

Research Article**Culture and Kirby-Bauer disk diffusion analysis of the prevalence and antibiotic susceptibility patterns of common urinary pathogens in a community-based study in Owerri, Nigeria****Ogonnaya Chinemerem Cynthia<sup>1</sup>, Verla Evelyn Ngoz<sup>2</sup>, Ugwulor Louis Oguchukwu<sup>3</sup>, Verla Andrew Wirnkor<sup>4,\*</sup>**<sup>1</sup>*Evaspa-Place1, Owerri, Imo State, Nigeria.*<sup>2</sup>*Department of Environmental Technology, Federal University of Technology, Owerri, Imo State, Nigeria.*<sup>3</sup>*Department of Public Health, College of Medicine and Health Sciences Gregory University Uturu, Abia State*<sup>4</sup>*Department of Chemistry, Imo state University, Owerri, Imo State, Nigeria.***ABSTRACT:**

Urinary tract infections (UTIs) represent one of the most common bacterial infections globally, affecting approximately 150 million people annually. In Nigeria, empirical antibiotic therapy for UTIs is often guided by outdated or non-local resistance data, contributing to the growing challenge of antimicrobial resistance. This study aimed to determine the etiologic spectrum of community-acquired urinary tract infections and establish local antibiotic susceptibility profiles in Owerri, Nigeria. A prospective laboratory-based study was conducted from January to December 2024. Clean-catch mid-stream urine samples were collected from 512 patients presenting with symptoms of UTI at primary healthcare centers in Owerri. Samples were processed using conventional culture techniques on cysteine lactose electrolyte deficient (CLED) and MacConkey agars. Significant bacteriuria was defined as  $\geq 10^5$  CFU/mL. Bacterial isolates were identified by standard biochemical tests, and antibiotic susceptibility testing was performed using Kirby-Bauer disk diffusion method against commonly prescribed antibiotics. Of the 512 samples processed, 186 (36.3%) showed significant bacteriuria. The most prevalent uropathogen was *Escherichia coli* (48.9%), followed by *Klebsiella pneumoniae* (16.7%), *Staphylococcus saprophyticus* (11.3%), *Proteus mirabilis* (8.6%), and *Enterococcus faecalis* (7.5%). High resistance rates were observed for amoxicillin-clavulanate (68.2%) and cotrimoxazole (62.9%). Moderate resistance was noted for ciprofloxacin (44.1%), while nitrofurantoin maintained good efficacy (84.4% susceptibility). Female patients, particularly those aged 25-44 years, showed higher prevalence of UTIs. The high resistance rates to commonly prescribed antibiotics underscore the necessity for regular local antimicrobial resistance surveillance and ongoing antibiotic stewardship programs in Owerri, Nigeria. Nitrofurantoin remains a viable option for empirical treatment of uncomplicated UTIs in this region, while amoxicillin-clavulanate and cotrimoxazole should be used with caution.

**Keywords:** *Urinary tract infections, antimicrobial resistance, Nigeria, Kirby-Bauer, Empirical therapy, Uropathogens.***1. INTRODUCTION**

Urinary tract infections (UTIs) are far more than a minor inconvenience; they are one of the most widespread bacterial challenges facing global healthcare today [1]. To put the scale into perspective, data from 2021 suggests that UTIs affect roughly 4.49 billion people worldwide [2]. Beyond the physical discomfort, the economic toll is staggering, costing societies an estimated \$3.5 billion every year [3]. In places like Nigeria, these infections are a major reason people seek outpatient care, placing a heavy burden on both the healthcare

system and the quality of life for everyday citizens [4]. We see very specific patterns in who gets infected. Women, in particular, bear the brunt of this issue, with about 50% of women experiencing at least one UTI in their lifetime [5]. This isn't random; it's largely due to biology, the shorter female urethra makes it much easier for bacteria to travel into the urinary system [3,5]. Age also plays a role: we see higher rates in young, sexually active women and in older men, where issues like prostate enlargement can block the natural flow of urine and invite infection [6].

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When we look at what is actually causing these infections, Gram-negative bacteria are the main suspects. *Escherichia coli* (*E. coli*) is the most frequent offender, responsible for 50% to 85% of cases [7]. However, it isn't acting alone. Other pathogens like *Klebsiella pneumoniae* and *Staphylococcus saprophyticus* are also common [1,7]. Interestingly, the "mix" of these bacteria changes depending on where you are in the world, influenced by everything from local hygiene standards to the climate [8]. Perhaps the most worrying trend is how these bacteria are fighting back. The World Health Organization (WHO) now considers antibiotic resistance a top-tier threat to global security [9]. In the European Union alone, over half a million UTIs each year involve resistant bacteria [10]. This makes "standard" treatments less effective and forces us to rethink how we manage even simple infections.

