

MULTI-DRUG RESISTANCE PROFILE OF BACTERIA ISOLATES FROM URINARY TRACT INFECTIONS AMONGST HOSPITALIZED FEMALE PATIENTS IN BENIN CITY, EDO STATE

ABSTRACT

Background: Urinary tract infections (UTIs) pose a significant public health burden, particularly among hospitalized female patients. Multidrug resistance (MDR) among UTI pathogens complicates treatment, necessitating continuous surveillance.

Objectives: This study aimed to isolate and phenotypically identify MDR bacterial isolates from UTIs in female patients at Edo Specialist Hospital, Benin City, Edo State, Nigeria.

Materials and Methods: Urine samples were collected and analyzed at the Medical Microbiology Laboratory, Benson Idahosa University. Bacterial isolation involved culturing on MacConkey and CLED agar, followed by Gram staining and biochemical tests. Antibiotic susceptibility was determined using the Kirby-Bauer disc diffusion method.

Results: The prevalence of bacterial infection in urine of participants was 60% in this study. *Escherichia coli* (50%), *Staphylococcus aureus* (43.3%), and *Pseudomonas aeruginosa* (6.7%) were isolated from samples. High resistance was observed against Ciprofloxacin, Cefuroxime, and Oxacillin, with sensitivity rates of 46.7%, 38.5%, and 13.3%, respectively. *P. aeruginosa* exhibited notable resistance to Oxacillin and Amoxicillin-clavulanic acid. Imipenem was the most effective antibiotic, with sensitivity rates of 73.3% (*E. coli*), 92.3% (*S. aureus*), and 100% (*P. aeruginosa*). Resistance mechanisms included extended-spectrum beta-lactamase (ESBL) production in *E. coli* and methicillin resistance in *S. aureus* (mediated by the *mecA* gene).

Conclusions: The findings highlight the urgent need for robust antibiotic stewardship programs, continuous surveillance, and the development of novel antibiotics to combat MDR UTI pathogens. Tailored treatment strategies based on local resistance patterns are essential for effective management and improved patient outcomes.

Keywords: Antibiotic, Bacteria, Multidrug resistance, Urinary tract infection, Women.

INTRODUCTION

Urinary tract infections (UTIs) represent a significant burden on public health globally, with varying prevalence rates across different regions and populations. Understanding the prevalence of UTIs in specific geographical areas is crucial for effective management and prevention strategies. UTIs are among the most common bacterial infections encountered in both healthcare and community settings, affecting individuals of all ages and genders, and are associated with increased treatment costs, morbidity, and mortality (Donkor *et al.*, 2012; Bader *et al.*, 2020; Mancuso *et al.*, 2023). The prevalence of UTIs varies depending on factors such as age, gender, socio-economic status, and geographical location. In Nigeria, several studies have been conducted to ascertain the prevalence of UTIs and identify associated risk factors.

A cross-sectional analysis in Calabar by Bassey *et al.* (2023) involving 227 patients with symptoms suggestive of UTIs reported a prevalence rate of 28.6%, with *Klebsiella pneumoniae* (23.1%) been the most predominant pathogen, followed by Coagulase-negative Staphylococci (16.9%) and *Escherichia coli* (12.3%). Another study by Jamiu *et al.* (2021) investigated the prevalence of UTIs among pregnant women attending antenatal clinics in Ibadan, reporting a prevalence rate of 8.7% and highlighting their vulnerability. Other similar studies also noted varying prevalence across the globe (Johnson *et al.*, 2021; Gebretensaie *et al.*, 2023). Several risk factors contribute to the prevalence of UTIs in Benin City, including poor hygiene practices, inadequate sanitation facilities, limited access to healthcare services, female gender, pregnancy, diabetes mellitus, and urinary catheterization (Ezugwu *et al.*, 2021).

