-squamous epithelial cells using the larger and the nuclear orientation of the latter. The white blood cells (WBC) were counted in the 4 or 2 large squares. A White Blood Cell count of 10 or more cells per cubic millimeter was considered significant [42].

2.7.3 Urine Culture-significant Bacteriuria Estimation

Well mixed urine specimens were cultured on Cystine Lactose Electrolyte Deficient (CLED) and blood agar for the primary isolation of uropathogens using a standard urine wire-loop (2 mm internal diameter) to deliver 0.05 ml of urine. Inoculated plates were incubated at 37°C for 24 hours. Bacteriuria was considered significant when at least 10^5 colony forming units of a single pathogen per milliliter of urine was counted (10^5 CFU/mL of urine). Observed colonies were sub-cultured until pure and distinct colonies were obtained [13].

2.8 Biochemical Testing

All biochemical tests were performed with pure, 24-hour old cultures. Positive and negative controls were included along with test specimens for the biochemical tests. The biochemical tests were carefully used to screen all isolates before further typing tests were carried out. The methods were based on standard procedures for the identification of bacterial pathogens [13, 43].

2.9 Antimicrobial Susceptibility Testing (AST) of Uropathogens

The antimicrobial susceptibility testing of all isolates was carried out using commercial disk following the standard disk diffusion method recommended by the National Committee for Clinical Laboratory Standards as

documented [44]. The drugs that were tested include Ceftazidime (30 µg), Amoxicillin-clavulanic acid (20 µg), Azetronam (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Nitrofurantoin (300 µg), Cefotaxime (30 µg), Gentamicin (20 µg), Imipenem (10 µg), Sulfamethoxazole-trimethoprim (1.25 µg) and Azithromycin (15 µg). All the antimicrobial drugs used for the research were purchased from Oxoid Limited, Cambridge, United Kingdom. All Culture media were tested for sterility and performance. Reference strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used during culture and antimicrobial testing of uropathogens from recruited subjects.

2.10 Statistical Analysis

Data from laboratory investigation and questionnaire was coded into Microsoft Excel Spreadsheet. The coded data was processed and analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Quantitative, clinical and laboratory variables were compare using Chi-square (χ^2) test and simple percentages. Statistical significant was set at $P \leq 0.05$.

3. Results

Urine samples collected from a total of 246 pregnant women and 100 non-pregnant women were examined for ASB that can cause UTIs, if not properly treated medically. The overall prevalence of ASB among the pregnant women in the two primary health care centers (PHC) was 29.7%.The

age of all subjects recruited ranged from 14 – 43 years. The prevalence of ASB was 32.2%, while 80.8% of pregnant women at Osuk Ediene primary health care center were married women, 48.6% attained primary school, 47.9% was monoparous, 52.7% are house wife and 79.5% have only one sex partner (Table 1). The prevalence of ASB was 26.2%, 82% of the pregnant women were married and 10% were singled in the primary health care center at barracks road, Uyo (Table 2).There was significant association of age, parity, marital status, trimester period, educational status, occupation and number of sexual partner with ASB in the two primary health care centers investigated (Table 1 and Table 2). The presence of abnormal conditions among the pregnant women at Osuk-Ediene primary health care center was 10.3%, 2.7%, 8.9% and 1.4% for pyuria, glycosuria, proteinuria and proteinuria/ glycosuria respectively (Table 3). Table 4 showed that pyuria was 5%, monomicrobial ASB and polymicrobial ASB was 22% and 4% respectively in the primary health care center at barracks road, Uyo.

The ASB Gram-negative isolated (n = 47) among pregnant women in the primary health care facility at Osuk Ediene were more prevalent 30(63.8%) than Gram-positive ASB 17(36.2%). The most common ASB isolates were *E. coli* 13(27.7%), followed by *Klebsiella pneumoniae* 7(14.9%) which was observed in the pregnant women examined (Table 5).

