

Original Research Article

Multidrug resistance and biofilm formation in methicillin-resistant *Staphylococcus aureus* from pregnant women attending antenatal clinic with asymptomatic bacteriuria in Rivers State, Nigeria

Oluchi J Osuala^{1*}, Chisom R Obasi¹, Terhemen Kasso², Chukwuemeka C Chukwuma³, Stephen N Ezekwueche⁴, Victor E Ike⁴, Eucharia Ekwegbalu⁵, Charles O Nnadi^{6,7}, Angus N Oli^{1,8}, Malachy C Ugwu^{8,9}

¹Department of Pharmaceutical Microbiology and Biotechnology, Madonna University, Elele 511101, ²Department of Obstetrics and Gynaecology, University of Port Harcourt, Port Harcourt 500004, ³Department of Biochemistry, Madonna University, Nigeria, Elele 511101, Rivers State, Nigeria, ⁴Department of Microbiology, University of Agriculture and Environmental Sciences, Umuagwo 464115, Imo State, ⁵Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli, 431124 Anambra State, ⁶Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka 410001, Enugu State, ⁷Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Madonna University, Elele, 511101, Rivers State, ⁸Department of Pharmaceutical Microbiology and Biotechnology, ⁹TETFUND, Centre of Excellence for Biomedical, Engineering and Agricultural Translational Studies, Nnamdi Azikiwe University, Awka 420001, Anambra State, Nigeria

*For correspondence: **Email:** osualaoluchioo@gmail.com; **Tel:** +234-8065844112

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Abstract

Purpose: To determine the prevalence of asymptomatic bacteriuria (ASB), methicillin-resistant *Staphylococcus aureus* (MRSA), and biofilm-forming *S. aureus* among pregnant women seeking antenatal care services in Rivers State, Nigeria.

Methods: A cross-sectional study was carried out at three tertiary hospitals, namely, the University of Port Harcourt Teaching Hospital (UPTH), Rivers State University Teaching Hospital (RSUTH), and Madonna University Teaching Hospital (MUTH), spanning from January 2024 to March 2024. Midstream urine samples of 10mL were collected from 423 consenting pregnant women and analysed microbiologically for bacterial isolation. Bacterial isolates were identified using standard biochemical methods, and MRSA detection was performed via disk diffusion. Analysis, including antibiotic susceptibility and biofilm production, was also evaluated.

Results: A high prevalence of bacteriuria (75.29 %) was recorded, with RSUTH recording the highest occurrence (43.70 %). MRSA prevalence varied across facilities, with MUTH showing the highest methicillin resistance (68 %). All bacterial isolates exhibited multidrug resistance (MDR), particularly against cephalosporins and fluoroquinolones. Biofilm production was detected in 2.5, 13.6, and 20 % of isolates from UPTH, RSUTH, and MUTH, respectively.

Conclusion: There was a high prevalence of bacteriuria and MRSA across the health facilities in Rivers State. This is a critical public health concern, and calls for targeted antimicrobial stewardship programmes in antenatal care.

Keywords: Antenatal clinics, Asymptomatic bacteriuria, Biofilm formation, Maternal health, Methicillin-resistant *Staphylococcus aureus*, Multidrug resistance

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INTRODUCTION

Urinary tract infections (UTIs) are common bacterial infections that affect different segments of the urinary tract in both men and women. When bacteria are present and proliferate in the lower urinary tract without the typical symptoms of UTI, like burning during urination, urgency, frequency, fever, or abdominal pain, the condition is referred to as asymptomatic bacteriuria (ASB) [1]. During pregnancy, anatomical and physiological changes in the uterus, such as significant enlargement that exerts pressure on the bladder and surrounding tissues, often contribute to ASB [1-3]. These changes make pregnant women more susceptible to infections in the urogenital tract [3,4]. UTIs during pregnancy increase the risk of complications, including preterm labour, neonatal sepsis, intrauterine growth restriction (IUGR), and intrauterine fetal death or stillbirth [3,5]. ASB is particularly important in pregnancy. This is because it progresses to symptomatic UTI in about one-third of cases and this condition is associated with complications [6].