Urinary tract infections (UTIs) are prevalent among both genders; however, there is a lack of documented research focusing specifically on the multidrug resistance (MDR) profile of

bacterial isolates from UTIs among hospitalized female patients in Edo Specialist Hospital, Benin City, Edo State, Nigeria. The clinical consequences of MDR in UTIs are substantial, leading to prolonged hospital stays, increased healthcare costs, and higher rates of treatment failure and recurrence. Patients infected with MDR uropathogens face a greater risk of developing complicated UTIs, necessitating more aggressive treatment regimens and potentially invasive interventions (Storme *et al.*, 2019). Understanding the prevalence of UTIs among hospitalized females in Benin City is crucial for monitoring antibiotic resistance patterns, as the overuse and misuse of antibiotics contribute to resistance, making infections more difficult to treat. Surveillance of prevalence rates can aid clinicians in prescribing appropriate antibiotics, thereby helping to combat antibiotic resistance. This study aims to isolate and employ phenotypic methods in identifying MDR bacterial isolates from UTIs among female patients in Edo Specialist Hospital, Benin City, Edo State. The specific objectives are to isolate and phenotypically identify bacterial isolates from UTIs among female patients, determine the prevalence of UTIs among hospitalized female patients, assess the antibiogram of bacterial isolates, and identify multidrug resistance in these isolates.

MATERIALS AND METHODS

Design of Study

This study employed a cross-sectional design that involved the collection and analysis of urine samples from hospitalized female patients at Edo Specialist Hospital, Benin City, Edo State. Ethical approval was obtained from the ethics committee of Edo Specialist Hospital (ESH) HA/737/24/D/0708308 before the commencement of this study. Informed consent was obtained from all participants before sample collection.

Study Location, Duration, and Patient Selection

The study was conducted at Edo Specialist Hospital (ESH), Benin City, Edo State, between November 2023 and June 2024. Urine samples were obtained from 50 hospitalized female

patients aged 18 years and above from the general surgery, obstetrics/gynaecology, and orthopedic wards.

Inclusion Criteria

- Only urine samples from hospitalized female patients were collected.

Exclusion Criteria

- Urine samples from hospitalized male patients were excluded.
- Non-hospitalized female patients were not included in the study.

Specimens

Urine samples were collected from 50 hospitalized female patients at Edo Specialist Hospital. The collected samples were transported immediately to the Medical Laboratory Department of Microbiology unit of Benson Idahosa University for processing under aseptic conditions.

Processing of Samples

Urine samples were inoculated onto MacConkey and Cystine Lactose Electrolyte Deficient (CLED) agar and incubated at 37°C for 24 hours. Bacterial isolates were identified phenotypically following standard microbiological procedures (Cheesbrough, 2005). Following incubation, a smear was prepared from the cultured samples, stained using the Gram staining technique, and examined under a light microscope (X100 objective) to determine bacterial morphology and Gram reaction.

Isolation and Identification of Bacteria

Colonial appearances on MacConkey and CLED agar were used for preliminary identification. Morphological characteristics such as size, form, elevation, opacity, odour, and edge were noted. Gram staining was performed to categorize isolates into Gram-positive and Gram-negative bacteria. Pure cultures of bacterial isolates were obtained by subculturing colonies onto nutrient agar and incubating at 37°C for 24 hours. Biochemical tests, including citrate

utilization, urease production, indole production, oxidase test, motility test, and sugar fermentation tests, were conducted for identification (Cowan and Steel, 1974).

Biochemical Tests

- **Catalase Test:** A loopful of bacterial isolate was placed on a glass slide, and 3% hydrogen peroxide was added. The presence of bubbling indicated a positive catalase test.
- **Coagulase Test:** A 0.5 ml aliquot of rabbit plasma was prepared in a test tube. A few colonies of the isolate were inoculated into the plasma and incubated at 37°C for up to 24 hours. Clot formation indicated a positive coagulase test.
- **Oxidase Test:** A strip of Whatman No.1 filter paper was soaked with 1% tetramethyl-p-phenylenediamine-dihydrochloride, and bacterial colonies were streaked onto it. A blue-purple colour change within a few seconds indicated a positive test.
- **Indole Test:** Isolates were inoculated into peptone water and incubated at 37°C for 24 hours. After incubation, 0.5 ml of Kovac's reagent was added. A red ring at the surface indicated a positive test, while a yellow colour indicated a negative test.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2020). The test isolates were inoculated onto Mueller-Hinton agar and incubated at 37°C for 24 hours. The antimicrobial disc used included Ceftriaxone (CRX 5ug), Imipenem (Imp, 30µg), Oxacillin (OX 5µg), Ciprofloxacin (CPR, 5µg), Erythromycin (ERY, 10ug), Cefuroxime (CRX, 30ug), Cefepime (Cro, 5ug), and Augmentin (Aug, 30ug). The inhibition zones were measured in millimetres using a ruler, and the results were interpreted according to CLSI guidelines (CLSI, 2020). Isolates resistant to at least three classes of antibiotics were classified as multidrug-resistant (MDR).

