

CARRIAGE OF MULTI-DRUG RESISTANT UROBACTERIA BY ASYMPTOMATIC PREGNANT WOMEN IN YENAGOA, BAYELSA STATE, NIGERIA

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Abstract

Background: Urinary Tract Infections (UTIs) in pregnancy are associated with significant morbidity for both mother and baby but its early detection and treatments can prevent unfavorable complications in pregnancy. This study therefore determined the prevalence of asymptomatic bacteriuria and antimicrobial resistance profile of the urinary bacteria among pregnant women in Yenagoa, Bayelsa State, Nigeria.

Materials and Methods: Mid-stream urine samples were collected from 201 apparently healthy pregnant women attending antenatal clinics in Yenagoa. The samples were analyzed and the organisms identified using standard microbiological methods. Haemolysin production by the organisms was screened and their antimicrobial susceptibility was performed using standard assay methods.

Results: A high significant bacteriuria of 119 (59.2%) was predominantly recovered from the pregnant women in their second and third trimesters. The bacteria isolated; *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus* species had 36 – 67% of haemolysin producers. The bacteria exhibited a very high resistance to most of the antibiotics tested but the resistance to ceftazidime, gentamicin and ciprofloxacin was 28 – 67%. All the *Staphylococcus* species exhibited 90% and 85% resistance to ceftazidime (methicillin resistant) and vancomycin respectively. An 89 - 100% of the bacteria exhibited multiple antibiotic resistance and 72.3% of the screened bacteria phenotypically expressed Extended-Spectrum Beta-lactamase production.

Conclusion: The high prevalence of significant bacteriuria with high multi-drug resistance among the study pregnant women call for regular education on proper personal hygiene and the need for early screening for UTIs during antenatal clinics as means of controlling the spread of antibiotic resistant organisms and complications in pregnancy.

Keywords: Carriage, Multidrug Resistance, Urobacteria, Asymptomatic, Pregnant women, Yenagoa

Introduction

Urinary Tract Infections (UTIs) are one of the most common bacterial infections affecting humans of all age groups regardless of their genders in both community and hospital settings (Colgan *et al.*, 2006). Urinary Tract Infections frequently occur due to the colonization and invasion of any part of the urinary tract by microorganisms with accompanying symptoms (Turpin *et al.*, 2007; Okonko *et al.*, 2009). The women particularly pregnant women are much more susceptible to UTIs partly because of their short and wide urethra which has close proximity to the anus thereby enhancing the upward movement of the rectal bacteria to the urethra thereby causing infections (Colgan *et al.*, 2006; Enayat *et al.*, 2008; Kolawole *et al.*, 2009).

Asymptomatic bacteriuria, a condition of significant growth of urobacteria (at least 10⁵ cfu/ml) in patient without urinary symptoms, commonly occurs with varying prevalence among people of different age groups, genders, sexual activities, socioeconomic status and with the presence of genitourinary abnormalities (Colgan *et al.*, 2006; Enayat *et al.*, 2008; Amin *et al.*, 2009; Demilie *et al.*, 2012). The development of urinary tract infection from asymptomatic bacteriuria in pregnancy has been associated with the increased risk of preterm delivery, preeclampsia, low birth weight infants, maternal anemia and foetal mortality if left untreated (Enayat *et al.*, 2008; Lane and Takhar, 2011; Girishbuabu *et al.*, 2011).

The infecting bacteria that have been implicated in people with asymptomatic bacteriuria include *Escherichia coli*, *Klebsiella* species, *Pseudomonas aeruginosa*, *Proteus* spp., *Streptococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Enterobacter* species, but Gram negative bacteria especially *E. coli* and *Klebsiella* species have been reported to be the most frequently encountered among healthy patients (Nicolle *et al.*, 2005; Macejko and Schaeffer, 2007; Parveen *et al.*, 2011; Demilie *et al.*, 2012).

Early antimicrobial treatment of significant bacteriuria in pregnancy even when the mother has no clinical symptoms has been reported to significantly reduce the incidence of acute pyelonephritis and its consequent effects on mother and foetus by 80-90% (Nicolle *et al.*, 2005; Turpin *et al.*, 2007; Schnarr and Smaill, 2008; Smaill and Vazquez, 2015). However, the increasing emergence of multi-drug resistant bacteria among these pathogens is a major public health problem in the treatment of UTI especially among pregnant women who are restricted to only few safe first line antimicrobials, which may now be ineffective on the resistant pathogens thus, making the women's treatment a difficult task (Loh and Sivalingam, 2007; Chakupurakal *et al.*, 2010; Demilie *et al.*, 2012).