A single bacterial species accounts for a large proportion of UTIs in pregnant women. The Kass criteria, established in 1956, provide a quantitative method for diagnosing bacteriuria by estimating bacterial counts in urine cultures. These parameters will help in determining whether there was just contamination or true colonization. Urinary colonization will be established if there is a count of more than or equal to 10^5 colony-forming units/mL (CFU/mL) in the urine sample [7]. However, pregnancy brings about unique physiological and immunological effects that may predispose women to hospital-acquired infections such as methicillin-resistant *Staphylococcus aureus* (MRSA) [8]. *Escherichia coli* is the most frequently observed microorganism in ASB, although other commonly isolated organisms belong to the Enterobacteriaceae group. Some other microorganisms that have been observed are *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Staphylococcus aureus* [1,3,9]. One of the major pathogens that is observed throughout the world is Methicillin-resistant *Staphylococcus aureus*. This organism is common among colonizers and is found to cause diseases like bacteraemia, endocarditis, skin and soft tissue infection, osteomyelitis, and nosocomial infections [10]. MRSA strains demonstrate a strong ability to form biofilms (structured microbial communities that are encased in a self-produced extracellular matrix that enables bacteria such as MRSA to persist on surfaces and resist antimicrobial therapy [11],

which enhances their persistence in clinical environments and resistance to antimicrobial therapy [12]. The initial attachment of MRSA cells to the host tissues or medical equipment is facilitated by adhesins and extracellular matrix proteins, causing the formation of microcolonies. The maturation of the biofilm occurs through the synthesis of polysaccharides and eDNA, thus forming a protective matrix that shields the bacterial population from the host's immune system and antibiotics [13].

Pregnant women are particularly vulnerable to ASB and MRSA, both of which can lead to severe maternal and neonatal complications [8,14]. Even though there is widespread recognition of such risks, there is little information available regarding their occurrence, antibiotic resistance profiles, and biofilm-forming abilities among antenatal subjects in Rivers State, Nigeria. In the absence of such localized studies, efforts toward infection prevention and antibiotic management will be hampered [15]. Lack of such studies will mean that pregnant women remain vulnerable to infections like pyelonephritis, premature births, and infant sepsis. This study, therefore, seeks to fill this critical gap by investigating the prevalence and resistance characteristics of ASB and MRSA among pregnant women in tertiary hospitals in Rivers State.

EXPERIMENTAL

Study area

The South-South geopolitical zone of Nigeria is composed of six states, including Rivers State. It is bordered by the Atlantic Ocean to the south, Bayelsa and Delta States to the west, Imo, Abia, and Anambra States to the north, and Akwa Ibom State to the east. Port Harcourt serves as the state capital. It is located at 4°45' N latitude and 6°50' E longitude. It has 11,077 square kilometres of land area. 5,198,716 people were living there as of the 2006 census, and the Nigerian population commission (NPC) reported a population density of ~255 persons/km² [16]. In Nigeria, the state is home to 3.7 % of the total population. The complex system of rivers, creeks, and swamps that makes up the Niger Delta region defines the state.

Study population

The study includes pregnant women aged 15 - 49 years attending the antenatal clinic or admitted to the hospital. The calculated sample size of 352 (minimum sample size of 320 plus 10 % for non-response) was estimated using the

Cochran formula (Eq 1) for cross-sectional studies [15].

$$\text{Sample Size} = \frac{Z^2 pq}{e^2} \dots\dots\dots (1)$$

Where, Z = 95 % confidence level converted to Z value of 1.96, p = prevalence of 29.5 % or 0.295 [13], q = (1- P), e = degree of precision (margin of error) = 5 %. Therefore, sample size = 320; resulting in a final sample size of 320 ± 32. The participant sample number was distributed based on the patient load of the different hospitals as follows: University of Port Harcourt Teaching Hospital (UPTH) and Rivers State University Teaching Hospital (RSUTH; 120 patients each); Madonna University Teaching Hospital (MUTH; 112 patients).

Study design

The cross-sectional hospital-based study was carried out at the selected tertiary hospitals in Rivers State, Nigeria. Madonna University Teaching Hospital (MUTH), Rivers State University Teaching Hospital (RSUTH), and the University of Port Harcourt Teaching Hospital (UPTH) over the course of three months spanning from January 2024 to March 2024.

Sampling method

Participants were selected using the convenience sampling approach [17]. All pregnant women who agreed to participate and met the selection requirements were enlisted. To gather their clinical history and sociodemographic information, a standard questionnaire was employed.