RESULTS

Out of the fifty (50) urine samples cultured from hospitalized female participants, thirty had growth of bacteria of different genera which gave a total prevalence rate of 60% (Figure 1). Bacteria isolates recovered from the urine sampled were *Escherichia coli* (50 %), *Staphylococcus aureus* (43.3%) and *Pseudomonas aeruginosa* (6.7%) (Table 1). Table 2 summarizes the Gram stain characteristics, biochemical reactions, and motility of the bacteria isolated.

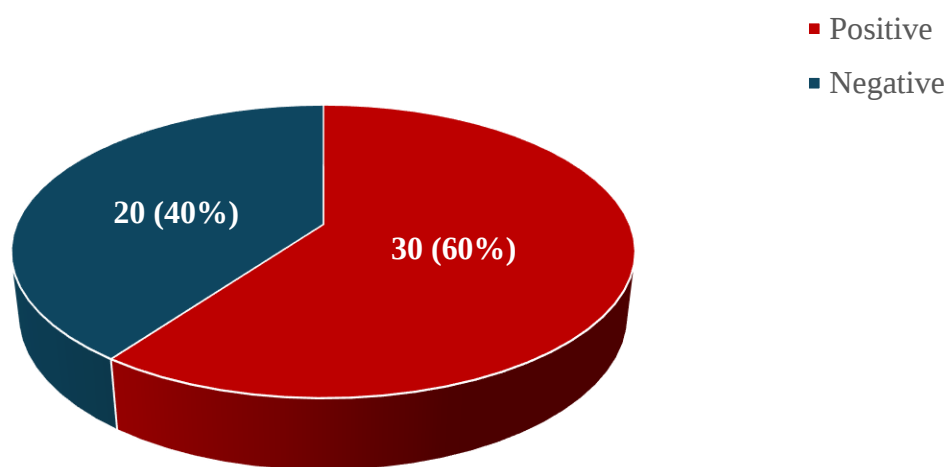


Figure 1. Prevalence of Urinary tract infection among participants

Table 1. Percentage of Bacteria Isolated

Organism	Frequency	Prevalence
<i>Escherichia coli</i>	15	50.0
<i>Staphylococcus aureus</i>	13	43.3
<i>Pseudomonas aeruginosa</i>	2	6.7
Total	30	100

Table 2: Phenotypic Identification of bacteria isolates from urine

Bacteria	Gram	Oxidase	Catalase	VP	MR	Indole	Citrate	Motility	Urease
<i>Escherichia coli</i>	-ve bacilli	-ve	+ve	-ve	+ve	+ve	-ve	Yes	-ve
<i>Staphylococcus aureus</i>	+ve cocci	-ve	+ve	+ve	-ve	-ve	+ve	No	-ve
<i>Pseudomonas aeruginosa</i>	-ve bacilli	+ve	+ve	-ve	-ve	-ve	+ve	Yes	-ve

Keys: -Ve = Negative, +Ve = Positive, VP: Voges-Proskauer test, MR: Methyl Red test, Indole: Indole test

Table 3 shows the antibiogram pattern of tested bacterial isolates, including *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Pseudomonas aeruginosa* (*P. aeruginosa*), against selected antibiotics. Among *E. coli* isolates (n = 15), the highest sensitivity was observed with imipenem (73.3%), followed by Amoxicillin-clavulanic acid (60.0%) and erythromycin (53.3%). Conversely, resistance was notably high against oxacillin (86.7%) and ciprofloxacin, cefuroxime, and ceftriaxone (each 53.3%). For *S. aureus* isolates (n = 13), imipenem exhibited the highest sensitivity (92.3%), followed by cefepime (69.2%) and erythromycin (61.5%). However, oxacillin demonstrated the highest resistance (92.3%), followed by ciprofloxacin and ceftriaxone (each 69.2%). *P. aeruginosa* (n = 2) showed complete sensitivity to imipenem and cefepime (100%) but exhibited full resistance to oxacillin and Amoxicillin-clavulanic acid (100%). Ciprofloxacin, cefuroxime, erythromycin, and ceftriaxone demonstrated equal sensitivity and resistance (50%) among *P. aeruginosa* isolates.

