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Prevalence and Antibiotic Resistance Patterns of Multidrug-Resistant Bacteriuria among Asymptomatic Pregnant Women in Awka, Nigeria

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Abstract

Multi-drug resistant (MDR) bacteria represent a significant public health threat, especially among vulnerable groups such as children and pregnant women. This study aimed to determine the prevalence of MDR Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, and Proteus mirabilis in asymptomatic pregnant women, as these organisms are known to cause urinary tract infections. A total of 100 participants were involved in the study, from whom midstream clean-catch urine samples were collected during their antenatal visit. Bacterial isolates were identified macroscopically and microscopically, and antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method. Results indicated a 33% *S. aureus* positive sample and 2% *S. epidermidis*. Among Gram-negative isolates, E. coli was the most prevalent (36%), and none of the urine samples was infected with *P. mirabilis*. Alarmingly, all isolates exhibited resistance to multiple antibiotic classes, with high rates of resistance to commonly used antibiotics, including amoxicillin-clavulanate, imipenem-cilastatin, cefuroxime, and cefotaxime. This study underscores the urgent need for enhanced antimicrobial stewardship and routine screening for asymptomatic bacteriuria (ASB) in pregnant women to reduce the risk of antibiotic-resistant infections.

Keywords: Multidrug resistance, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, pregnancy, asymptomatic bacteriuria

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Introduction

Asymptomatic bacteriuria (ASB) is defined basically as the presence of bacteria in urine without clinical symptoms. This has been reported to be common in 2.5 -10% of pregnant women (Ayoyi *et al.,* 2017; Schneeberger *et al.,* 2014). Untreated ASB, often overlooked, can progress to pyelonephritis and increase the risk of preterm delivery, low birth weight, and maternal sepsis (Bello, 2023). Globally, there has been a reported rise in the threat of antibiotic resistance, driven by inappropriate antibiotic use, self-medication, substandard products, and limited access to diagnostic testing (WHO, 2022). Pregnant women represent a particularly vulnerable group due to physiological changes that alter immune function, thus predisposing them to urinary tract infections (UTIs).

In pregnancy, urinary tract infections account for the majority of bacterial infections reported and have been linked to cases of preeclampsia, low birth weight, intrauterine growth restriction, and premature delivery, among other issues (Mazor-Dray *et al*, 2009). Early treatment of infection reduces the likelihood of complications, but in low-resource settings such as Nigeria, empirical antibiotic use is widespread, often with little regard to antimicrobial susceptibility profiles (Nicolle, 2016).

The most common pathogen implicated in UTIs is *Escherichia coli*, accounting for 60-80% of cases in pregnancy (Azami *et al*., 2019; Belete & Saravanan, 2020). Other pathogens include *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas*

aeruginosa, *Enterococcus spp.*, *Enterobacter spp.*, *Bacillus spp.*, and others (Belete & Saravanan, 2020). Antimicrobial resistance (AMR) has been increasing, especially in underdeveloped countries, and poses a significant concern, particularly when organisms are resistant to multiple antibiotics, including third-generation cephalosporins, carbapenems, and 2nd-, 3rd-, and 4th-generation fluoroquinolones.

Given the limited interest in antimicrobial susceptibility testing in some parts of Nigeria and the rising prevalence of self-medication, there is a need to study patients' resistance profiles to guide treatment and support national antimicrobial stewardship goals.

Materials and Methods

Study design and area

This study employed a cross-sectional descriptive design to examine the prevalence and antimicrobial resistance profiles of selected uropathogens isolated from pregnant women. The study was conducted at two major healthcare facilities in Awka, the capital of Anambra State: Regina Caeli Specialist Hospital and Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH). The hospital was chosen because it serves a large and diverse population of pregnant women across urban, semi-urban, and, in some cases, rural communities.

All microbiological analyses were conducted in the Department of Pharmaceutical Microbiology and Biotechnology Laboratory at Nnamdi Azikiwe University, Awka, which is equipped for microbial culture, characterisation, and antibiotic susceptibility testing.

Sample size determination

The minimum required sample size was estimated using the formula described by Naing *et al.* (2006) for prevalence studies, with 95% confidence and an expected prevalence of 5%, based on previous studies. A 25% attrition rate was accounted for to cover unusable or contaminated samples; we ended up with an adjusted sample of 100 participants.