Inclusion and exclusion criteria

Pregnant women between the ages of 15 and 49 who presented and gave their consent to participate in the sampling hospitals were included [15]. Pregnant women at risk, who needed welfare or care services, with a history of mental illness or serious illness, with renal disease, who refused to disclose information about their medication use, with missing demographics, and pregnant women receiving chronic antibiotic therapy (72 h of antibiotic therapy) who showed up at the study centres during the study were all excluded.

Instrument

The data utilised were collected using a self-developed questionnaire. The questionnaire was

chosen as the tool to obtain quantitative data since it is dependable for collecting social data. By distributing the questionnaire to the population under study, information relevant to the analysis was gathered. A categorical rating scale (ordinal scale) serves as the framework for the two portions of the structured questionnaire, A and B. The respondents' sociodemographic information was gathered through the questions in Section A, and their clinical history in Section B.

Validity of the instrument

In order to make sure that the tool collected valid information, it underwent a validation procedure. The developed survey form was reviewed by an experienced clinical pharmacist from the MUTH clinical pharmacy department in terms of content validity regarding the objectives of the study. Content validity assessment is a common practice in expert reviews when the judgment of domain experts about whether survey items reflect the construct being assessed were sought [18,19].

Reliability of questionnaire

A statistical internal consistency coefficient called Cronbach's Alpha is the test tool utilised in this instance to assess dependability [20]. It calculates how closely a group of questionnaire items measures the same thing. The coefficients of Cronbach's Alpha for the Antibiotic Use Scale, UTI Symptoms Scale, and Medical History Scale are 0.70, 0.76, and 0.70, respectively. From the findings, it is evident that the reliability of the questionnaire is high, indicating high internal consistency.

Ethical considerations

Ethical approval was given by the Research Ethics Committee of the various hospitals (UPT/ADM/90/S. II/VOLXI/1718; MAU/DRC/HD/E/2024/008; and NHREC/21/033/2022, MAU/DRC/HD/E/2024/002 for UPTH, MUTH, and RSUTH, respectively). Data were collected after obtaining informed, willing, and written consent from every study participant while maintaining the confidentiality of subjects' information.

Sample collection

The standard protocol was followed to aseptically take samples. Each patient received a sterile, dry, leak-proof, wide-mouthed container to collect 10–20 millilitres of urine midstream. After the sample was received at not later than 9 am, one

millilitre of it was seeded into a nutrient broth test tube and allowed to incubate for subsequent steps [21].

Bacteriological culture of clinical specimens

Sample examination for bacteriuria

The two broths containing the inoculated urine were thoroughly combined and diluted into sterile normal saline in a 1:100 ratio. A quantity of 0.1 mL of the diluted sample was then carefully transferred into plates of nutrient agar (L: S-BIOTECH, USA) that had been previously dried. At 37 °C, plates were incubated overnight. Agar plate colonies; colony-forming unit (CFU) were counted, and the average number of colonies was then noted. Any plate that had an enumeration of 10⁵ CFU/ml or higher was considered to have bacteriuria [22].

Isolation and identification of isolates

Samples were plated on appropriate selective and differential media, such as Mannitol Salt Agar (MSA; FLU MEDIA, Berkshire, UK), a selective medium for *Staphylococcus aureus* (*S. aureus*) that distinguishes *S. aureus* based on mannitol fermentation (yellow colony formation) [23]. Following incubation, plate cultures were observed, and typical colonies of *S. aureus* (e.g., yellow-coloured colonies on MSA) were subjected to additional tests to ascertain the presence of *S. aureus*, including Gram stain (*S. aureus* is Gram-positive), catalase test (*S. aureus* is catalase-positive), and coagulase test (*S. aureus* is coagulase-positive) [24].

Antimicrobial susceptibility testing

The antibiotic susceptibility of the *S. aureus* isolates was evaluated by the Kirby-Bauer method. The isolates were cultivated overnight in

a nutrient broth medium. The 0.5 McFarland standard was matched by the bacterial solution. On a Mueller-Hinton agar plate, the standardised bacterial suspension was evenly swabbed. The swabbed agar plate was covered with commercially available antibiotic discs (Celtect Diagnostics, Belgium; Table 1). The plates were incubated for 18 h at 35°C, and the zone of inhibition surrounding each antibiotic disc was measured and classified as sensitive, moderate, or resistant following the Clinical and Laboratory Standards Institute (CLSI) [25].