Table 3. Antibigram Pattern of Antibiotics Against Tested Isolates.

Antibiotics	<i>E. coli</i> (n=15)		<i>S. aureus</i> (n=13)		<i>P. aeruginosa</i> (n=2)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Ciprofloxacin	7 (46.7%)	8 (53.3%)	4 (30.8 %)	9 (69.2%)	1 (50 %)	1 (50%)
Cefuroxime	7 (46.7%)	8 (53.3%)	5 (38.5 %)	8 (61.5%)	1 (50 %)	1 (50%)
Oxacillin	2(13.3 %)	13(86.7%)	1(7.7 %)	12(92.3%)	0 (0%)	2 (100%)
Amoxicillin- clavulanic acid	9 (60.0 %)	6 (40.0 %)	7 (53.8%)	6 (46.2%)	0 (0%)	2 (100%)
Erythromycin	8 (53.3%)	7 (46.7 %)	8 (61.5%)	5 (38.5%)	1 (50.0%)	1 (50.0%)
Imipenem	11 (73.3%)	4 (26.7 %)	12 (92.3%)	1 (7.7 %)	2 (100%)	0 (0%)
Ceftriaxone	7 (46.7%)	8 (53.3%)	4 (30.8%)	9 (69.2%)	1 (50.0%)	1 (50.0%)
Cefepime	8 (53.3%)	7 (46.7%)	9 (69.2%)	4 (30.8%)	2 (100%)	0 (0%)

Key: S=Sensitive, R=Resistant.

DISCUSSION

Urinary tract infections (UTIs) are among the most common bacterial infections encountered in community healthcare settings, contributing to increased treatment costs, morbidity, and mortality (Bader *et al.*, 2020; Mancuso *et al.*, 2023). UTIs are generally classified into two categories based on their mode of acquisition: community-acquired and hospital-acquired infections (Mancuso *et al.*, 2023). *Escherichia coli* is the most frequently implicated uropathogen in UTIs, although other bacterial species such as *Klebsiella* spp., *Staphylococcus* spp., *Enterococcus* spp., *Enterobacter* spp., and *Citrobacter* spp. have also been identified as causative agents (Mancuso *et al.*, 2023).

The primary aim of this study was to isolate and phenotypically identify bacterial pathogens associated with UTIs among hospitalized female patients at Edo Specialist Hospital, Benin City, Edo State. The findings revealed a diverse range of bacterial isolates including *E. coli*,

Staphylococcus aureus, and *Pseudomonas aeruginosa*. This microbial profile aligns with previous studies that consistently report these organisms as major uropathogens. *E. coli* was the most prevalent isolate. This finding supports existing literature that highlights *E. coli* as the leading cause of UTIs worldwide, primarily due to its virulence factors such as adhesins, toxins, and iron-acquisition systems, which facilitate its colonization and persistence within the urinary tract (Flores-Mireles *et al.*, 2015). Additionally, *E. coli*'s ability to form biofilms further enhances its pathogenic potential by enabling evasion of the host immune response and antimicrobial treatments (Terlizzi *et al.*, 2017). *Staphylococcus aureus* was identified as the second most prevalent isolate. Although *S. aureus* is not traditionally regarded as a primary uropathogen, its increasing association with hospital-acquired UTIs is noteworthy. The presence of *S. aureus* in UTIs among hospitalized patients suggests a potential nosocomial origin, often linked to invasive procedures such as catheterization. The pathogenicity of *S. aureus* in UTIs is attributed to its ability to produce biofilms and an array of virulence factors, including surface proteins and toxins, which enhance its ability to colonize and persist within the urinary tract (Vandenesch *et al.*, 2012). *Pseudomonas aeruginosa* was the least frequently isolated pathogen and despite its lower prevalence, *P. aeruginosa* remains clinically significant due to its intrinsic resistance to many antibiotics and its role in complicated UTIs (Pang *et al.*, 2019). This pathogen's ability to survive in harsh environments, form biofilms, and resist antimicrobial treatments makes it a formidable cause of persistent infections, particularly among immunocompromised patients. The detection of *P. aeruginosa* in this study is consistent with global trends, reinforcing its role in healthcare-associated infections and underscoring the need for vigilant antimicrobial stewardship and infection control measures (Bonomo *et al.*, 2018).