Table 1: Prevalence of ASB and demographic characteristics of subjects in primary health center Osuk-Ediene

Variables	Pregnant women (N=146)			Non-pregnant women (N=100)			X ²	p-value
	No tested (%)	No negative (%)	No positive (%)	No tested (%)	No negative (%)	No positive (%)		
Age (years)								
14 – 19	14(9.6)	7(4.8)	7(4.8)	11(11)	9(9)	2(2)	1.581	0.639
20 – 25	47(32.2)	35(24.0)	12(8.2)	36(36)	32(32)	4(4)		
26 – 31	61(41.8)	43(29.5)	18(12.3)	30(30)	25(25)	5(5)		
32 – 37	21(14.4)	12(8.2)	9(6.2)	13(13)	10(10)	3(3)		
38 – 43	3(2.1)	2(1.4)	1(0.7)	10(10)	9(9)	1(1)		
Total	146(100)	99(67.8)	47(32.2)	100(100)	85(85)	15(15)		
Marital status								
Married	118(80.8)	79(54.1)	39(26.7)	98(98)	83(83)	15(15)	0.341	0.861
Single	20(13.7)	14(9.6)	6(4.1)	2(2)	2(2)	0(0)		
Divorced	5(3.4)	4(2.7)	1(0.7)	0(0)	0(0)	0(0)		
Widowed	3(2.1)	2(1.4)	1(0.7)	0(0)	0(0)	0(0)		
Educational status								
Informal	8(5.5)	5(3.4)	3(1.8)	3(3)	2(2)	1(1)	2.62	0.98
Primary	71(48.6)	48(32.9)	23(4.8)	21(21)	16(16)	5(5)		
Secondary	60(41.1)	40(27.4)	20(14.8)	56(56)	50(50)	6(6)		
Tertiary	7(4.8)	6(4.1)	1(0.7)	20(20)	17(17)	3(3)		
Parity								
Nullipara	31(21.2)	23(15.8)	8(5.5)	48(48)	42(42)	6(6)	1.57	0.681
Monopara	70(47.9)	51(34.9)	19(13.0)	36(36)	28(28)	8(8)		
Multipara	45(30.8)	25(17.1)	20(13.7)	16(16)	15(15)	1(1)		
Thrimester								
1 st	19(16.1)	14(9.6)	5(3.4)				2.173	0.534
2 nd	56(30.4)	39(26.7)	17(11.6)					
3 rd	71(53.5)	46(31.5)	25(17.1)					
Occupation								
Housewife	77(52.7)	58(40.0)	19(13.0)	36(36)	32(32)	4(4)	1.49	0.032
Self-employed	53(36.3)	30(20.5)	23(15.8)	52(52)	44(44)	8(4)		
Employed	13(8.9)	9(6.2)	4(2.7)	10(10)	8(8)	2(2)		
Unemployed	3(2.1)	2(1.4)	1(0.7)	2(2)	1(1)	1(1)		
No. of Sexual partners								
One	116(79.5)	89(61.0)	27(18.5)	84(84)	79(79)	5(5)	1.87	0.052
Multiple	30(20.5)	10(6.8)	20(13.7)	16(16)	6(6)	10(10)		

Table 2: Prevalence of ASB and demographic characteristics of subjects in primary health center barracks road-Uyo

Variables	Pregnant women (N=100)			Non-pregnant women (N=100)			X ²	p-value
	No tested (%)	No negative (%)	No positive (%)	No tested (%)	No negative (%)	No positive (%)		
Age (years)								
14 – 19	4(4)	2(2)	2(2)	11(11)	9(9)	2(2)	1.471	0.639
20 – 25	31(31)	23(23)	8(8)	36(36)	32(32)	4(4)		
26 – 31	40(40)	30(30)	10(10)	30(30)	25(25)	5(5)		
32 – 37	23(23)	18(18)	5(5)	13(13)	10(10)	3(3)		
38 – 43	2(2)	1(1)	1(1)	10(10)	9(9)	1(1)		
Total	100(100)	74(74)	26(26)	100(100)	85(85)	15(15)		
Marital status								
Married	82(82)	63(63)	19(19)	98(98)	83(83)	15(15)	0.341	0.861
Single	10(10)	6(6)	4(4)	2(2)	2(2)	0(0)		
Divorced	5(5)	3(3)	2(2)	0(0)	0(0)	0(0)		
Widowed	3(3)	2(2)	1(1)	0(0)	0(0)	0(0)		
Educational status								
Informal	0(0)	0(0)	0(0)	3(3)	2(2)	1(1)	1.98	0.98
Primary	13(13)	8(8)	5(5)	21(21)	16(16)	5(5)		
Secondary	58(58)	46(46)	12(12)	56(56)	50(50)	6(6)		
Tertiary	29(29)	20(20)	9(9)	20(20)	17(17)	3(3)		
Parity								
Nullipara	46(46)	37(37)	9(9)	48(48)	42(42)	6(6)	1.57	0.681
Monopara	19(19)	14(14)	5(5)	36(36)	28(28)	8(8)		
Multipara	35(35)	23(23)	12(12)	16(16)	15(15)	1(1)		
Trimester								
1 st	4(4)	2(2)	2(2)				2.021	0.534
2 nd	27(27)	17(17)	10(10)					
3 rd	69(69)	55(55)	14(14)					
Occupation								
Housewife	27(27)	21(21)	6(6)	36(36)	32(32)	4(4)	1.27	0.032
Self-employed	34(34)	23(23)	11(11)	52(52)	44(44)	8(4)		
Employed	23(23)	17(17)	6(6)	10(10)	8(8)	2(2)		
Unemployed	16(16)	13(13)	3(3)	2(2)	1(1)	1(1)		
No. of Sexual partners								
One	80(80)	63(63)	17(17)	84(84)	79(79)	5(5)	1.64	0.052
Multiple	20(20)	11(11)	9(9)	16(16)	6(6)	10(10)		

