
Asymptomatic Uropathogenic Bacteriuria Among Pregnant and Non-pregnant Women at St Luke's Hospital Anua, Offot Ukwa District Uyo: A Reassessment Case-Control Approach

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Abstract: Asymptomatic bacteriuria is the presence of multiplying bacteria in the absence of any symptoms. The relevance of ASB lies in the insight it provides into symptomatic infections. Physiological and anatomical alterations during pregnancy make women more predisposed to urinary tract infection. This study seeks to determine the prevalence, risk factors, and bacteria profile among pregnant and non-pregnant women. A total of 230 pregnant women and 100 age-matched non-pregnant women were recruited. All pregnant women were recruited from individuals attending antenatal clinic and the controls recruited within the same hospital. Clean catch mid-stream urines ample was collected and microbial analysis done immediately. Significant ASB was identified and antibiotic sensitivity determined by conventional protocols. The overall prevalence of ASB in this study was 29.1% and 15% among pregnant and non-pregnant women respectively. The mean age was 25.3±5.2 and 24.2±5.6 years for pregnant and non-pregnant women. Based on their parity among pregnant women, 112 (48.7%), 61 (26.5%) and 57 (24.8%) were nulliparous, monoparous and multiparous respectively. Also, 37 (16.1%), 70 (30.4%) and 123 (53.5%) of the pregnant women were in the 1st, 2nd and 3rd trimester. Sixty-seven (29.1%), 125 (54.3%) and 37 (16.1%) pregnant subjects were housewives, self-employed and civil servants in their occupation. Trimester was a risk factor for asymptomatic bacteriuria in the 2nd and 3rd trimester. There was association between age, parity, trimester and ASB. The most common isolate in this study was *Escherichia coli* (28.4%), followed by *Klebsiella pneumoniae* (23.9%). The *Escherichia coli* and other uropathogens isolates were multiple drug sensitive between 50-100%. Previous bacteriuria treatment seeking pattern among the pregnant women was 138 (60%), 42 (18.3%), 32 (13.9%) and 1 (0.4%) for individuals who had sought treatments in hospitals, patent drug dealers (chemists), multi-centres and traditionally respectively. It is recommended that routine urine culture screening be conducted for all pregnant women at least in the second and third trimesters and positive ASB promptly treated.

Keywords: Asymptomatic Bacteriuria, Prevalence, Uropathogens, Pregnant Women, Offot Ukwa District

1. Introduction

Urinary tract infection (UTI) is one of the commonest health problems among women due to shorter urethra, pathogens entry facilitated by sexual intercourse and close proximity of the anus with vagina [1-4]. Researchers have reported that approximately one in three female human subjects within child bearing age contracts urinary tract infection, that may present signs or remain asymptomatic [5, 6]. Pregnant women are more vulnerable to UTI because of the alteration in anatomical and physiological state during pregnancy [2]. Firstly, the weight of the gravid uterus on the renal system often leads to the accumulation of fluid in the ureter, known as hydronephrosis. Also, there is decrease in the bladder tone which may lead to the accumulation of urine up to twice the normal urinary volume without discomfort [7]. Gestational glycosuria, proteinuria and elevated levels of progesterone can decrease the muscle tone of the ureter and bladder. It can result in vesico-ureteric reflux [7]. These provide enriched culture media for bacteria pathogens that may invade the urinary system.

Bacteriuria during pregnancy may be classified as asymptomatic bacteriuria, infection of the lower urinary tract (cystitis), or pyelonephritis (infection of the upper urinary tract) according to Glaser and Schaeffer [8]. Lower tract bacterial infection is associated with an increased risk of developing pyelonephritis in pregnancy, which is linked to adverse maternal and obstetric outcomes [8]. Asymptomatic bacteriuria (ASB) refers to the presence of significant quantity of uropathogenic bacteria in a properly collected urine sample from an individual without signs or symptoms of UTIs [9]. Asymptomatic bacteriuria can progress to uncomplicated cystitis and subsequently to acute pyelonephritis if not properly diagnosed and treated [9, 10]. Treatment of UTI is imperative in keeping with the safe goal of motherhood initiative; that women safely go through pregnancy period and child birth and deliver healthy babies. Untreated ASB is a risk factor for acute cystitis up to 40% and pyelonephritis (between 25-30%) in pregnant women in some populations; and could lead to adverse obstetric and maternal outcomes like prematurity, low-birth weight, abnormalities in babies, and higher fetal mortality rates in several documented researches [11-13].

UTI is mostly caused by a wide range of Gram-negative aerobic pathogens found in gastrointestinal tract of mammals like *E. coli*, *Klebsilla pneumoniae*, *Proteus aeruginosa*, *Proteus mirabilis*, *Ectrobacter*, *Enterobacter*, etc. Other pathogens that cause UTI include *Enterococcus species*, *Serratiaspecies*, *staphylococcus epidermidis*, *Staphylococcus saprophyticus*, etc [14] due to colonization of the genito-urinary tract. UTI has become more complicated and difficult to treat because of appearance of mutant uropathogens that are resistant to the commonly used antimicrobial drugs [14].

The prevalence of ASB or UTI is influenced by several factors like socio-economic status of patients, increased maternal age, high parity, poor perineal hygiene, sexual activity, anatomic /functional urinary tract abnormality,

history of recurrent UTIs, diabetes mellitus, etc as reported by investigators [4, 15-17]. The prevalence and epidemiological distribution of ASB uropathogens varies geographically; like in Ethiopia it is between 7% to 18.8% [12, 18-19] and a lower prevalence rate of 3.6% in Sri Lanka [20].