The increasing changing rate of bacterial resistance to common anti-infective agents has generated varied recent literatures on the asymptomatic bacteriuria in pregnancy globally, but similar report in this part of the country is very scarce. Therefore, we sought to determine the prevalence of asymptomatic bacteriuria and antimicrobial resistance profile of the urinary bacteria among pregnant women in Yenagoa, Bayelsa State, as a means of providing relevant health strategies for the management of pregnant women in this area.

Materials and Methods

Study Population

The pregnant women attending ante-natal clinics from June to September, 2015 in three General Hospitals/Health Centres in Yenagoa Metropolis of Bayelsa state were recruited into this cross sectional study. Yenagoa is the capital city of Bayelsa State in the South Southern part of Nigeria, which is situated on 4.92° North latitude, 6.26° East longitude and 86 meters elevation above the sea level. The major occupations of its inhabitants are civil service, farming, fishing, trading and schooling.

Settings and Ethical Approval

The study involved the collection of early morning catch of midstream urine from the antenatal women who were not on any antibiotic therapy for two weeks prior to the time of survey. The volunteers gave written informed consent and provided socio-demographic data such as age, stage of pregnancy, parity and history of UTIs by filling questionnaire. The study was approved by the ethical committees of the institution and hospitals prior to the commencement of the study.

Samples collection and Bacteriology

Each volunteer collected clean mid-stream urine samples into wide opened sterile universal bottles after being properly instructed on the procedure of collection without touching the skin. The urine samples were labeled appropriately and transported within two hours of collection in iced packs carrier to the laboratory. A 10 µL of each sample was aseptically transferred using a standard 10 µL micropipette onto a sterilized nutrient agar plate for the purpose of bacterial quantification and properly spread on the agar before incubated at 37°C for 24 hours for discrete colonies. The recovered colony count per sample was multiplied by 100 to get the total colony forming unit per ml (CFU/ml) and a sample with a total of 10⁵ CFU/ml or more was regarded as having significant bacteriuria but otherwise was considered insignificant. The discrete colonies arising from each sample plate were separately streaked on Cysteine lactose electrolyte deficient agar (CLED), Eosin methylene blue agar (EMB) and Mannitol salt agar plates for proper identifying features of the various uropathogens. The isolates with characteristic features on these agar plates were identified using standard bacteriological protocols like Gram's stain reaction and biochemical tests for Gram positive and Gram negative bacteria, before storing at 4°C for further investigations.

Haemolysin Testing

The confirmed uropathogens were investigated for haemolysin production as a virulence trait by spot-inoculating each strain on blood agar plate which was prepared by mixing 5 ml of fresh human blood gently with sterile 100 ml of molten Nutrient agar before distributing into various plates. The spot-inoculated blood agar plates were incubated at 37°C for 24 hours. Thereafter the plates were observed for haemolytic effect of each of the strain on the red blood cells.

Antimicrobial Susceptibility Testing

The overnight culture of each confirmed bacterial isolate was standardized to the turbidity of 0.5 McFarland standard suspension (1.5 x 10⁸ CFU/ml) using colony suspension method and the resultant microbial suspension was used

to swab the surface of a dried Mueller-Hinton agar (MAST, UK). The antimicrobial susceptibility of each bacterial isolate was determined using the modified Kirby-Bauer disc diffusion method as specified by Clinical and Laboratory Standards Institute (CLSI, 2014). The following antimicrobial discs from MAST, UK were placed firmly on the surface of the inoculated Mueller Hinton agar; Amoxicillin/clavulanate 20/10 µg, Cefuroxime 30 µg, Ceftazidime 30 µg, Cefotaxime 30 µg, Gentamicin 10 µg, Ciprofloxacin 5 µg, Levofloxacin 5 µg, Ertapenem 10 µg, Co-trimoxazole 25 µg and Nitrofurantoin 300 µg for Gram negative bacteria while Ceftazidime and Cefotaxime were replaced with Cefoxitin 30 µg and Vancomycin 2–16 µg/ml (using agar dilution method) for Gram positive bacteria. All the Mueller Hinton agar plates containing Gram negative bacteria were incubated at 37°C while those containing *Staphylococcus* species were incubated at 35°C for 24 hr. The zone diameter of inhibition around each antimicrobial disc was measured and interpreted using the Clinical and Laboratory Standards Institute chart while the *Staphylococcus* isolates that were resistant to vancomycin at 2 µg/ml were subjected to 4–16 µg/ml concentrations (CLSI, 2014).