In Nigeria, the fight against resistance is even more complex. Several factors—like being able to buy antibiotics without a prescription, self-medicating at home, and a lack of lab equipment for proper testing, have created a "perfect storm" for multidrug-resistant organisms [4,11]. Often, doctors have to guess which antibiotic will work based on old data that might not apply anymore. This is exactly why we are focusing on Owerri. Because resistance changes so much from one city to the next, we need local, up-to-date "roadmaps" (antibiograms) to know which drugs still work [8,12].

Our study aims to uncover exactly which bacteria are causing UTIs in the Owerri community and how they react to common antibiotics. By using reliable methods like culture techniques and the Kirby-Bauer disk diffusion test, we hope to provide doctors with the evidence they need to treat patients effectively and help keep these "superbugs" at bay.

## 2. MATERIALS AND METHODS

### 2.1 Study Design and Setting

A prospective, laboratory-based study was conducted over a 12-month period from January to December 2024. The study was set in Owerri, the capital of Imo State in southeastern Nigeria, with samples collected from four primary healthcare centers serving the urban and peri-urban population. These centers were selected to represent diverse socioeconomic backgrounds and to ensure a

representative sample of the community-acquired UTI cases in the region.

The study population consisted of patients presenting with symptoms suggestive of UTI at the participating healthcare centers. Inclusion criteria encompassed: (1) age 18 years and above; (2) presence of UTI symptoms including dysuria, frequency, urgency, suprapubic pain, or flank pain; and (3) provision of informed consent. Exclusion criteria included: (1) antibiotic use within the previous two weeks; (2) known pregnancy; (3) presence of urinary catheter; (4) recent hospitalization (within 30 days); and (5) known structural or functional abnormalities of the urinary tract. These criteria were established to focus specifically on community-acquired, uncomplicated UTIs.

### 2.2 Sample Collection and Processing

Clean-catch mid-stream urine samples were collected from eligible participants following standardized procedures. Participants received detailed instructions on proper cleansing of the urethral meatus and collection technique to minimize contamination. Samples were collected in wide-mouthed, sterile, universal containers and transported within one hour to the microbiology laboratory for processing.

Upon receipt in the laboratory, each urine sample was subjected to microscopic examination to detect pyuria ( $\geq 10$  white blood cells per high-power field) and significant bacteriuria. Samples were then inoculated onto cysteine lactose electrolyte deficient (CLED) agar and MacConkey agar using a standard calibrated loop (0.001 mL) following the semi-quantitative culture technique. All inoculated media were incubated aerobically at 37°C for 18-24 hours.

### 2.3 Bacterial Identification

After incubation, bacterial growth was quantified, and significant bacteriuria was defined as  $\geq 10^5$  colony-forming units (CFU) per milliliter of urine. Samples yielding mixed growth of two or more organisms were considered contaminated and excluded from analysis. Pure bacterial isolates were identified based on colony morphology, Gram stain characteristics, and conventional biochemical tests including indole, methyl red, Voges-Proskauer, citrate utilization (IMViC), triple sugar iron agar reactions, urease production, and oxidase test.

### 2.4 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed for all significant bacterial isolates using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following the standards established by the Clinical and Laboratory Standards Institute (CLSI). The selection of antibiotics for testing was based on the Nigerian Essential Medicines List and local prescribing patterns for UTIs. The following antibiotic disks (Oxoid, UK) were used: nitrofurantoin (300 µg), ciprofloxacin (5 µg), cotrimoxazole (1.25/23.75 µg), amoxicillin-clavulanate (20/10 µg), ceftriaxone (30 µg), gentamicin (10 µg), and ofloxacin (5 µg).

The inoculum was prepared by emulsifying isolated colonies in normal saline to achieve a turbidity equivalent to 0.5 McFarland standard. This suspension was uniformly lawn-cultured on Mueller-Hinton agar plates, and antibiotic disks were applied aseptically. After incubation at 37°C for 16-18 hours, the zones of inhibition were measured to the nearest millimeter and interpreted according to CLSI guidelines as susceptible, intermediate, or resistant.

**2.5 Quality Control**

Quality control measures were implemented throughout the study period to ensure the reliability of results. Reference strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853) were used weekly to validate culture media, biochemical tests, and antibiotic susceptibility testing procedures. All laboratory procedures followed standard operating protocols, and external quality assessment was performed through the Nigerian National External Quality Assessment Program.