Table 3: Bacteriuria and the presence of abnormal conditions among pregnant women investigated in primary health center Osuk-Ediene

Abnormal conditions	No of positive samples (%)
Pyuria	15(10.3)
Glycosuria	4(2.7)
Proteinuria	13(8.9)
Proteinuria and glycosuria	2(1.4)
Monomicrobial ASB	33(22.6)
Polymicrobial ASB	14(9.6)

Table 4: Bacteriuria and the presence of abnormal conditions among pregnant women investigated in primary health center barracks road-Uyo

Abnormal conditions	No of positive samples (%)
Pyuria	5(5)
Glycosuria	0(0)
Proteinuria	0(0)
Proteinuria and glycosuria	0(0)
Monomicrobial ASB	22(22)
Polymicrobial ASB	4(4)

Table 5: Frequency of ASB isolated among women in OsukEdiene

Bacterial isolates	Pregnant women No of positive samples (%) (n = 47)	Non-pregnant women No of positive sample (n = 15)
<i>Escherichia coli</i>	13(27.7)	7(47.0)
<i>Staphylococcus aureus</i>	11(23.4)	2(13.3)
<i>Klebsiella pneumonia</i>	7(14.9)	3(20.0)
<i>Pseudomonas aeruginosa</i>	5(10.6)	1(6.7)
<i>Proteus spp</i>	2(4.3)	0(0.0)
<i>Klebsiella spp</i>	3(6.4)	0(0.0)
<i>S. saprophyticus</i>	6(12.8)	2(13.3)

Asymptomatic pathogenic bacterial profile isolated among pregnant women in primary health care facility in barracks road, Uyo revealed that Gram- negative ASB were more prevalent 20(76.9%) than Gram-positive ASB 6(23.1%). The most common ASB isolates was *E. coli* 8(30.8%), followed by *Klebsiella pneumoniae* 7(26.9%) and *Proteus spp.* was 1(3.8%) as identified. In non-pregnant women, bacteria isolated (n = 15) recorded 7(47%) and 3(20%) for *E. coli* and *K. pneumoniae* respectively (Table 6). The antimicrobial susceptibility pattern of Gram-negative and Gram- positive ASB isolates from pregnant women in both primary health care facilities investigated are displayed on Table 7. All bacterial isolates had high resistance to Gentamicin (ranging from 53% to 100%) and Imipenam

(ranging from 67% to 93%). *E. coli* predominantly caused ASB displaying high percentage resistance (greater than 51%) to all the antibiotics tested except Azetronam (14.3%). Multi drug resistance (MDR) i.e., isolate resistance to more than two antimicrobial drugs was discovered in all ASB isolated (100%). All isolates of Gram-negative and Gram-positive ASB were resistance for more than two or more drugs (Table 7). The previous bacteriuria treatment seeking pattern of the pregnant women in the two primary health care facilities was 33(45.2), 24(32.9), 8(11.0) and 3(4.1) for individuals who had sought treatments in hospital, patent drug dealers (chemists), multi-centres and traditional herbs providers respectively; but were still positive for asymptomatic bacteriuria (Table 8).