The phenotypic identification of bacterial pathogens in this study was based on conventional biochemical tests and cultural characteristics, which remain standard practices in clinical

microbiology. These methods provide timely and reliable diagnoses that guide appropriate treatment decisions. However, incorporating molecular techniques such as polymerase chain reaction (PCR) could improve specificity and sensitivity, particularly in detecting resistance genes and virulence factors (Zhang *et al.*, 2021). In addition to the identification of prevalent pathogens, the study's findings highlight the need for individualized patient care and the importance of antimicrobial stewardship. Effective management of UTIs requires accurate identification of causative agents and susceptibility testing to guide appropriate antibiotic therapy (Zhang *et al.*, 2021). The diverse microbial profile observed in this study indicates that empirical treatment strategies must be informed by local epidemiological data to optimize therapeutic outcomes and minimize the development of resistance. Moreover, the high prevalence of UTIs in this study reflects broader trends observed globally. UTIs are among the most common infections worldwide, with significant morbidity and healthcare costs (Amiri *et al.*, 2025). The findings from this research contribute to the growing body of evidence highlighting the persistent and pervasive nature of UTIs in various populations and settings.

The antibiogram analysis highlighted varying levels of antibiotic resistance among UTI pathogens, emphasizing the need for targeted antibiotic stewardship. Ciprofloxacin, a commonly prescribed fluoroquinolone, showed moderate effectiveness against *Escherichia coli* in this study, with resistance patterns consistent with global trends. Iqbal *et al.* (2021) similarly reported increasing resistance among UTI pathogens, particularly *E. coli*, which raises concerns about the continued efficacy of fluoroquinolones in empirical therapy. Cefuroxime exhibited inconsistent susceptibility across bacterial isolates in this study, reinforcing the necessity of local resistance data to guide antibiotic selection. This underscores the importance of routine antimicrobial surveillance to optimize treatment protocols. Oxacillin demonstrated minimal effectiveness, particularly against *S. aureus*, which showed a high resistance rate. The widespread resistance observed in this study suggests the presence of

methicillin-resistant *S. aureus* (MRSA) strains, a well-documented challenge in hospital settings. Lakhundi and Zhang (2018) similarly highlighted the increasing prevalence of MRSA, emphasizing the need for stringent infection control measures and alternative treatment options. Amoxicillin-clavulanic acid remained a viable treatment option for *E. coli* and *S. aureus* in this study, despite observed resistance. Huttner *et al.* (2020) similarly noted that while amoxicillin-clavulanic acid retains effectiveness in UTI management, emerging resistance necessitates cautious use. The observed resistance patterns reinforce the need for periodic susceptibility testing to ensure optimal antibiotic selection.

Erythromycin demonstrated moderate effectiveness against *S. aureus* in this study, whereas *E. coli* and *P. aeruginosa* showed variable susceptibility. These findings align with those of Saginur and Suh (Asim *et al.*, 2017), who reported fluctuating erythromycin resistance among UTI pathogens, reinforcing the necessity of routine antimicrobial testing to guide clinical decisions.

Imipenem exhibited high effectiveness across all tested pathogens, particularly against *P. aeruginosa*, which showed complete sensitivity. This supports its role as a potent treatment for severe or complicated UTIs. Similarly, Chen *et al.* (2021) highlighted the critical role of carbapenems in managing multidrug-resistant infections, cautioning against overuse to prevent the emergence of carbapenem-resistant strains. Ceftriaxone displayed significant resistance among *S. aureus* and *E. coli* in this study, indicating potential limitations in its empirical use for UTI treatment. Such trends highlight the need for alternative therapeutic options in resistant cases.

Cefepime demonstrated strong activity against *S. aureus* and *P. aeruginosa* in this study, making it a reliable option for treating UTIs caused by multidrug-resistant organisms. This aligns with the observations of Bonnin *et al.* (2025), who reported cefepime as a potent treatment choice against resistant Gram-negative bacteria.

CONCLUSION

This study found that the main bacterial isolates causing UTIs in female patients at Edo Specialist Hospital were *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The results make clear how serious the problem of multidrug resistance is and how important it is to create novel treatment approaches, efficient antibiotic stewardship practices, and focused prevention measures.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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