From the findings of the AST, the Multiple Antibiotic Resistance Index (MARI) was calculated to indicate the multidrug resistance potential of the isolates. The calculation formula is modified from Sandhu *et al* [26]. The Multiple Antibiotic Resistance (MAR) index for every isolate was computed using Eq 2.

$$\text{MAR} = \text{Nr/Tn} \dots\dots\dots (2)$$

Where Nr is the Number of antibiotics to which the isolate had resistance, while Tn is the Total number of antibiotics tested for resistance.

Resistance to methicillin

The Mueller-Hinton agar plates were seeded with the test organisms. The methicillin discs were placed on the agar surface, and the plates were incubated at 35 °C ± 2 °C for 24 h. After growth, the zone of inhibition surrounding the antibiotic disc was measured. Resistance was indicated by a narrow zone of inhibition (typically ≤ 10 mm) or none, showing that the organism was resistant to methicillin [27].

Biofilm detection

The procedure adopted a modification of the tube adherence test [28].

Table 1: Standard antibiotics profile

Antibiotics	Group of antibiotics	Breakpoints (S/I/R; mm)
AUG-Amoxicillin clavulanate (30 µg)	Penicillin and β-lactamase	≥20/15–19/≤14
CTX- Cefotaxime(25 µg)	Cephalosporin 3 rd generation	≥26/23–25/≤22
CRO-Ceftriaxone sulbactam (45 µg)	Cephalosporin	≥21/14–20/≤13
ZEM -Cefexime (5 µg)	Cephalosporin	≥19/16–18/≤15
LBC- Levofloxacin (5 µg)	Quinolone	≥17/14–16/≤13
CIP- Ciprofloxacin (5 µg)	Fluoroquinone	≥21/16–20/≤15
IMP- Imipenem/cilastatin (10/10 µg)	Carbapenem	≥23/20–22/≤19
CXM- Cefuroxime (30 µg)	Cephalosporin	≥18/15–17/≤14
OFX- Ofloxacin (30 µg)	Fluoroquinone	≥16/13–15/≤12
ER- Erythromycin (15 µg)	Macrolide	≥23/14–22/≤13
GN- Gentamycin (10 µg)	Aminoglycoside	≥15/13–14/≤12
AZN- Azithromycin (15 µg)	Macrolide	≥18/14–17/≤13

Mueller-Hinton (MH) medium was used, and test tubes were seeded with a 24 h bacterial culture. The test tubes were incubated at 37°C for 48 h. After incubation, the content was discarded, and the tubes were stained with 0.1 % safranin. They were washed thrice with sterile water and dried. A positive test was exhibited by a stain residue on the inside of the tube. Only a stained ring on the liquid-air interface indicated no biofilm formation.

Analysis of data

All the information gathered for this study was kept private and controlled. Participants' privacy was protected by removing or encrypting personal identifiers. Data was placed into a password-protected database that was regularly backed up to prevent data loss. Both descriptive and inferential statistics were used. To summarise the data, descriptive statistics such as percentages and frequencies were employed. By dividing the number of positive MRSA cultures by the total number of samples, the prevalence of MRSA in the study population was determined. The correlation in the demography and association between MRSA infection and various factors (e.g., age, stage of pregnancy, etc.) was established by appropriate statistical tests, such as the Chi-square test for categorical

variables. P -value < 0.05 was taken as statistically significant. One-way ANOVA was utilised in comparing Tables of GraphPad Prism 5.

RESULTS

Demographic and clinical characteristics of the respondents

The distribution of selected demographic, obstetric, and educational characteristics of the participants of three teaching hospitals: UPTH, RSUTH, and MUTH is presented in Table 2. Data collected included participants' age, gestation weeks, highest educational level, and number of pregnancies.

Prevalence of bacteriuria among pregnant women

The occurrence of bacteriuria conditions among pregnant women, focusing on critical parameters such as facility, age, gestational weeks, education level, and number of pregnancies are shown in Table 3. The results indicate how various issues affect the prevalence of bacteriuria in particular communities.