Detection of Extended Spectrum Beta-lactamase (ESBL) Producing Organisms

The detection of ESBL enzymes was investigated phenotypically in *E. coli* and *K. Pneumoniae* isolates that were resistant to the tested third generation cephalosporins (ceftazidime and cefotaxime) by subjecting them to combination discs of cefotaxime/clavulanic acid, ceftazidime/clavulanic acid, single discs of cefotaxime and ceftazidime on the same Mueller Hinton agar plates, and then incubated at 37°C for 24 h. The isolate is said to be an ESBL producing organism, if the zone diameter difference between each antibiotic combination disc and its single disc on the organism's plate is ≥ 5 mm (CLSI, 2014).

Statistical analysis

The groups differences were tested using the Chi-square test (or Fisher's exact test when expected frequencies were too low), with the assumed level of statistical significance at a $P < 0.05$ while the strength and direction association or relationship was measured using the phi coefficient/Cramer's V tests where applicable. Data was performed with SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL).

Results

A total of 201 asymptomatic pregnant women belonging to age group range of 16 – 45 years with mean age of 28.7 years were investigated in this study. Majority of the study subjects were in the age group of 26 -35 years (60.7%) and the distribution of the foetal gestational age across the subjects' age group is shown in Table 1.

Table 1: The distribution of foetal gestational age across the subjects' age group

Age Group (years)	No of Samples	Number (%) of subjects in different gestational age		
		1-3 Months (First Trimester)	4-6 Months (Second Trimester)	7-9 Months (Third Trimester)
16-25	58 (28.9)	2 (3.4)	21 (36.2)	35 (60.3)
26-35	122 (60.7)	4 (3.3)	26 (21.3)	92 (75.4)
36-45	21 (10.4)	0 (0.0)	5 (23.8)	16 (76.2)
TOTAL	201	6 (3.0)	52 (25.9)	143 (71.1)

Of all the 201 screened samples of the asymptomatic pregnant women, 192 (95.5%) yielded positive bacterial growth and 119 (59.2%) of them had significant bacteriuria. The distribution of UTI (significant bacteriuria) across the subjects' age groups was observed to be significantly higher among the 16 -25 and 26 – 35 age groups ($P = 0.02$ and 0.01 respectively) (Table 2). However, it is significantly lower during the first trimester period ($P = 0.042$) but higher during the last two trimesters (Table 3).

Table 2: The distribution of significant bacteriuria among the subjects' age groups

Age Group (Years)	No. of Samples	No. (%) with significant bacteriuria	Chi- Square	P - value
16-25	58	27	5.403	0.020*
26-35	122	81	6.643	0.010*
36-45	21	11	0.452	0.501
Total	201	119 (59.2)		

*Statistically significant ($P < 0.05$)

Table 3: The distribution of significant bacteriuria among subjects' gestational age

Gestational Age (Months)	No. of Samples	No. (%) with significant bacteriuria	Chi- Square	P - value
1 - 3	6	1 (16.7)	4.633	0.042**
4 - 6	52	28 (53.8)	0.834	0.361
7 - 9	143	90 (62.9)	2.859	0.091
Total	201	119 (59.2)		

**Statistically significant ($P < 0.05$), P -value calculated using Fisher exact method.

Notable bacteria were recovered from the samples with significant bacteriuria and a total of 60 (50.4%) of them were haemolysin producers as shown in Table 4.

Table 4: The Isolation and haemolysin production of common urinary bacteria

Urinary bacteria	No. of isolates	No. (%) of haemolysin Producers
<i>Escherichia coli</i>	18	8 (44.4)
<i>Klebsiella pneumoniae</i>	45	16 (35.6)
<i>Pseudomonas aeruginosa</i>	36	24 (66.7)
<i>Staphylococcus</i> species	20	12 (60.0)
TOTAL	119	60 (50.4)

The antimicrobial susceptibility tests revealed that all the bacteria generally exhibited high resistance to all the tested antimicrobial agents. However, 60 -72% of *E. coli* isolates were observed to be susceptible to ceftazidime and ciprofloxacin. The *Staphylococcus* species showed 90% and 85% resistance to cefoxitin (methicillin resistant) and vancomycin respectively (Table 5).