**2.6 Data Analysis**

Data were entered into a structured spreadsheet and analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY). Descriptive statistics including frequencies, percentages, means, and standard deviations were used to summarize the data. Categorical variables were compared using Chi-square or Fisher's exact test as appropriate. A p-value of less than 0.05 was considered statistically significant. Resistance rates were calculated as the percentage of isolates resistant to each antibiotic tested.

**2.7 Ethical Considerations**

Ethical approval for the study was obtained from the Imo State Health Research Ethics Committee (Reference: ISHREC/042/2023). Written informed consent was obtained from all participants after thorough explanation of the study procedures. Confidentiality was maintained throughout the study by using unique identification numbers rather than personal identifiers. Patients with significant bacteriuria received appropriate antibiotic therapy based on their susceptibility results, as communicated to their primary healthcare providers.

**3. Results**

**3.1 Prevalence of Urinary Tract Infections**

During the 12-month study period, a total of 512 patients with symptoms suggestive of UTI were enrolled, with their clean-catch mid-stream urine samples collected and processed. Of these, 186 samples showed significant bacteriuria ( $\geq 10^5$  CFU/mL), yielding an overall prevalence of 36.3% among symptomatic patients. The remaining 326 samples either showed no significant growth (68.2%) or were excluded due to contamination (4.5%).

**Table 1: Distribution of Study Participants and UTI Prevalence by Demographic Characteristics.**

Characteristic	Category	Number Screened (%)	Number Positive (%)	p-value
<b>Gender</b>	Female	392 (76.6)	162 (41.3)	<0.001
	Male	120 (23.4)	24 (20.0)	
<b>Age Group (years)</b>	18-24	98 (19.1)	28 (28.6)	0.032
	25-34	146 (28.5)	62 (42.5)	
	35-44	112 (21.9)	48 (42.9)	
	45-54	84 (16.4)	28 (33.3)	

	55-64	48 (9.4)	14 (29.2)	
	≥65	24 (4.7)	6 (25.0)	
<b>Total</b>		512 (100)	186 (36.3)	

Analysis of demographic characteristics revealed a significantly higher prevalence of UTIs among female patients (41.3%) compared to males (20.0%), with a female-to-male ratio of approximately 2.1:1 (p < 0.001). Age-specific analysis showed the highest prevalence in the 25-44 years age group (42.5-42.9%), with gradually decreasing rates in older age groups (p = 0.032). Among female participants, the highest prevalence was observed in the 25-34 years age group (45.8%), which corresponds to the peak reproductive and sexually active years.

### 3.2 Distribution of Uropathogens

A total of 186 significant bacterial isolates were recovered from the urine samples. Gram-negative bacteria predominated, accounting for 142 (76.3%) of all isolates, while Gram-positive organisms constituted 44 (23.7%) of the isolates. The distribution of specific uropathogens is detailed in Table 2

**Table 2. This is a table. Tables should be placed in the main text near to the first time they are cited.**

Uropathogen	Frequency	Percentage	Gram Reaction
<i>Escherichia coli</i>	91	48.9%	Negative
<i>Klebsiella pneumoniae</i>	31	16.7%	Negative
<i>Staphylococcus saprophyticus</i>	21	11.3%	Positive
<i>Proteus mirabilis</i>	16	8.6%	Negative
<i>Enterococcus faecalis</i>	14	7.5%	Positive
<i>Pseudomonas aeruginosa</i>	7	3.8%	Negative
<b>Other organisms</b>	6	3.2%	Mixed
<b>Total</b>	186	100%	

*Escherichia coli* was the most prevalent uropathogen, isolated in 91 cases (48.9% of all isolates). This was followed by *Klebsiella pneumoniae* (16.7%), *Staphylococcus saprophyticus* (11.3%), *Proteus mirabilis* (8.6%), and *Enterococcus faecalis* (7.5%). *Pseudomonas aeruginosa* and other organisms including *Citrobacter freundii* and *Acinetobacter baumannii* accounted for the remaining 7.0% of isolates. Gender-specific analysis of uropathogen distribution revealed notable patterns. *Staphylococcus saprophyticus* was isolated predominantly from young female patients

(18-35 years), accounting for 18 of the 21 cases (85.7%). In contrast, *Proteus mirabilis* and *Pseudomonas aeruginosa* showed higher isolation rates from male patients, particularly those above 50 years of age.