Table 2: Demographic and clinical characteristics of the participants

Parameter	Occurrence n (%)	X ² -Chi-square p-value
Facility		
UPTH	168 (39.71)	(X ²): 4.32 p-value: 0.1152
RSUTH	155 (36.64)	
MUTH	100 (23.64)	
Total	423(100 %)	
Age		
≤20 years	45 (10.63)	(X ²): 0.78 p: 0.0792
21-30	151 (35.69)	
31-40 years	138 (32.62)	
>40 years	89 (21.04)	
Total	423 (100 %)	
Gestation weeks		
≤12 weeks	57 (13.47)	(X ²): 2.21 p: 0.531
13-27 weeks	112 (26.47)	
28-40 weeks	189 (44.68)	
>40 weeks	65 (15.36)	
Total	423(100 %)	
Highest education level		
No Formal Education	143 (13.40)	(X ²): 1.99 p: 0.574
Basic School Level	39 (9.20)	
Secondary school level	57 (33.80)	
Tertiary Level	184 (43.49)	
Total	423 (100 %)	
Number of pregnancies		
1	148 (43.49)	(X ²): 3.67 p: 0.146
2-4	221 (52.24)	
≥5	54 (12.76)	
Total	423(100 %)	

In order to ascertain the level of significance, chi-square (χ^2) tests were used, and p-values were used to determine associations between the variables observed.

Prevalence of *S. aureus* isolates among pregnant women

The proportion of *S. aureus* isolates to the total number of samples collected from each of the facilities showed that 100 samples were collected from each site, with the following isolates: 40, 44, and 25 for UPTH, RSUTH, and Madonna University Teaching Hospital (MAUTH), respectively. There was no significant difference demonstrated from the result of analyses down the column using one-way ANOVA ($P=0.9761$).

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Antibiotic susceptibility of *S. aureus* isolates

The variability in the effectiveness of different antibiotics showed that some isolates exhibited high levels of resistance (such as imipenem with 100 % resistance), and others (such as ciprofloxacin and cefuroxime) showed less resistant isolates at UPTH (Figure 2A). The antibiotic susceptibility of *S. aureus* isolates from RSUTH indicates the disparity in effectiveness of different antibiotics, with ciprofloxacin (CIP) having relatively greater susceptibility (43 % susceptible isolates), compared with cefotaxime (CTX), while azithromycin (AZN) showed greater resistance (41 % and 23 % resistance, respectively; Figure 2B).

Table 3: Correlation between the demography and the bacteriuria condition

Parameter	Occurrence (%)	Bacteriuria (%)	X ² -Chi-square (p-value)
Facility			
UPTH	168 (39.71)	106 (33.33)	(X ²): 4.59 p-value: 1.574
RSUTH	155 (36.64)	139 (43.70)	
MUTH	100 (23.64)	73 (22.95)	
Total	423(100 %)	318(100 %)	
Age			
≤20 years	45 (10.63)	35 (11.00)	(X ²): 6.78 p-value: 0.574
21-30	151 (35.69)	119 (37.42)	
31-40 years	138 (32.62)	120 (37.73)	
>40 years	89 (21.04)	44 (13.83)	
Total	423(100 %)	318(100 %)	
Gestation weeks			
≤12 weeks	57(13.47)	44 (13.83)	(X ²):2.99 p-value: 0.574
13-27 weeks	112 (26.47)	97 (30.50)	
28-40 weeks	189 (44.68)	126 (39.62)	
>40 weeks	65 (15.36)	51 (16.03)	
Total	423(100 %)	318(100 %)	
Highest education level			
No Formal Education	143 (13.40)	122 (39.36)	(X ²):1.93 p-value: 0.574
Basic School Level	39 (9.20)	28 (8.80)	
Secondary school level	57 (33.80)	44 (13.83)	
Tertiary Level	184 (43.49)	124 (38.99)	
Total	423(100 %)	318(100 %)	
Number of pregnancies			
1	148 (43.49)	127 (39.93)	(X ²):3.67 p-value: 0.174
2-4	221 (52.24)	143 (44.96)	
≥5	54 (12.76)	48 (15.09)	
Total	423(100 %)	318(100 %)	