Inclusion and exclusion criteria

Eligible participants were pregnant women in any trimester who attended antenatal clinics at the selected study sites. Only women who were clinically asymptomatic for urinary tract infection, had not received any form of antibiotic treatment within the two weeks preceding recruitment, and who provided written informed consent were enrolled. Exclusion applied to women presenting with clinical features suggestive of urinary tract infection, such as dysuria, urinary frequency, or fever, those currently receiving antibiotic therapy, and individuals who declined participation or later withdrew consent.

Sample collection

From each participant, midstream clean-catch urine samples were collected to minimize contamination from periurethral flora. Before sample collection, participants were instructed in proper urine collection techniques, including cleaning the periurethral region with sterile wipes, discarding the initial stream of urine, and collecting approximately 10-20 ml in a sterile, wide-mouthed, leak-proof universal container. Samples were labelled to protect patients' confidentiality and transported to the microbiology laboratory within 2 hours of collection in a cold box to preserve microbial integrity. Samples that did not meet quality requirements were excluded from analysis.

Laboratory analysis

The urine samples were inoculated onto Mannitol Salt Agar (MSA) to isolate Staphylococcus spp. and onto MacConkey Agar to isolate and identify Gram-negative bacilli, such as *E. coli* and *Proteus mirabilis.* Following inoculation, the colonial morphology of each isolate was observed, and the isolates were then subjected to preliminary microscopic tests, such as Gram staining, for differentiation. Isolate identification was further confirmed using biochemical tests, as described by Cheesbrough (2006).

Antibiotic susceptibility testing

Using the Kirby-Bauer disk diffusion assay, antimicrobial susceptibility testing was carried out on Mueller-Hinton agar. Antibiotics commonly used in the clinical management of urinary tract infections, as identified in our brief review with healthcare providers, were included in the testing. The antibiotics include: cefotaxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), and imipenem-cilastatin (10 µg). The inoculum was then standardised to a 0.5 McFarland standard before the commencement of the analysis, after which the plates were incubated at 37 °C for 18 to 24 hours, and the inhibition zone diameter was measured. Results were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2016) performance standards. An isolate was classified as multidrug-resistant if it showed resistance to at least one of the tested antibiotics.

Data analysis

All data were entered into IBM SPSS version 23.0 for statistical analysis. To present the prevalence rates and antibiotic profiles, descriptive statistics, specifically frequencies and percentages, were used. Associations between bacterial isolates and maternal demographic variables were analysed using the Chi-square test, with statistical significance set at <0.05.

Results

The study analysed 100 urine samples, and *E. coli* was the most frequently isolated organism (36% of total isolates), confirming its dominance as the primary uropathogen (Fig. 1). This were followed by *S. aureus* (31%) and *S. epidermidis* (2%), which were rarely isolated, consistent with their opportunistic nature, while *P. mirabilis* was absent. These findings highlight *E. coli* as the primary pathogen and underscore the clinical significance of *S. aureus* in community-acquired urinary infections.

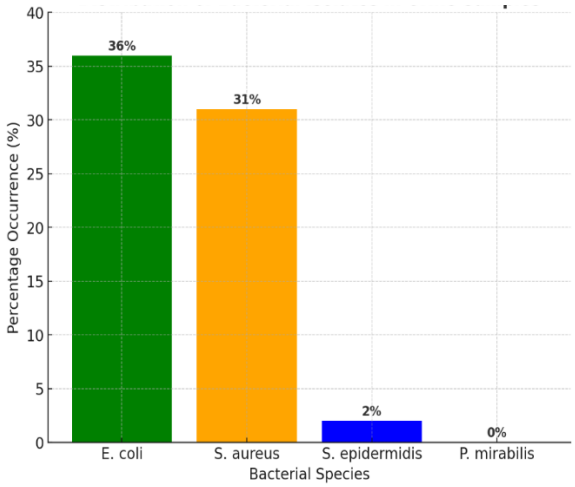

Figure 1: Presence and prevalence of the isolates

Multiple Antibiotic Resistance (MAR) Index

All three isolates were multidrug-resistant, resistant to at least three antibiotic classes and are a cause for concern, suggesting limited treatment options for UTIs amongst the study population. Calculated MAR values ranging from 0.833 to 1.000 for the majority of isolates emphasise their resistance burden. Krumperman's interpretation of MAR index values states that values above 0.2 indicate a high exposure to environments with frequent or indiscriminate antibiotic use. The study results are consistent with Krumperman's interpretation of MAR, supporting the unregulated sale and misuse of antibiotics in the study area. Figure 2 illustrates the distribution of MAR indices among isolates, with clustering at the upper end indicating widespread, near-complete resistance.