Table 6: Frequency of ASB isolated in women at primary health center Barracks road-Uyo

Bacterial isolates	Pregnant women No of positive samples (%) (n = 26)	Non-pregnant women No of positive sample (n = 15)
<i>Escherichia coli</i>	8(30.8)	7(47.0)
<i>Staphylococcus aureus</i>	4(15.4)	2(13.3)
<i>Klebsiella pneumonia</i>	7(26.9)	3(20.0)
<i>Pseudomonas aeruginosa</i>	2(7.7)	1(6.7)
<i>Proteus spp</i>	1(3.8)	0(0.0)
<i>Klebsiella spp</i>	2(7.7)	0(0.0)
<i>S. saprophyticus</i>	2(7.7)	2(13.3)

Table 7: Resistant profile of ASB among pregnant women isolates in two primary health centers

Bacteria agent (n)	Antimicrobial agent tested										
	CAZ NO (%)	AMC No (%)	AZT No (%)	CRO No (%)	CIP No (%)	NIT No (%)	CTX No (%)	GEN No (%)	IPM No (%)	SXT No (%)	AZM No (%)
<i>E. coli</i> (21)	11(52)	13(62)	11(52)	11(52)	15(71)	11(52)	14(67)	19(90)	15(71)	11(52)	3(14.3)
<i>K. pneumonia</i> (14)	7(50)	8(57)	7(50)	6(43)	10(71)	7(50)	6(43)	12(86)	11(79)	8(57)	6(43)
<i>P. aeruginosa</i> (7)	4(57)	4(57)	2(28)	3(43)	4(57)	4(57)	4(57)	5(71)	5(71)	4(57)	2(29)
<i>Klebsiellasp</i> (5)	3(60)	3(60)	1(20)	1(20)	3(60)	1(20)	2(40)	4(80)	4(80)	3(60)	1(20)
<i>Proteus spp</i> (3)	2(67)	1(33)	0(0)	1(33)	2(67)	1(35)	1(33)	3(100)	2(67)	1(33)	2(67)
<i>S. aureus</i> (15)	8(53)	7(47)	5(33)	7(47)	11(73)	8(53)	7(47)	8(53)	14(93)	5(33)	6(40)
<i>S. saprophyticus</i> (8)	5(63)	4(50)	0(0)	3(38)	6(75)	4(50)	2(25)	7(88)	7(88)	4(50)	0(0)

CAZ = ceftazidime, AMC = Amoxicillin - Clavulinic acid, AZT = Azetronam, CRO = Ceftriaxone, CIP = Ciprofloxacin, NIT = Nitrofurantoin, CTX = Cefotaxime, GEN = Gentamicin, IPM = Imipenam, SXT = Sulphamethoxazole-trimethoprim, AZM = Azithromycine.

Table 8: Previous bacteriuria treatment seeking pattern of the pregnant women in two primary health care centers

Mode of treatment	No examined (%)	No of positive ASB (%)
Hospital	103(41.9)	33(45.2)
Chemists	73(29.7)	24(32.9)
Prayer/faith healing	20(8.1)	5(6.8)
Orgnaizations		
Traditional herbs	8(3.3)	3(4.1)
Multi-centre	42(17.1)	8(11.0)
Total	246(100)	73(29.8)

4. Discussion

In several developing countries, UTIs are common health problems affecting women in their reproductive ages; especially pregnant individuals and some remain asymptomatic [1-4, 18, 20-21, 23-24, 45]. Pregnant women are more susceptible to UTIs due to physiological and hormonal alterations that predispose them to bacterial agents [46]. In this present study, the overall prevalence of asymptomatic bacterial agent was 29.7% among pregnant women in the two primary health centers studied, which is in tandem with the results reported in Middle East, precisely Iran [20] and in Uyo [45]. The result of this present research is higher than the prevalence of 9.9% in Qatar; carried out using four primary health centers [46], 8.9% in Kilimanjaro Christian Medical Centre in Northern Tanzania [47], 3.6% in Sri Lanka [48], 4.7% in India [49], 6%- 10.6% in Addis Ababa, Ethiopia [50], 2.3% and 4.7% documented among ASB pregnant women without and with DM or GDM respectively [51], 8% and 11% for ASB and UTI respectively by Kumar and colleagues [52] in other geographical environment. Also 9% in Nsukka; eastern part of Nigeria, [53], 10.7% in Ibadan; western part of Nigeria [54], 15.7% [55] and 21.5% [56] in different antenatal clinics located in Nairobi, Kenya, 18% to 20% in some developing central and West African countries [4, 32, 34-35], 23.9% in Sagamu, Nigeria [21] and 23.6% in Cape Coast, Ghana [57] which are lower in their prevalence when compared with the 29.7% observed in this present study among pregnant women in South-south Nigeria. In contrast, higher prevalence of 45.3% [58] and 55% [59] was observed in Benin city; South-south Nigeria, 54% in Akwa metropolis, South eastern Nigeria [60], 72.5% and 86.6% in western states; Nigeria [33, 61], and 78.7% in eastern Nigeria; Ebonyi State [62]. In pregnancy there is a great relation between ASB and UTI. UTI. It has been established that untreated ASB develop into symptomatic UTI [63, 64]. Although the earlier study looked at symptomatic UTI and our current study looked at ASB, they both used similar subjects (pregnant women). However, there are differences in the prevalence rate of asymptomatic bacteriological agents from the same geographical regions of the same countries or other regions may be due to variations in their environmental conditions, risk factors, social habits, personal hygiene, educational attainment of populace and research methodology adopted in carry out some of the study involving extended spectrum producing uropathogens in those populations investigated; agreeing with other studies [4, 15-17].