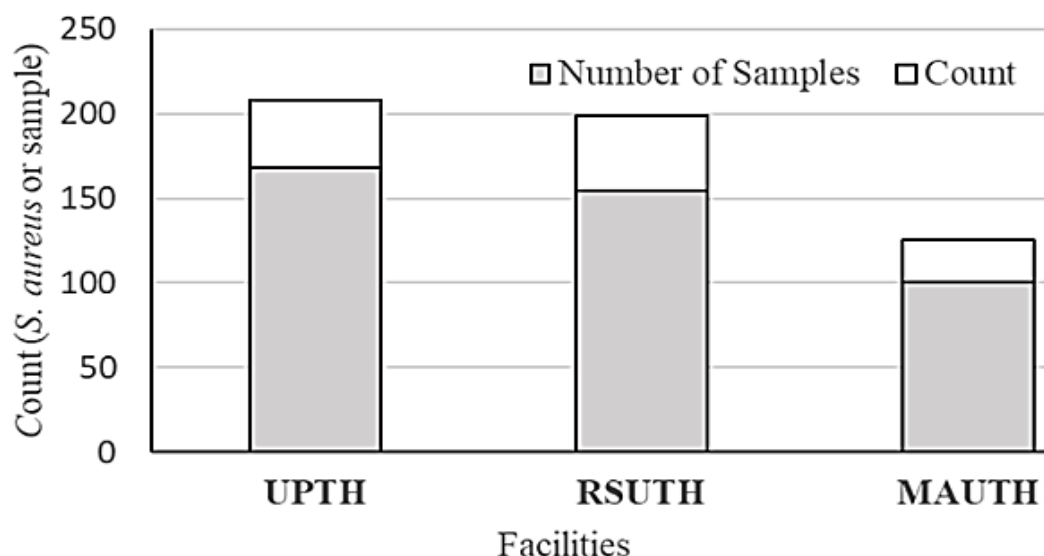


Figure 1: Distribution of *S. aureus* isolates

Table 4: Association between the isolates and demography

Parameter	<i>S. aureus</i> (Isolates)	X ² -Chi-square (df); <i>p</i> -value
Facility		
UPTH	40	8.28(2)0.0159
RSUTH	44	
MUTH	25	
Total	109	
Age		
≤20 years	25	28.90(3) < 0.0001
21-30	44	
31-40 years	30	
>40 years	10	
Gestation weeks		
≤12 weeks	28	3.07(3)0.3809
13-27 weeks	26	
28-40 weeks	33	
>40 weeks	22	
Highest education level		
No Formal Education	18	6.20(3)0.1027
Basic School Level	28	
Secondary school level	33	
Tertiary Level	30	
Number of pregnancies		
1	34	4.96(2)0.0840
2–4	40	
≥5	35	

df- Degree of freedom

Of the *S. aureus* isolates collected from the MUTH, a total of 25 isolates were tested against several antibiotics, whose responses were categorised as susceptible, intermediate, or resistant (Figure 2C). The susceptibility difference to the antibiotics showed that the isolates are more susceptible to antibiotics such as levofloxacin (LBC) and ciprofloxacin (CIP), and more resistant to amoxicillin-clavulanic acid (AUG) and cefotaxime (CTX).

Methicillin-resistant, biofilm-producing *S. aureus*

The results of the resistance patterns of the *S. aureus* to methicillin show that all the isolates from the three facilities were multidrug resistant (MDR), with the MAR index varying between 0.35 and 1.00. The methicillin-resistant, biofilm-producing *S. aureus* (MRSA) isolates identified in the three healthcare facilities are shown in Table 5.