In Nigeria, 18.21% was reported in South Eastern Nigeria [21] while 19% [4] and 20% were reported in South-South Nigeria [22]. However, a higher prevalence of 55% among pregnant women in a traditional birth home, Benin City, Nigeria has been documented [1]. Therefore, periodic evaluation of asymptomatic uropathogenic bacterial profile among pregnant women, etiology, risk factors and characteristics are needed to update information. There is dearth of information on case-control research of asymptomatic uropathogenic bacteriuria among pregnant and non-pregnant women in Offot Ukwa district, Anua, Uyo metropolis, Akwa-Ibom State Nigeria. Thus, this study seeks to investigate the prevalence, risk factors, and asymptomatic uropathogenic bacteria profile among participants visiting St Luke's Hospital Anua, Offot Ukwa district using a case-control approach.

2. Materials and Methods

2.1. Study Design

This study was designed as a retrospective survey, made up of registered pregnant women at all stages of pregnancy and non-pregnant women (the control group) attending antenatal clinics of St Luke's hospital Anua, Offot Ukwa district, Uyo, Akwa-Ibom State, Nigeria. This hospital is one of the referral government hospitals located in Anua, Offot Ukwa district, which provides medical services to 22 villages within the district.

2.2. Ethical Considerations and Participants Recruitment

The study adhered strictly to the tenet of the declaration of Helsinki by obtaining full ethical approval from the Ethical Review Board of St Luke's Hospital Anua, under the auspices of Akwa-Ibom State Ministry of Health Research Ethical Review Committee, Uyo, Akwa-Ibom State, Nigeria, before subjects were recruited. Informed written consent was obtained from the study participants. Participants were given a 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 duration were referred to attending physicians and treated accordingly with appropriate drugs in line with the national guidelines for treatment of pregnant women. Urine samples were collected from a total of 330 participants, comprising 230 pregnant women between the ages of 14-43 years and 100 age-matched non-pregnant women as control subjects. All pregnant women were recruited from individuals attending antenatal clinics in St Luke Hospital Anua, Uyo, Akwa-Ibom State and the controls were also recruited within the same hospital. The study lasted over a

six month period.

2.3. Inclusion and Exclusion Criteria

All pregnant women not on antibiotics therapy, without clinical signs and symptoms of urinary tract infections (UTIs) and willing to participate were included. All pregnant women currently on antibiotics therapy for urinary tract infection or any type of infection in this hospital were excluded. In addition, the control subjects who refused to participate and/or fully cooperate with the guidelines of the study were excluded. Asymptomatic bacteriuria 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.*, [16].

2.4. Sample Collection

Clean catch early morning mid-stream urine samples of 5ml were collected in sterile universal containers as described by the protocols of Karlowsky *et al.* [23] and Solberg *et al.* [24]. They 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, midstream urine samples were collected [25]. The clinic matrons, senior and junior nursing staff assisted in the proper instruction of all the patients (in Pidgin English, Annang, Efik, Igbo and Ibibio languages for the educationally less-privileged ones) and supervision of those needing assistance. The patients were given well-structured questionnaire to fill. Information such as age (Age at last Birthday), number of years married, number of children, spacing of children, and gestational age derived from the last menstrual period (LMP) of the patients. Based on gestational age information, patients were classified into 3 trimesters namely: first trimesters between 0-13 weeks, second trimesters between 14-27 weeks and third trimesters between 28-40 weeks. Also, the educational background and occupation of both the patients and their husbands were obtained.

From these information obtained, cases were grouped into social classes 1-5 according to the method documented [26]. 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 (between 1-2 hours of collection). This was carried out to prevent bacterial population growth and proliferation in specimens [27].

2.5. Microbiological Examination of the Samples

2.5.1. Macroscopic Examination

All samples were macroscopically examined for colour,

turbidity and odour.

2.5.2. 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 [28]. 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 [29].

2.5.3. 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 0.05ml 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 [30].

2.6. 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 [30, 31].

2.7. Antimicrobial Susceptibility Testing (AST) of Uropathogens

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

for Clinical Laboratory Standards as documented [32]. The drugs that were tested include Ceftazidime (30µg), Amoxicillin-clavulanic acid (20µg), Azetronam (30µg), eftriaxone (30µg), Ciprofloxacin (5µg), Nitrofurantoin (300µg), Cefotaxime (30µg), Gentamicin (20µg), Imipenem (10µg), Sulfamethoxazole-trimethoprim (1.25µg) and Azithromycin (15µg). All the antimicrobials used for the research were purchased from Oxoid Limited, Cambridge, United Kingdom.

2.8. Quality Control

All Culture media were tested for sterility and performance. Reference strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were utilized during culture and antimicrobial testing of uropathogens from recruited participants.

2.9. Statistical Analysis

Data from laboratory investigation and questionnaire was entered into Microsoft Excel Spread sheet. The coded data was processed and analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Quantitative, clinical and laboratory variables were compared using Chi-square (χ^2) test and simple percentages. Statistical significance was set at 5% ($P \leq 0.05$).