Table 5: Antimicrobial Resistance Patterns of the volunteers' urinary bacteria isolates

Antimicrobial Agent	Number (%) of Resistant Isolates			
	<i>Escherichia coli</i> (N = 18)	<i>Klebsiella pneumoniae</i> (N = 45)	<i>Pseudomonas aeruginosa</i> (N = 36)	<i>Staphylococcus</i> spp. (N = 20)
Amoxicillin/Clavulanate	15 (83.3)	36 (80.0)	29 (80.6)	15 (75.0)
Cefuroxime	13 (72.2)	37 (82.2)	31 (86.3)	11 (55.0)
Ceftazidime	5 (27.8)	30 (66.7)	21 (58.3)	
Cefotaxime	13 (72.2)	37 (82.2)	27 (75.0)	
Gentamicin	10 (55.6)	25 (55.6)	21 (58.3)	10 (50.0)
Ciprofloxacin	7 (38.9)	23 (51.1)	16 (44.4)	10 (50.0)
Co-trimoxazole	18 (100)	42 (93.3)	34 (94.4)	18 (90.0)
Tetracycline	18 (100)	42 (93.3)	36 (100)	18 (90.0)
Cefoxitin				18 (90.0)
Vancomycin				17 (85.0)

The phenotypic screening for ESBL producers among *E. coli* and *Klebsiella pneumoniae* isolates that exhibited resistance to both ceftazidime and cefotaxime revealed a total of 34 (72.3%) and its distribution is showed in Table 6. Multiple antibiotic resistance as defined by Magiorakus *et al.* (2012) was screened for among the bacterial isolates. The antimicrobial agents used in this study can be grouped into seven classes of antibiotics which include the penicillins, cephalosporins, aminoglycosides, fluoroquinolones, folate antagonists, tetracyclines and glycopeptides. The rate of multiple antibiotic resistance among the studied isolates was 89 -100% (Table 7).

Table 6: The frequency of ESBL producer among the isolated enterobacteria

Urinary enterobacteria	Number of isolates	Number (%) of ESBL Producers
<i>Escherichia coli</i>	5	4 (80.0)
<i>Klebsiella pneumoniae</i>	42	30 (71.4)
Total	47	34 (72.3)

Table 7: Multi-drug resistance pattern of isolated Urinary bacteria

Resistance to classes of antibiotics	Number (%) of resistant Isolates			
	<i>Escherichia coli</i> N = 18	<i>Klebsiella pneumoniae</i> N = 45	<i>Pseudomonas aeruginosa</i> N = 36	<i>Staphylococcus spp.</i> N = 20
0	0	2	1	2
1	0	1	1	0
2	0	1	2	0
3	3	3	2	1
4	6	12	9	2
5	6	6	6	5
6	3	20	15	0
7	-	-	-	10
≥ 3	18 (100)	41 (91.1)	32 (88.9)	18 (90)

Discussion

Urinary tract infections are probably the most widely spread infections of the world which require proper investigations and prompt treatments in order to prevent serious life threatening complications and morbidity among vulnerable human populations like pregnant women and their unborn children (Musbau *et al.*, 2013).

The study revealed that the pregnant women poorly attended antenatal clinics in the first trimester periods of their pregnancies but majority of them only visited the clinics in their third trimester periods irrespective of whether they had previous birth delivery or not. This observation cut across all the age groups but was more prevalent among those in the 36 – 45 years age group. The reason for this behaviour might be partly due to the fact that many of them never discovered that they were pregnant probably until when the signs became more obvious around 3 – 4 months of the pregnancy. It may also be that many deliberately waited till the pregnancy is advanced before seeking medical attention because of personal reasons and lack of finance. In fact, there are a number of women in the community who never visited antenatal clinics but only come to hospital to deliver their babies when they can no longer manage the situation in their various homes because of poverty. The consequences of this observed behaviour is enormous leading to stillbirth, foetal and maternal mortality.