In this research conducted using primary health centers within Uyo and Osuk Ediene, the highest incidence of ASB was observed in the 3rd trimester pregnancy, which is in agreement with other findings [4, 18, 35, 59] in southern, northwest and central Ethiopia, and traditional birth home in Benin city but not in tandem with others studies [3, 45, 55, 59] that reported highest prevalence in the second trimester pregnancy in Akwa Ibom using both secondary health center, tertiary hospital and also in Kenya. Hormonal changes and several anatomical alterations occurred in pregnancy lead to urethral dilation and urinary stasis which contributed to increased risk of developing urinary tract infection [24, 16, 65]. It was reported that urinary stasis increases with advancing in gestational period [46], which is in harmony with the result of this study where highest ASB was seen in the third trimester period. Also the high prevalence of ASB uropathogens observed among pregnant women in this study and may be due to bad clean-up of

genital organ and further complicated by heavily distended belly during 3rd trimester pregnancy. Age, occupational, parity, number of sexual partners and educational status were significantly associated with the prevalence of ASB uropathogens except marital status in this present study. This result is in agreement to the finding in Uyo using recruited pregnant women in a tertiary hospital [3] but not in harmony with other studies [18, 48] in northwest Ethiopia and Sri Lanka. Highest prevalence of ASB was observed in the age range of 26 – 31 years for pregnant women, which was similar to the result documented in Kenya, Ethiopian cities, Benin city, Ibadan, Uyo [3-4, 18, 45, 50-51, 54-55, 59] but disagrees with the published result in Qatar [46]; where highest prevalence of ASB was observed in the age range of 35-39 years. The number sexual partners was significantly associated with ASB statistically in this present study where considerably number of pregnant women have multiple sex partners, which is concomitant with the documented study in Kenya [50]. The increased prevalence observed within this age range may be attributed to multiparity which has been recorded as one of the risk factors for ASB in pregnant women [3-4, 14, 35, 55, 45]. This agrees with the result of this present study in Uyo and Osuk Ediene using primary health centers.

Pyuria, glycosuria, proteinuria and bacteriuria showed no association statistically in this study. However, it was noticed that pyuria, proteinuria and glycosuria were higher in women without significant bacteriuria, pointing that these conditions may not be used as predictive parameters of significant bacteriuria. For instance, pyuria may be observed in women with urogenital tuberculosis, renal mycobacterial infectious and Chlamydia [66]. Also significant bacteriuria may be present without pyuria because of contamination or inappropriate sample collection, production of leucocyte destroying enzyme by bacteria (example *Staphylococcus aureus* producing leucocidin), neutropaenia; while gestational proteinuria and glycosuria may be due to physiological challenges gestation that are not related to the presence of bacterial infection.

In this study, Gram-negative bacteriological agents isolated were more prevalent (69.0%) than Gram-positive bacteria (31.5%) found in pregnant women examined. This result is in harmony with studies carried out in Tanzania, Kenya, India and Ethiopia and Uyo, Nigeria [3-4, 18, 34-35, 45, 47, 49-51, 55]. This may be due to the presence of unique structure in Gram-negative bacteria which assist them in anchoring to the uroepithelial cells; and prevent these micro-organisms from urinary lavage, allowing for multiplication and tissue invasion. This result in invasive infection and pyelonephritis in pregnancy [67]. *E. coli* was the commonest bacteriological agent causing ASB which recorded 28.4% of the uropathogen isolated. This result is harmony with the results from central African countries, Qatar, Sri Lanka and India [4, 18, 35, 46-51, 55-56], Nnewi [32], Benin City [59], Uyo [3, 34, 45] and Arab populations [1-2]. *E. coli* and *Klebsiella* (coliforms) were the predominant pathogens isolated with the highest frequency in Ibadan [54] and Sri Lanka [48] which was in tandem with our studies.