3. Results

3.1. Socio-demographic Variables of Participants

Urine samples were collected from a total of 230 and 100 pregnant women and non-pregnant women (control group) respectively were examined for ASB. The age of all participants recruited ranged from 14-43 years, with the mean age of 25.3±5.2 years and 24.2±5.6 years for pregnant women and non-pregnant women (control group) respectively. In the population investigated 227 (98.7%) and 98 (98.0%) were married pregnant women and non-pregnant women respectively. Two hundred and three (88.3%) had educational status of secondary school and above among the pregnant women, while 76 (76%) of the non-pregnant women had secondary education and above. Based on parity among the pregnant women, 112 (48.7%), 61 (26.5%) and 57 (24.8%) were nulliparous, monoparous and multiparous respectively, while in the non-pregnant women, 48%, 36% and 16% were nulliparous, monoparous and multiparous respectively. Additionally, 37 (16.1%), 70 (30.4%) and 123 (53.5%) of the pregnant women were in the 1st, 2nd and 3rd trimester period of pregnancy. Sixty-seven (29.1%), 125 (54.3%), 37 (16.1%) and 1 (0.4%) of the pregnant subjects were housewives, self-employed, civil servants and applicant, respectively in terms of their occupational status. Furthermore, in the non-pregnant

subjects (control), 36%, 52%, 10% and 2% were housewives, self-employed, civil servants and applicants respectively based on their occupation (Table 1).

3.2. Prevalence of ASB Uropathogens and the Presence of Abnormal Conditions

The overall prevalence of ASB uropathogens among the pregnant women in the study population was 29.1%. There was significant association of age, parity and trimester to the ASB among the pregnant women while marital status, educational status and occupation of the pregnant women did not have any significant association with ASB uropathogens in the population investigated (Table 1). The presence of abnormal conditions among the pregnant women was 17 (7.4%), 3 (1.3%), 16 (7.0%) and one (0.4%) for pyuria, glycosuria, proteinuria and proteinuria/glycosuria respectively (Table 2).

3.3. Isolation and Identification of ASB Uropathogens

From the uropathogens isolated (n=67), Gram-negative ASB were more prevalent 46 (68.7%) than Gram-positive ASB uropathogens 21 (31.3%) among the pregnant women. The most commonly isolated ASB were *E. coli* 19 (28.4%), followed by *Klebsiella pneumoniae* 16 (23.9%) and the least was *Proteus spp* 2 (3%) which was detected in the pregnant women investigated. In non-pregnant women, the Gram-positive bacteria isolated (n=15) recorded 7 (47%), 3 (20%) for *E. coli* and *K. pneumoniae* respectively, while the Gram-negative bacteria was 2 (13.3%) for both *S. aureus* and *S. saprophyticus* (Table 3).

3.4. Antimicrobial Susceptibility Pattern of ASB Uropathogens

The results of antimicrobial susceptibility testing pattern of ASB uropathogens in pregnant women revealed that Gram-negative isolates displayed high resistance pattern in comparison to Gram-positive isolates for most of commonly prescribed antibiotics. *E. coli* which predominantly cause UTI showed high percentage (> than 50%) to all the antibiotics except Azetronam (16%). Multiple drug resistance (MDR) that is, isolate resistant to more than two antimicrobial drugs was discovered in all ASB uropathogens isolated (100%). All isolates of Gram-negative and Gram-positive ABS were resistant to more than two or more drugs (Table 4). The previous bacteriuria treatment seeking pattern of the pregnant women was 138 (60%), 42 (18.3%), 32 (13.9%) and 1 (0.4%) for individuals who had sought treatments in hospitals, patent drug dealers (chemists), multi-centres and traditional herbs respectively but were still positive with ASB uropathogens after medical examinations (Table 5).

Table 1. The prevalence of ASB and demographic variables of the study population.

Variables	Pregnant women (N=230)			None pregnant women (N=100)			X ²	p-value
	No tested (%)	No negative (%)	No positive (%)	No tested (%)	No negative (%)	No positive (%)		
Age (years)								
14-19	10 (4.3)	9 (3.9)	1 (0.4)	11 (11)	9 (9)	2 (2)		
20-25	91 (39.6)	66 (28.7)	25 (10.9)	36 (36)	32 (32)	4 (4)		
26-31	100 (43.5)	71 (30.9)	29 (12.6)	30 (30)	25 (25)	5 (5)	1.720	0.731
32-37	25 (10.9)	14 (6.1)	11 (4.8)	13 (13)	10 (10)	3 (3)		
38-43	4 (1.7)	3 (1.3)	1 (0.4)	10 (10)	9 (9)	1 (1)		
Total	230 (100)	163 (70.9)	67 (29.1)	100 (100)	85 (85)	15 (15)		
Marital status								
Married	227 (98.7)	160 (69.6)	67 (29)	98 (98)	83 (83)	15 (15)	0.341	0.861
Other	3 (1.3)	3 (1.3)	0 (0)	2 (2)	2 (2)	0 (0)		
Educational status								
Informal	4 (1.7)	4 (1.7)	0 (0)	3 (3)	2 (2)	1 (1)		
Primary	23 (10)	12 (5.2)	11 (4.8)	21 (21)	16 (16)	5 (5)	0.268	2.72
Secondary	134 (58.3)	100 (43.5)	34 (14.8)	56 (56)	50 (50)	6 (6)		
Tertiary	69 (30.)	47 (20.4)	22 (9.6)	20 (20)	17 (17)	3 (3)		
Parity								
Nullipara	112 (48.7)	104 (45.2)	8 (3.5)	48 (48)	42 (42)	6 (6)		
Monopara	61 (26.5)	32 (13.9)	29 (12.6)	36 (36)	28 (28)	8 (8)	1.57	0.889
Multipara	57 (24.8)	28 (12.2)	29 (12.6)	16 (16)	15 (15)	1 (1)		
Thrimester								
1 st	37 (16.1)	29 (12.6)	8 (3.5)					
2 nd	70 (30.4)	38 (16.5)	32 (13.9)				2.173	0.534
3 rd	123 (53.5)	96 (41.7)	27 (11.7)					
Occupation								
Housewife	67 (29.1)	49 (21.3)	18 (7.8)	36 (36)	32 (32)	4 (4)		
Self-employed	125 (54.3)	86 (37.4)	39 (17)	52 (52)	44 (44)	8 (4)	0.006	1.653
Civilservant	37 (16.1)	27 (11.7)	10 (4.4)	10 (10)	8 (8)	2 (2)		
Applicant	1 (0.4)	1 (0.4)	0 (0)	2 (2)	1 (1)	1 (1)		