The prevalence of significant bacteriuria among the study volunteers was significantly higher among the 16 – 35 years age group than those of the 36 – 40 years age group ($P < 0.05$). The women in the 26 – 35 years age group had a stronger statistical significance with a prevalence rate of 66.4% significant bacteriuria. This suggests that high sexual activities of this group might have highly predisposed them to significant bacteriuria being the most sexually active group among the study subjects since it has been reported that rate of bacteriuria in women increases with increase in sexual activities and use of contraceptives (Parveen *et al.*, 2011; Amala and Nwokah, 2015). This findings support previous reports of positive cases of bacteriuria in pregnancy among women age group of 26 – 35 years (Rajshekhkar and Umashenkar, 2013; Amala and Nwokah, 2015). This observed condition might develop into a disease condition termed “honey moon cystitis” which is used to describe a high association between bacteriuria and sexual activities in young married women that can lead to unfavorable pregnancy outcomes and complications (Colgan *et al.*, 2006; Demilie *et al.*, 2012).

Significant bacteriuria was most prevalent among the women in their second and third trimester periods of their pregnancy. This might be due to the combination of mechanical, hormonal and physiological changes during pregnancy which make the ureters to dilate progressively till delivery period thereby exposing them to urinary tract infections (Delzell and Lefevre, 2000; Taher, *et al.*, 2009; Kawser *et al.*, 2011). This observation supports the findings of many researchers who reported higher prevalence of UTI in pregnancy during the second and third trimester of pregnancy (Al- Haddad, 2005; Okonko *et al.*, 2009; Parveen *et al.*, 2011). However, the findings of Turay *et al.* (2014) who reported a higher prevalence of bacteriuria during the first trimester period of pregnancy disagreed with our findings. This might be as a result of the difference in the personal hygiene conditions of the individuals in the study areas. Hence, the education of pregnant women on the importance of proper personal hygiene and starting antenatal clinic at the early stage of pregnancy is crucial to the avoidance of infections and complications in pregnancy.

Notable bacteria that are commonly implicated in urinary tract infections were recovered from the asymptomatic volunteers with the enterobacteriaceae being the commonest group of bacteria as it has been widely reported (Demilie *et al.*, 2012; Kerure *et al.*, 2013; Sujatha and Nawani, 2014). The relatively high detection of *Pseudomonas aeruginosa*, a widely encountered opportunistic bacterium suggests the possibility of poor personal hygiene among the pregnant women. Also, the high prevalence of haemolysin production (a virulence trait) observed among all the isolated bacteria suggests the possibility of appreciable proportion of these pregnant women developing symptomatic UTIs.

The antimicrobial susceptibility screening of these bacteria revealed their exhibition of very high resistance to the commonly used antibiotics and this has been widely reported in many developing countries (Biadglegne and Aber, 2009;

Anyadoh *et al.*, 2010; Rene *et al.*, 2012; Gyasi-Sarpong *et al.*, 2014). The moderately high resistance (39 – 58%) of these bacteria to gentamicin and ciprofloxacin, the antibiotics which were among the last resort in the treatment of multidrug resistant bacterial infections in this environment, calls for a serious health concern especially among these individuals in whom the drugs are contraindicated because of the drugs' serious side effects to the foetus. The reports on the increasing resistance of these organisms to these agents is however on the increase and this call for the need to search for more effective antimicrobial agents either as crude or pure herbal medicaments against multidrug resistant organisms (Demilie *et al.*, 2012; Gyasi-Sarpong *et al.*, 2014; Anyadoh-Nwadike *et al.*, 2015; Onanuga and Selekere, 2016). The observed high multiple antibiotic resistance (89 – 100%) in this study might probably be due to a very high proportion (72.3%) of phenotypically expressed ESBL enzymes among the screened Gram negative bacteria isolates since reports have shown that ESBL producing bacteria possess resistance determinants to many other important groups of antibiotics (Hyle *et al.*, 2005; Melzer and Petersen, 2007; Akinjogunla *et al.*, 2010; Onanuga and Selekere, 2016). Thus, the need for concerted efforts to control the spread of antibiotic resistance through education on rational use of antibiotics in both human and agricultural endeavours, and proper personal hygiene cannot be overemphasized.

In conclusion, the high prevalence of significant bacteriuria in this study necessitate the need to include routine screening for asymptomatic bacteriuria and treatment for UTIs as an essential part of antenatal care in pregnancy in order to prevent obstetric complications, whilst the high prevalence of multidrug resistance in the study show the need to constantly educate the pregnant women during their antenatal health talk on the practice of proper hand washing techniques and rational use of antibiotics as a lifestyle, as a means of controlling antibiotic resistance in our society.

Conflict of Interests: The authors declare no conflict of interests regarding the publication of this paper.

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