Antibiotic resistance of ASB to some commonly used antimicrobial agents have become high, leaving clinicians with few choices of drugs for the treatment of UTIs [50, 68-69]. In this study, susceptibility pattern of bacteria showed that most of the isolated etiological agents were sensitive to gentamicin (53% - 100%), imipenam (67% - 93%),

ciprofloxacin (between 57% - 75%) and ceftazidime (between 50% - 67%). In other researches, similar reports were observed where bacterial isolates were susceptible to some antimicrobial agents used in susceptibility pattern testing, but with varying degree above 50% [3, 18, 45, 50, 67]. Multi-drug resistance was observed in approximately 100% of the bacterial agents isolated among pregnant women using the primary health centers in Uyo and Osuk Ediene. This is tandem to some documented results in northwest Ethiopia at 95% [18], 74% in Anbessa specialist hospital Addis Ababa [50] and between 50% - 100% in Uyo [3, 45] for multi-drug resistance. This showed that multi drug resistance was high in some commonly utilized antimicrobial drugs. Antibiotic resistance has been known because of factors including self-medication without prescription; unnecessary use of antibiotics, such as intake for viral illness; incomplete course of treatment and reuse of leftover antibiotics according to documented studies [2, 69-71]. Over-the-counter sales of antibiotics without clinicians' guidance and prescription are widespread in different developing countries, especially in rural areas [71]. This may be another possible reason for increased multidrug resistance. However, it was seen that some participants recruited for this research had sought previous treatment(s) by patronizing patent drug dealers, traditional healers, prayer/faith healing centers, etc. These patronage may have exposed them to over-the-counter usages of antibiotics, leading to drug abuse; pointing to the increasing multi-drug resistance observed in this present research. This is concomitant with other previous studies [3, 45, 50, 59, 68].

6. Conclusions

Conclusively, the research reported on the prevalence, bacterial etiological agents and risk factors of ASB among the pregnant women attending antenatal care in two primary health centers (PHC) located in Uyo metropolis (urban) and Osuk Ediene (rural) setting, South-south Nigeria. The prevalence of 29.7%, coupled with the low sensitivity of microorganisms isolated in this study to first line antibiotics warrant placement of national guidelines to screen and treat ASB in this population, especially among pregnant women; since they more susceptible to UTIs.

5. Limitations of the Study

Molecular methods for identification were not carried out in this research.

Abbreviations

ASB: Asymptomatic bacteriuria, AST: Antimicrobial susceptibility testing, AMC: Amoxicillin-Clavulanic acid, AZT: Azetronam, AZM: Azithromycin, CFU/mL: Colony forming units per milliliter, CRO: Ceftriaxone, CIP: Ciprofloxacin, CAZ: Ceftazidime, CLED: Cystine lactose electrolyte deficient, CTX: Cefotaxime, GEN: Gentamicin, DM: Diabetes millitus, GDM: Gestational diabetes millitus, SPSS: Statistical packages for social sciences, IPM: Imipenam, UTIs: Urinary tract infections, LMP: last menstrual period, PHC: primary health center, WBC: White blood cells, MDR: Multiple drug resistance, NIT: Nitrofurantoin, SXT: Sulphamethoxazole-trimethopime.

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Ethical Approval

All authors hereby declare that all experiments have been examined and approved by the appropriate ethical committee and have therefore been performed in accordance with the ethical standards laid down by the Akwa Ibom State Ministry of Health Research Ethical Review Board, Uyo, Akwa Ibom State.

Competing Interests

Authors' contributions

This work was carried out in collaboration between all the authors. Author NGA was the principal investigator, designed the original work plan, carried out the field work and laboratory work for the research. Author AJU conducted data analysis, interpreted the results, draft and finalized the manuscript for publication. Authors TTL, ISE and UEA assisted in data analysis and presentations. Authors TTL and ASO managed the literature searches and read the first draft of the manuscript. All authors read and approved the final manuscript.

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