Table 2. Bacteriuria and the presence of abnormal conditions in pregnant women investigated.

Abnormal conditions	No of positive samples (%)
Pyuria	17 (7.4)
Glycosuria	3 (1.3)
Proteinuria	16 (7.0)
Proteinuria and glycosuria	1 (0.4)
Monomicrobial ASB	49 (21.3)
Polymicrobial ASB	18 (7.8)

Table 3. Frequency of ASB uropathogens isolated from the study population.

Bacterial isolates	Pregnant women	Non-pregnant women
	No of positive samples (%) (n=67)	No of positive sample (n=15)
<i>Escherichia coli</i>	19 (28.4)	7 (47.0)
<i>Staphylococcus aureus</i>	14 (21.0)	2 (13.3)
<i>Klebsiella pneumonia</i>	16 (23.9)	3 (20.0)
<i>Pseudomonas aeruginosa</i>	5 (7.5)	1 (6.7)
<i>Proteus spp</i>	2 (3.0)	0 (0.0)
<i>Klebsiella spp</i>	4 (6.0)	0 (0.0)
<i>S. saprophyticus</i>	7 (10.4)	2 (13.3)
Total	67 (100)	15 (100)

Table 4. Antimicrobial susceptibility pattern of ASB uropathogens among pregnant women isolates.

Bacteria agent (n)	Antimicrobial agents tested										
	CAZ	AMC	AZT	CRO	CIP	NIT	CTX	GEN	IPM	SXT	AZM
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
<i>E. coli</i> (19)	10 (53)	13 (68)	12 (63)	10 (53)	13 (68)	10 (53)	13 (68)	12 (63)	10 (53)	11 (58)	3 (16)
<i>K. pneumonia</i> (16)	11 (69)	10 (63)	11 (69)	9 (56)	11 (69)	9 (56)	10 (63)	12 (75)	10 (63)	6 (68)	5 (31)
<i>P. aeruginosa</i> (5)	3 (60)	4 (80)	0 (0)	3 (60)	3 (60)	1 (20)	3 (60)	4 (80)	4 (80)	4 (40)	0 (0)
<i>Klebsiella spp</i> (4)	2 (50)	3 (75)	0 (0)	0 (0)	3 (75)	0 (0)	2 (50)	2 (50)	2 (50)	1 (25)	1 (25)
<i>Proteus spp</i> (2)	2 (100)	1 (50)	0 (0)	0 (0)	1 (50)	1 (50)	1 (50)	1 (50)	1 (0)	0 (0)	0 (0)
<i>S. aureus</i> (14)	7 (50)	13 (92)	5 (36)	6 (43)	12 (86)	4 (29)	12 (86)	13 (92)	7 (50)	8 (57)	4 (29)
<i>S. saprophyticus</i> (7)	4 (57)	5 (71)	3 (43)	0 (0)	4 (57)	3 (43)	4 (57)	5 (71)	5 (71)	4 (57)	7 (71)

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

Table 5. Previous bacteriuria treatment seeking pattern of the pregnant women.

Mode of treatment	No examined (%)	No of positive uropathogen (%)
Hospital	138 (60)	36 (15.7)
Chemists	42 (18.3)	15 (6.5)
Prayer/faith healing	17 (7.4)	5 (2.2)
Organizations		
Traditional herbs	1 (0.4)	0 (0)
Multi-centre	32 (13.9)	11 (4.8)
Total	230 (100)	67 (29.1)

4. Discussions

Pregnant women are at increased risk of UTI but in many cases uropathogenic bacterial infections remain asymptomatic [1, 4, 8, 10, 12, 18, 21, 33-34]. In this research, the overall prevalence of ASB uropathogens among pregnant women was 29.1%, which is in harmony with the documented research from Iranian population [35], which reported the same prevalence of 29.1%. The result of this study is higher than the prevalence of 3.6% in Sri Lanka [20], 6%-10.6% in north west and central Ethiopia [19], 2.3% and 4.7% documented among ASB pregnant women without and with DM or GDM respectively [34], 8% and 11% for ASB and UTI respectively by Kumar *et al.* [36] in other populations. Also 9% in Nsukka [37], 10.7% in Ibadan [33], approximately between 18 to 19% in some developing central and West African countries [4, 12, 16, 21] and 23.9% in Sagamu, Nigeria [38] which are lower prevalence rates. In contrast, higher prevalence of 45.3% [39] and 55% [1] in Benin, 54% in Akwa metropolis [40], 72.5% in Lagos State [41], 78.7% in Ebonyi State [42] and 86.6% in Oyo State [43], all Nigerian populations. The variations in the prevalence of ASB or UTIs from the same geographical regions of the same countries or different countries might be due to differences in the environment, associated risk factors, social habits of the community, the standard of personal hygiene, educational status of individuals as well as some specifically documented extended spectrum producing uropathogens in ASB (methodology adopted).