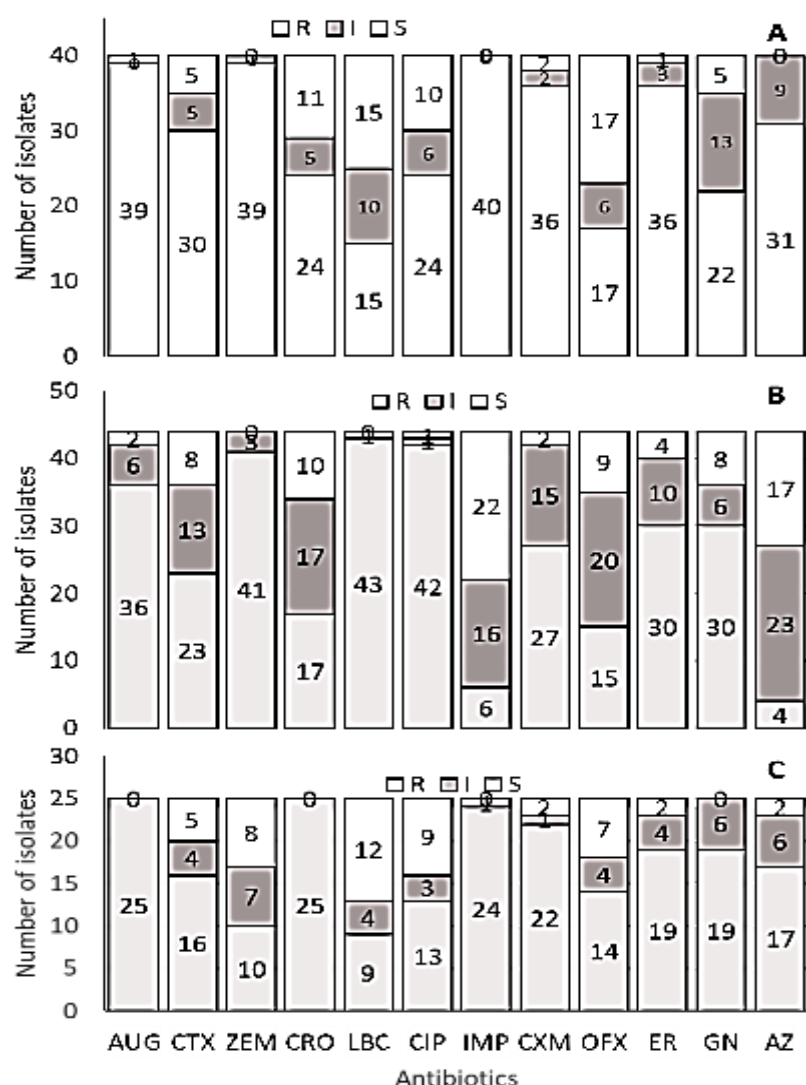


Figure 2: Antibiotic Susceptibility of isolates from UPTH (A), RSUTH (B), and MUTH (C); Intermediate (I), Resistant (R), and Susceptible (S). amoxicillin clavulanate (AUG), ceftriaxone (CTX), Imipenem/cilastin (IMP), gentamicin (GN), ofloxacin (OFX), cefuroxime (CXM), ceftriaxone sulbactam (CRO), cefixime (ZEM), ciprofloxacin (CIP), erythromycin (ER), azithromycin (AZ), levofloxacin (LBC)

Table 5: Methicillin-resistant, biofilm-producing, and the combinations

Facility	Number of isolates	METR n (%)	Biofilm producers n (%)	MDR+METR n (%)	MDR+biofilm n (%)	METR+ biofilm n (%)
UPTH	40	24 (60)	1 (2.5)	23(57.5)	13(32.5)	3(7.5)
RSUTH	44	12 (27.2)	6 (13)	12(27.3)	37(84.1)	3 (6.8)
MUTH	25	17 (68)	5 (20)	17(68.5)	13 (52)	0 (0)

MDR+METR = multi-drug resistance + methicillin-resistant; MDR + biofilm = multi-drug resistance + biofilm-producer; METR + biofilm = Methicillin-resistant + biofilm producer

There was no significant difference reported from the result of analyses by one-way ANOVA ($p = 0.6508$).

DISCUSSION

This study demonstrates a markedly high burden of bacteriuria among pregnant women across three tertiary hospitals, with RSUTH showing the

highest prevalence (43.7 %), followed by UPTH (33.33 %) and MUTH (22.95 %). These inter-site differences followed the pattern reported by Onemu and coworkers and pointed to local epidemiologic, diagnostic, and health-system factors that shape infection rates [22]. The overall prevalence of 75.29 % is significantly higher compared to previous findings in Jos (10.3 %), Calabar (9.1 %), and Abuja (29.5 %) [1-3],

implying either an actual rise in the prevalence of bacteriuria among pregnant females or variability in study populations, methodology, and criteria used in the laboratories. The physiological and hormonal changes during pregnancy, favoring retention of urine in the bladder, sugar in the urine and altered immunity may contribute to the increased incidence of UTI in the current investigation.

Age stratification found that the highest prevalence of bacteriuria was among females in the age range 21 – 30 (37.42 %) and 31 – 40 (37.73 %), which agrees with the findings discussed by Onemu *et al*, who noted an increase in risk during reproductive ages [22]. There was an increasing trend in prevalence with increasing gestational ages, with the peak occurring between 28 and 40 weeks of gestation in the third trimester of pregnancy, agreeing with trimester-based risk factors mentioned earlier by Onemu *et al* [22]. The risk was increased by sociodemographic and obstetric factors, such as lack of education and an unexpectedly increased risk in tertiary education, parity in the range of two to four.