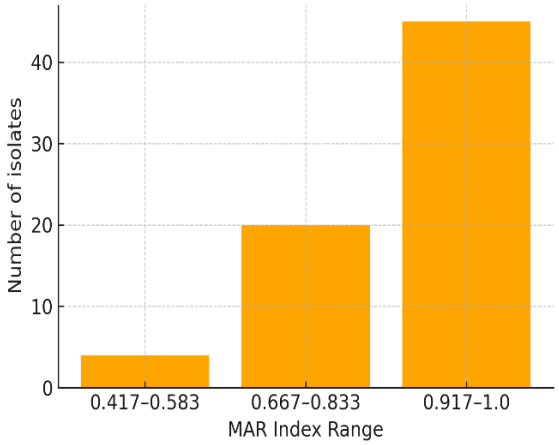

Figure 2: MAR Index distribution of isolates

Demographic Associations

Prevalence, as reported in Figure 3, was highest among women aged 25-29 years, aligning with the peak reproductive age group, suggesting a high vulnerability to infections amongst this population, possibly due to their physiological changes associated with frequent or successive pregnancies. Conversely, the lowest prevalence was observed among women aged ≥ 40 years, which could reflect smaller sample sizes in older age groups or reduced sexual activity that might lower exposure risk.

Another significant factor in the study group's gestational age prevalence. Bacteriuria in the study was predominantly higher amongst participants in their second trimester. This observation is biologically plausible, consistent with physiological dilation of the urinary tract and decreased bladder tone, which likely lead to urinary stasis and predispose to bacterial colonisation.

Interestingly, no significant association was observed between bacteriuria prevalence and maternal education level or occupation, suggesting that the high risk of bacteriuria is attributable to biological and physiological factors of pregnancy.

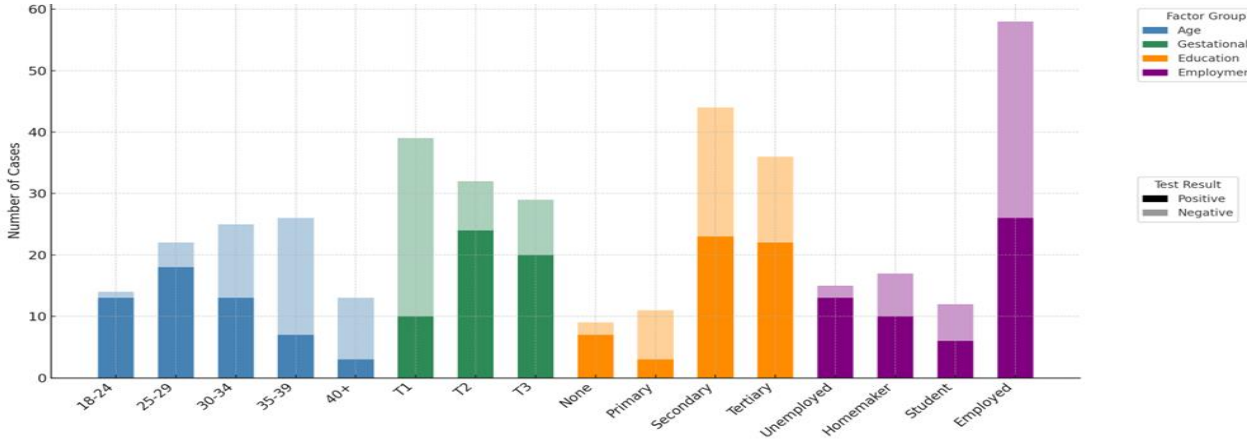

Figure 3: Distribution of Bacteriuria by Demographic Factors

Antibiotic Susceptibility Profiles

The antibiogram of the isolates revealed a concerning pattern of resistance across the pathogens. Staphylococcus aureus showed limited sensitivity, with only 41.9% of isolates susceptible to gentamicin and 38.7% to levofloxacin, as shown in Figure. 4. High resistance was also observed among commonly used antibiotics, including cefotaxime, imipenem-cilastatin, and amoxicillin-clavulanate. Of particular concern is the decreased effectiveness of imipenem, often considered a last-line therapy. *Escherichia coli* as a whole showed near-complete resistance to the tested antibiotics, further indicating a narrowing therapeutic window for the management of UTIs in pregnancy.