Highest prevalence of ASB uropathogens was observed in the age range of 26-31 years for both pregnant and non-pregnant women and the mean ages of 23.3±5.2 and 24.2±5.6

years were stipulated for pregnant and non-pregnant women respectively in this study, which was similar to that reported in Hawassa referral hospital [12], Ambo town [16] Gondar tertiary teaching hospital [6] in Ethiopian cities, Benin city [1, 39], Ibadan [33] and University of Uyo teaching hospital, Nigeria [4]. The increased prevalence observed within this age range may be attributed to multiparity which has been documented as one of the risk factors for ASB in pregnant women in many populations [4, 15-17, 44]. This is in tandem with the result of this present study.

In this present study conducted at Anua, Offot Ukwu district, the highest rate of ASB uropathogens was observed in the third trimester pregnancy, which is in harmony with other researches [1, 12, 16, 20] in Ibadan, Ethiopia and Sri Lanka but disagrees with others [4, 6] that reported highest prevalence in the first trimester and second trimester pregnancy. Several anatomical and hormonal changes in pregnancy lead to urethral dilation and urinary stasis which contributed to increased risk of developing urinary tract infection [2, 45]. Moyo *et al* [46] documented that urinary stasis increases with advancing pregnancy. The high prevalence of ASB uropathogens observed among pregnant women in this study may be due to those reason stated above, coupled with bad clean up of genital and further complicated by heavily distended belly during 3rd trimester pregnancy. Occupational, marital and educational status did not significantly associate with the prevalence of ASB uropathogens, which is concomitant to previous findings [47-48].

Pyuria, glycosuria, proteinuria and bacteriuria showed no association statistically in this study. However, it was observed that pyuria, proteinuria and glycosuria were higher in women without significant bacteriuria. This may imply

that these conditions may not be used as predictive parameters of significant bacteriuria. For example, pyuria may be observed in women with urogenital tuberculosis, renal mycobacterial infectious and Chlamydia [49]. 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 (individual with poor cell-mediated immune response); while gestational proteinuria and glycosuria may be due to physiological challenges of pregnancies that are not related to the presence of bacterial infection. Rahimkhani *et al.*, [35] reported that the utilization of microscopic urinalysis is not an effective method of determining ASB pathogens.

In this present research, Gram-negative uropathogens isolates were more prevalent (68.7%) than Gram-positive bacteria isolates (31.3%) among pregnant women investigated. This result is in harmony with studies carried out in Gondar [6], Ambo town [16], Addis Ababa [19], Dire Dawa [50] all Ethiopian cities, Tanzania [51] and Uyo, Nigeria [22]. This may be due to the presence of unique structure in Gram-negative bacteria which aids in attachment to the uroepithelial cells and prevent bacteria from urinary lavage, allowing for multiplication, proliferation and tissue invasion, resulting in invasive infection and pyelonephritis in pregnancy [52]. *E. coli* was the most frequent etiological agent causing ASB which recorded 28.4% of the uropathogen isolated. This result is in harmony with the findings from Ethiopian populations [6, 16, 19, 51], Tanzania [51], Nnewi [21], Benin City [1], Uyo [4, 22] and other populations [19, 35, 39]. *E. coli* and *Klebsiella* (coliforms) were the predominant pathogens isolated with the highest frequency in Ibadan [33] and Sri Lanka [20] which was consistent with our findings in Offot Ukwa district, Uyo. The pathogen named *E. coli* is known as the most prominent uropathogenic bacteria because of its number of virulence factors specific for colonization and invasion of the urinary epithelium [53]; this may be another reason why we observed high and predominant frequency of *E. coli*, coupled with microorganisms ascending from the peri-urethral areas contaminated by fecal flora due to the close proximity to anus, warm and moist environment [7, 54].

Antimicrobial resistance of uropathogens to some commonly used antibiotics have become high, making doctors with few choices of drugs for the treatment of UTIs [19, 55]. In this study, susceptibility pattern of bacteria revealed that most of the isolates were sensitive to amoxicillin-clavulanic acid (ranged from 58% to 92%), ciprofloxacin (ranged from 53% to 86%), cefotaxime (ranged from 50% to 86%), gentamicin (ranged from 50% to 92%), imipenam (ranged from 50% to 71%) and ceftazidime (ranged from 50% to 100%). In other studies, similar reports were documented that bacterial isolates were susceptible to some antibiotics utilized in this study, but with varying frequency above 50% [44, 6, 19, 55]. Multiple drug resistance was seen in approximately 100% of the bacterial isolates. This is similar to some reports in Gondar at 95% [6],

74% in Addis Ababa [19] and between 80%-100% in Uyo [4] for multi drug resistance. This showed that multi drug resistance was high in some commonly used antibiotics. Antibiotic resistance has been known as the consequence of frequent antibiotic usage, self medications and abuse according to Albrich *et al.*, [56] and Moyo *et al.*, [46]. Prescription of antibiotics without laboratory guidance as well as over-the-counter sales of antibiotics without prescription is wide spread in Nigeria, especially in village setting [57]. This may be adduced as a possible reason for increased multi drug resistance for antibiotics. Also it was observed that some participants recruited for this study had sought previous treatment(s) by patronizing traditional healers (traditional birth homes, etc), patent drug dealers (locally called "chemists"), prayer/faith healing centers, etc. These actions may have exposed them to over-the-counter usages of antibiotics, leading to drug abuse; pointing to the increasing antimicrobial multi drug resistance observed in this study. This is in tandem with other documented researches in Nigeria [1, 4, 57] and other populations [19, 55].