The results from microbiological tests indicated that *S. aureus* was a frequent pathogen isolated in all study locations, especially RSUTH (44 %) and UPTH (40 %). This finding is consistent with the carriage levels reported [4]. The rate of occurrence is higher than the low rate of *S. aureus* bacteriuria reported by Bale *et al* [5]. The frequency rate of MRSA was very high in MUTH (68 %), UPTH (60 %), and RSUTH (27.2 %). This is much higher than the 12.5 % rate reported by Bale *et al* [5] and similar to the Sokoto study findings [6]. The antimicrobial susceptibility profiles were quite disturbing. UPTH isolates displayed complete resistance to imipenem, ceftazidime, and amoxicillin clavulanate, whereas MUTH isolates displayed complete resistance to amoxicillin clavulanate, ceftriaxone, and imipenem. RSUTH isolates displayed intermediate sensitivity to imipenem and azithromycin but were highly resistant to fluoroquinolones. This trend is in accordance with the national and international findings regarding the development of resistance towards cephalosporins and fluoroquinolones [7]. This trend is also a consequence of improper use of antibiotics in healthcare settings [8].

All the isolates were multidrug-resistant, surpassing the 70 % found earlier [4]. The ability to form biofilms was also site-specific: UPTH (2.5 %), RSUTH (13.6 %), MUTH (20 %). The rates are much lower than the very high prevalence seen in Oche *et al* [9]; however, the presence of

MRSA, MDR, and biofilm producers, especially the 84 % MDR and biofilm-producing strains at RSUTH, denotes a very pathogenic and resistant bacterium. This study highlights the pressing need for enhanced surveillance, infection control efforts, and antibiotic stewardship specific to the local resistance profile. Antenatal screening, customized treatment protocols, enhanced laboratory facilities, and population-based measures targeting socio-economic factors are crucial steps toward lowering UTIs in both mothers and newborns. Nevertheless, the use of molecular techniques and surveillance systems for understanding transmission patterns merits consideration.

Limitations of the study

There are several limitations associated with this study that need to be highlighted. Firstly, due to the cross-sectional design, it was not possible to determine the causality between bacteriuria, MRSA occurrence and maternal/neonatal health. A longitudinal study would have indicated the progression from bacteriuria to symptoms of infection. Secondly, since a convenience sample was used, there was a risk of selection bias because only women who agreed and were available during that period participated in the study. This may affect the generalization of the results to all antenatal women in Rivers State. Thirdly, due to the lack of molecular typing techniques, it was difficult to understand the diversity and virulence factors related to the bacteria, along with the transmission patterns. Fourthly, antibiotic resistance patterns were tested based on a particular range of antibiotics and not all newer or local antibiotics. Fifthly, the detection of biofilms was based on the modified tube adhesion method, but this test is less sensitive when compared to others.

CONCLUSION

The study shows that there is a high rate of asymptomatic bacteriuria, methicillin-resistant, and biofilm-producing *S. aureus* in Rivers State, especially during the second and third trimesters of pregnancy, as well as among multiparous and illiterate women. All the isolates of MRSA strains were multidrug resistant, which presents challenges in treatment from the point of public health. The resistance of these antimicrobials to carbapenems and cephalosporins is a call for the appropriate utilization of antimicrobials and regular susceptibility testing. Therefore, stricter infection control, frequent screening for bacteriuria and MRSA, and public awareness are necessary.

DECLARATIONS

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Ethical approval

Ethical approval was given by the Research Ethics Committee of the various hospitals (UPT/ADM/90/S. II/VOLXI/1718; MAU/DRC/HD/E/2024/008; and NHREC/21/033/2022, MAU/DRC/HD/E/2024/002). Data were collected after obtaining informed, willing, and written consent from every study participant. Confidentiality of subjects' information was ensured.

Availability of data and materials

We indicate that the datasets used and analysed during the study are available from the corresponding author.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Use of research reporting tools

Grammarly was used for English language editing.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Conceptualization: OJO, Methodology: OJO, Investigation: OJO, CRO, Writing – Original Draft: OJO, CRO, Writing – Review & Editing: TK, CCC, SNE, VEI, EE, CON, ANO, MCU, Supervision: TK, ANO, MCU, OJO. All authors approved the final version of the manuscript.

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