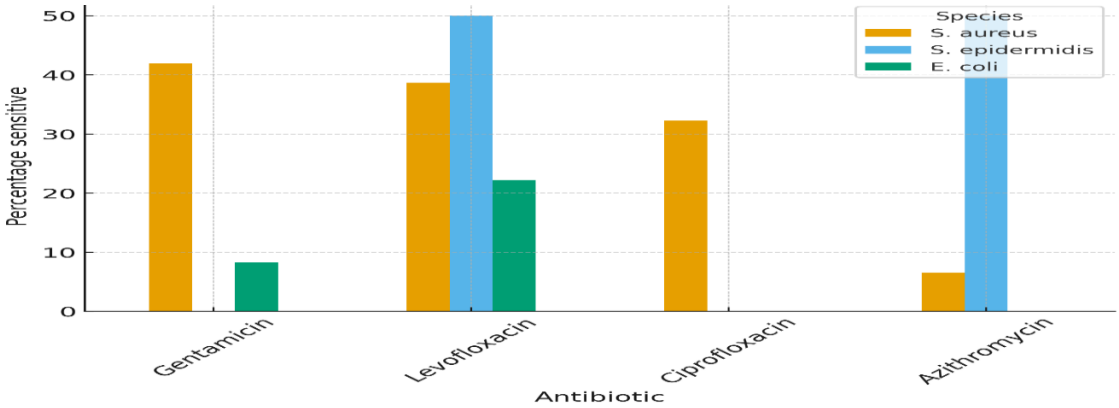

Figure 4: Antibiotic susceptibility of isolates (%)

Discussion

The study provides crucial insights into the burden and resistance of urinary pathogens among asymptomatic pregnant women attending antenatal clinics at two major centers in Awka, Nigeria. Following laboratory analysis, a 100% MDR rate was observed among the isolates. This exceeds levels reported in Nigeria; studies by Okoani et al. (2024) and Bello et al. (2023) in Enugu and Ilorin, respectively, documented a high prevalence of MDR E. coli and *S. aureus* among pregnant women, but did not report complete resistance. This indicates insufficient antimicrobial surveillance and underscores the need to strengthen regulation of antibiotic use and misuse. Studies from Saudi Arabia (Ghady & Shahrani, 2024) reported aminoglycoside and fluoroquinolone activity against MDR pathogens, similar to those observed in the present study.

Similar observations noted in this study regarding isolates' susceptibility to imipenem and β-lactam/β-lactamase inhibitor combinations have also been reported in research from Kano and Yola on community-acquired infections (Durowaiye et al., 2023; Sale & Kanu, 2024), underscoring the widespread presence of antimicrobial resistance. This study also documented the absence of Proteus mirabilis. While this is good, it contrasts with reports from northern Nigeria, which may reflect regional variations in microbial ecology and differences in environmental and host factors. Demographic data revealed that the pregnancy trimester was a significant determinant of bacteriuria, with the highest prevalence recorded in women within their second trimester. Studies such as that by Onanuga *et al.* (2018) are in agreement with the study. The absence of a significant association between bacteriuria and maternal education or occupation suggests that socioeconomic variables do not meaningfully contribute to the presence of bacteriuria.

Poor antibiotic surveillance at the study site is evident, as indicated by high MAR indices (≥0.833) for most isolates. These findings underscore the need for improved antibiotic stewardship programmes that educate the public about the risks of indiscriminate antibiotic use. The results of this study highlight a public health crisis of growing antimicrobial resistance in the study area and across the country, with direct implications for maternal and neonatal health.

Conclusion

This study revealed a significant health burden of MDR E. coli and *S. aureus* among asymptomatic pregnant women in Awka, with all isolates resistant to more than 3 antibiotic classes and a high MAR index. It further confirms *E. coli* as the predominant uropathogen. Overall, the study highlights the need for routine screening and improved antibiotic stewardship and education to reduce the burden of resistant urinary infections.

Conflict of Interests

The authors declare no conflict of interest.

Funding

This research did not receive specific funding.

Ethical Approval

Ethical approval was obtained from the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Ethics Committee (Ref: COOUTH/HREC/ETH.C/VOL.1/FN.04/358). Written or oral informed consent was obtained from all participants.

Authors Contributions

ECK, Concept and study design; EJN and NMU, data collection; ABO, data analysis; AEC, drafting and editing of manuscript, and ECE, data collection and manuscript draft. All authors approved the final manuscript.

Availability of Data and Materials

Data is available upon reasonable request.

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