5. Conclusions

This study have provided current asymptomatic bacterial profile, prevalence and associated risk factors among pregnant and non-pregnant women in a peri-urban area (Anua, Offot Ukwa district), Akwa-Ibom state. The overall prevalence of ASB uropathogens was 29.1% and the prevalence was significantly associated with age, parity and trimester period. *E. coli* was the most predominant bacterial uropathogen, followed by *Klebsiella pneumonia* and most of the uropathogens isolated were sensitive to some common antimicrobial agents utilized in our locality for treatment. Multiple drug resistant bacteria were common in the isolates. The high prevalence of ASB in pregnant women warrant the need to screen all pregnant women and treat those infected with appropriate antibiotic regimens. Health education of the pregnant women by relevant intervention agencies is strongly advocated. Also periodic and continuous follow up are mandatory to reduce the consequences of ABS and multiple drug resistant bacteria in pregnancy.

Abbreviations

ASB: Asymptomatic bacteriuria, AST: Antimicrobial susceptibility testing, AMC: Amoxicillin-Clavulanic acid, AZT: Azetronam, AZM: Azithromycin, CFU/mL: colony forming units permilliliter, CRO: Ceftriaxone, CIP: Ciprofloxacin, CAZ: Ceftazidime, CLED: Cystine lactose electrolyte deficient, CTX: Cefotaxime, GEN: Gentamicin, DM: Diabetes mellitus, GDM: Gestational diabetes mellitus, SPSS: Statistical packages for social sciences, IPM: Imipenam, UTI: Urinary tract infection, LMP: Last menstrual period, WBC: White blood cells, MDR: Multiple drug resistance, NIT: Nitrofurantoin, SXT: Sulphamethoxazole-trimetopin.

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Authors' Contributions

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

References

- [1] Oladeinde B. H, Omoregie R, Oladeinde O. B. (2015). Asymptomatic urinary tract infection among pregnant women receiving ante-natal care in a traditional birth home in Benin City, Nigeria. *Ethiop Journal of Health Science* 25(1): 3-8.
- [2] Abdullah A. A, Al-Moslih, M. I. (2005). Prevalence asymptomatic bacteriuria in pregnant women in Sharjah, United Arab Emirates. *East Mediterr Health Journal* 11: 5-6.
- [3] Feitosa D. C. A, Silva M. G, Parada C. M. G. L (2009). Accuracy of simple urine tests for diagnosis of urinary tract infections in low-risk pregnant women. *Rev Latino-Am Enfermagem* 17(4): 507-513.
- [4] Akpan N. G, Onwuezobe I. A, Antia U. E. (2017). Asymptomatic bacteriuria among pregnant women at University Hospital in Uyo, Nigeria: Prevalence risk factors and characteristics. *Asian Journal of Medicine and Health* 3(3):1-9.
- [5] Duarte G, Marcolin A. C, Quintana S. M., Cavalli, R. C. (2008). Urinary tract infection in pregnancy. *Rev Bras Gynecology Obstet.* 30: 93-100.
- [6] Alemu A, Moges F, Shiferaw Y, Tafess K, Kassu A, Anagaw B, Agegn A. (2012). *BMC Research Notes* 5(197): 1-7.
- [7] Chaliha C, Stanton S. L. (2002). Urological problems in pregnancy. *British Journal of Urology International* 89: 469-476.
- [8] Glaser AP, Schaeffer A. J. (2015). Infection and bacteriuria in pregnancy. *Urol Clin North America* 42 (4): 547-560.
- [9] Small F, Vazquez J. C. (2007). Antibiotics for asymptomatic bacteriuria in pregnancy. *Cochane Database of Systematic Reviews* 2.
- [10] Schnarr J, Small F. (2008). Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy. *Eur Journal of Clin Invest.* 38(2): 50-57.
- [11] Ade-Ojo IP, Oluyeye A. O, Adegun PT, Akintayo AA, Aduloju OP, Olofinbiyi BA (2013). Prevalence and antimicrobial susceptibility of asymptomatic significant bacteriuria among new antenatal enrollees in south west Nigeria *International Research Journal of Microbiology* 4(8): 197-203.
- [12] Tadesse E, Teshome M, Merid Y, Kibret B, Shimelis T. (2014). Asymptomatic urinary tract infection among pregnant women attending the antenatal clinic of Hausa referral hospital, Southern Ethiopia. *BioMed Cent Res Note* 7(155):1-5.
- [13] Alfred A. O, Chiedozi I, Martin D. U. (2013). Pattern of asymptomatic bacteriuria among pregnant women attending antenatal clinic at a private health facility in Benin, South Nigeria. *Ann Afr Med.* 12(3):160-164.
- [14] Beyene G, Tsegaye W. (2011). Bacterial uropathogens in UTI and antibiotic susceptibility pattern in Jimma University specialized hospital. *Ethiopia Journal of Health Sci.* 21(2):141-146.
- [15] Ezechi O. C, Gab-Okafor C. V, Oladele DA, Kalejaiye O. O, Oke B. O, Ekama S. O, Audu RA, Okoye RN, Ujab I. A. (2013). Prevalence and risk factors of asymptomatic bacteriuria among pregnant Nigerians infected with HIV. *Journal of Matern Fetal Neonatal Med.* 26(4):402-406.
- [16] Gessese Y. A, Damessa D. L, Amare M. M, Bahta Y. H, Shifera A. D, Tasent F. S, Gebremedhin E. Z (2017). Urinary pathogenic bacteria profile, antibiogram of isolates and associated risk factors among pregnant women in Ambo Town, Central Ethiopia: A cross-sectional study. *Antimicrobial Resistance and Infection Control* 16 (132): 1-10.
- [17] Fatima N, Ishrat S. (2006). Frequency and risk factors of asymptomatic bacteriuria during pregnancy. *Journal of Coll Physicians Surg Park.* 16: 273-275.
- [18] Gabre-Selassie S. (1998). Asymptomatic bacteriuria in pregnancy: Epidemiological clinical and microbiological approach. *Ethiop Med Journal* 36: 185-192.
- [19] Assefa A, Asrat D, Woldeamanuel Y, G/Hiwot Y, Abdella A, Melesse T. (2008). Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at Tikur Anbessa Specialist Hospital, Addis Ababa, Ethiopia. *Ethiopia Medical Journal.* 46: 227-235.
- [20] Jennifer P, Cyril R, Piyumi P, Nimesha G, Renuka J. (2012). Asymptomatic bacteriuria in pregnancy; Prevalence, risk factors and causative organism. *Sri Lanka Journal of Infectious Diseases* 1(2): 42-46.
- [21] Oli A. N, Okafor C. I, Ibezim E. C, Akujiobi C. N, Onwunzo M. C (2010). The prevalence and bacteriology of asymptomatic bacteriuria among antenatal patients In Nnamdi Azikiwe University Teaching Hospital, Nnewi, South eastern Nigeria. *Nigeria Journal of Clinic Practice* 13(4): 409-412.
- [22] Onwuezobe A. I, Orok F. E. (2015). Extended spectrum beta-lactamase producing uropathogens in asymptomatic pregnant women attending antenatal care in an urban community secondary health facility. *African Journal of Clinical and Experimental Microbiology* 16(1): 49-53.
- [23] Karlowsky J. A, Hoban D. J, Decorby M. R, Laing N. M, Zhanel G. G (2012). Fluoroquinolone-resistance urinary isolates of *Escherichia coli* from outpatients are frequently multidrug; Results from the North American Urinary tract infection collaborative alliance-quinolone resistance study. *Antimicrobial Agent and Chemotherapy* 50: 2251-2254.

- [24] Solberg O. O, Ajiboye R, Riley L. W (2006). Origin of class 1a integron and genecassettes in a population-based sample of uropathogenic *Escherichia coli*. *Journal of Clinical Microbiology* 44: 1347-1357.
- [25] Agency of Clinical Innovation (ACT) Urology Network (2012). Collection of urine. Data accessed 2018. Available: http://www.aci.health.nsw.gov.au/data/assets/pdffile/005/165920/collection_of_urine_midstream_toolikit.pdf.
- [26] Ogunlesi T. A, Dedeké I. O. F., Kuponiyi O. T (2008). Socioeconomic classification of children attending specialist paediatric centres in Ogun State, Nigeria. *Nigerian Medical Practitioner* 54: 21-25.
- [27] Cheesbrough M (2000). *District laboratory practice in tropical countries, part 2*. Cambridge University Press, UK, 69-90.
- [28] Hoberman A, Wald E. R, Reynolds E. A, Penchansky L, Charron M. (1994). Pyuria and bacteriuria in urine specimens obtained by catheter from young children with fever. *Journal of Pediatrics* 124: 513-519.
- [29] Graham J. C, Galloway A. (2001). *The laboratory diagnosis of urinary tract infection: Clinical microbiology laboratory, Royal Victoria infirmary Queen Victoria Road, New Castle, Upon Tyne UK*, 54: 911-9.
- [30] Bryan C (2011). Infectious disease, urinary infections from infectious disease section of microbiology and immunology on-line. University of South Carolina. Data accessed: 21st August, 2018. Available: <http://pathmicro.med.sc.edu/infectious%infectioushtm>.
- [31] World Health Organization (2003). *Manual for the laboratory identification and antibiotic susceptibility testing of bacteria pathogens of public health importance in the developing world*. Geneva.
- [32] National committee for Clinical Laboratory Standards (Clinical Laboratory Standard Institutes) (2012). Performance of standard for antimicrobial disk susceptibility test: Approved standards. M2-A7. PA USA: NCCL, 32 (1), Villanova.
- [33] Awonuga D. O, Dada-Adegbola H. O, Fawole A. O, Olala F. A, Onimisi-Smith H. O (2011). Asymptomatic bacteriuria among an obstetric population in Ibadan. *West African Journal of Medicine* 30 (20): 89-93.
- [34] Schneeberger C, Erwisch J. J. H. M, vanden Heuvel E. R, Mol B. W. J, Ott A, Geerlings S. E. (2018). Asymptomatic bacteriuria and urinary tract infection In pregnant women with and without diabetes. *Eur Journal of Obstet Gynecol Reprod Biol*. 222: 176-181.
- [35] Rahimkahni M, Khavari-Daneshvar H, Sharifian R. (2008). Asymptomatic bacteriuria and pyuria in pregnancy. *Acta Med Iran* 46(5): 409-412.
- [36] Kumar C. T, Carbnneau M, Keough A, Ma M, Tandon P (2013). Asymptomatic bacteriuria is an independent predictor of urinary tract infections in an ambulatory cirrhotic population: Prospective evaluation. *Liver International* 34(6).
- [37] Afunwa R. A, Odimegwu D. C, Iroha R. I, Esimone C. O. (2011). Antimicrobial resistance status and prevalence rate of extended spectrum beta-lactamase (ESBL) producers isolated from a mixed human population. *Basic Medical Science* 11(2): 91-96.
- [38] Olusanya O, Ogunledum A, Fakoya T. A. (1993). Asymptomatic significant bacteriuria among pregnant and non-pregnant women in and non pregnant women in Sagamu Nigeria. *WAJM*. 12(1): 27-33.
- [39] Imade P. E, Izekor P. E, Eghafona N. O, Enabulele O. I, Onyeagba O. I, Ophori E. (2010). Asymptomatic bacteriuria among pregnant women. *North American Journal of Medical Science* 2: 263-266.
- [40] Obiogbolu C. H, Okonko I. O, Anyamere C. O, Adedeji A. O. (2009). Incidence of urinary tract infection amongst pregnant women within Akwa Metropolis, South eastern Nigeria. *Scientific Research and Essay* 4:820-824.
- [41] Egbebi A. O Famurewa O. (2011). Prevalence of extended spectrum beta-lactamase (ESBL) Production among *Klebsiella* isolated in some part of south west Nigeria. *Journal of Microbiology Biotechnology and Research* 16(2): 64-68.
- [42] Amadi E. S, Enemuo O. B, Uneke C. J, Nwosu O. K, Onyeagba R. A, Ugboju O. C. (2007). Asymptomatic bacteriuria among pregnant women in Abakaliki, Ebonyi State, Nigeria. *Journal of Med Science* 7(4): 698-700.
- [43] Kehinde A. O, Adedapo K. S, Aimaikhu C. O, Odukogbe A. A, Olayemi O, Salako B. (2011). Significant bacteriuria among asymptomatic antenatal clinic attendees in Ibadan. *Nigeria Tropical Med and Health* 39: 73-76.
- [44] Akinloye O, Ogbobu D. O, Akinloye O. M, Terry Alli O. A (2006). Asymptomatic bacteriuria of pregnancy in Ibadan, Nigeria: A reassessment. *British Journal of Biomedical Science* 63: 109-12.
- [45] Le J, Briggs G. G, McKeown A, Bustillo G. (2004). Urinary tract infections during pregnancy. *Ann Pharmacotherapy* 38(10): 1692-1701.
- [46] Moyo S. J, Aboud S, Kasubi M, Maselle S. Y. (2010). Bacterial isolates and drug susceptibility pattern of urinary tract infection among pregnant women at Mihimbili National Hospital in Tanzania. *Tanzania Journal of Health Research* 12(4): 233-236.
- [47] Kovavisarch E, Vichairpruck M, Kanjarahareutai S. (2009). Risk factors related to asymptomatic bacteriuria in pregnant women. *Journal of Med Assoc Thailand*. 9(5): 606-610.
- [48] Sheikh M. A, Khan M. S, Khatoon A, Arain G. M (2000). Incidence of urinary tract infection during pregnancy. *East Mediterr. Health Journal* 6(2-3): 265-271.
- [49] Hwang J. H, Park H. C, Jeong J. C, Baek B, Han M. Y, Bang K. (2013). Chronic asymptomatic pyuria proceeds overt urinary tract infection and deterioration of renal function in autosomal dominant polycystic kidney disease. *BMC Nephrology* 14.
- [50] Derese B, Kedir H, Teklemariam Z, Weldegebrea I. F, Balakrishnan S. (2016). Bacterial practice of UTI and antimicrobial susceptibility pattern among pregnant women attending at antenatal clinic in Dil Chora referral Hospital, Dire Dawa, Eastern Ethiopia. *Ther. Clin. Risk Manag.* 12(1): 251-260.
- [51] Sabrina J, Said A, Mabula K, Samuel Y (2010). Bacterial isolates and drug susceptibility patterns of urinary tract infection among pregnant women at Muhimbili National Hospital in Tanzania. *Journal of Health Research* 12: 4-14.

- [52] Amiri F. N, Rooshan M. H, Ahmady MH, Soliamani M. J. (2009). Hygiene practices and sexual activity associated with urinary tract infection in pregnant women. *East Mediterr Health* 15: 105-108.
- [53] Lavigne J. P, Boutet-Dubois A, Lquini D, Combescure C, Bouziges N, Mares P, Sotto A. (2011). Virulence potential of *E. coli* strains causing asymptomatic bacteriuria during pregnancy. *Journal Clin Microbiol.* 49(1): 3950-3953.
- [54] Ramos NL, Sekikubo M, Dzung D. T, Kosnopfel C, Kironde F, Mirembe F, Brauner A. (2012). Uropathogenic *E coli* isolates from pregnant women in different countries. *Journal of Clin Microbiol.* 50(11): 3569-3574.
- [55] Mohammed M, Mahdy Z. A, Omar J, Maan N, Jamic MA (2002). Laboratory aspects of asymptomatic bacteriuria in pregnancy. *Southeast Asian Journal Trop. Med. Public Health* 33: 575-580.
- [56] Albrich W. C, Monnet D. L, Harbarth S. (2004). Antibiotics selection pressure and resistance in *Streptococcus pneumoniae pyogenes*. *Emerging Infectious Disease* 38: 363-371.
- [57] Omoregie R, Eghafona N. O. (2009). Urinary tract infection among asymptomatic HIV patients in Benin City, Nigeria. *British D. J Biomed Sci.* 166(4): 190-193.