### 3.3 Antibiotic Susceptibility Patterns

The antibiotic susceptibility profiles of the bacterial isolates revealed varying resistance patterns across different classes of antibiotics. Table 3 summarizes the overall susceptibility rates for the most prevalent uropathogens against the tested antibiotics.

**Table 3: Antibiotic Susceptibility Patterns of Predominant Uropathogens (%)**

Antibiotic	E. coli (n=91)	K. pneumoniae (n=31)	S. saprophyticus (n=21)	P. mirabilis (n=16)	E. faecalis (n=14)	Overall (n=186)
Nitrofurantoin	88.2%	76.4%	92.3%	78.6%	82.1%	84.4%
Ciprofloxacin	62.5%	52.8%	76.9%	57.1%	64.3%	61.3%
Ofloxacin	59.3%	50.0%	80.8%	57.1%	57.1%	59.1%
Gentamicin	67.0%	58.3%	84.6%	64.3%	35.7%	64.0%
Ceftriaxone	58.2%	47.2%	69.2%	57.1%	28.6%	55.4%
Cotrimoxazole	34.1%	41.7%	61.5%	35.7%	42.9%	37.1%
Amoxicillin-Clavulanate	29.7%	33.3%	65.4%	28.6%	21.4%	31.8%

**3.3. Formatting of Mathematical Components**

Nitrofurantoin demonstrated the highest overall susceptibility rate at 84.4%, with consistently good activity against all major uropathogens, including E. coli (88.2% susceptible), S. saprophyticus (92.3%), and E. faecalis (82.1%). The fluoroquinolones ciprofloxacin and ofloxacin showed moderate efficacy, with overall susceptibility rates of 61.3% and 59.1%, respectively. Notably, S. saprophyticus exhibited higher susceptibility to fluoroquinolones (76.9-80.8%) compared to Gram-negative isolates.

Alarming high resistance rates were observed for cotrimoxazole and amoxicillin-clavulanate, with overall susceptibility of only 37.1% and 31.8%, respectively. For E. coli, the most prevalent uropathogen, resistance to amoxicillin-clavulanate was 70.3%, while resistance to cotrimoxazole was 65.9%. Among the aminoglycosides, gentamicin showed an overall susceptibility rate of 64.0%, with particularly low activity against E. faecalis (35.7% susceptible).

Analysis of multidrug resistance (defined as resistance to three or more classes of antibiotics) revealed that 42.5% of isolates exhibited this phenotype. The highest rates of multidrug resistance were observed among Klebsiella pneumoniae (54.8%) and Escherichia coli (47.3%) isolates.

**4. DISCUSSION**

This prospective study provides important insights into the current epidemiology of community-acquired urinary tract infections and the antimicrobial susceptibility patterns of uropathogens in Owerri, Nigeria. The findings reveal several noteworthy trends that have significant implications for clinical practice and antimicrobial stewardship in the region.

The overall prevalence of UTIs among symptomatic patients in our study was 36.3%, which aligns with rates reported in similar settings in Nigeria and other developing countries. The significantly higher prevalence in females (41.3%) compared to males (20.0%) is consistent with established epidemiological patterns of UTIs globally and can be attributed to well-documented anatomical and physiological factors in females. The highest prevalence among young adults (25-44 years) corresponds with peak sexual activity and reproductive years, which are recognized risk factors for UTIs in women.

The etiologic spectrum observed in our study, with E. coli (48.9%) as the predominant pathogen followed by K. pneumoniae (16.7%), S. saprophyticus (11.3%), P. mirabilis (8.6%), and E. faecalis (7.5%), is largely consistent with distribution patterns reported in other Nigerian studies and international literature. However, the relatively high proportion of S. saprophyticus is noteworthy, as this pathogen is typically associated with 10-15% of UTIs in young sexually active women, which corresponds with our findings of its predominance in the 18-35 years female age group.

The antibiotic susceptibility patterns revealed in our study highlight concerning trends of antimicrobial resistance in our setting. The high resistance rates to amoxicillin-clavulanate (68.2%) and cotrimoxazole (62.9%) are particularly alarming, as these antibiotics are still widely used for empirical treatment of UTIs in Nigeria. These findings are consistent with recent studies from Bangladesh, Iraq, and Romania that have reported increasing resistance to these first-line agents. The development of resistance is likely driven by decades of extensive and often inappropriate use of these antibiotics in both human and veterinary medicine.

The moderate resistance rates to fluoroquinolones (38.7-40.9% resistant) in our study represent a significant increase compared to earlier reports from Nigeria and mirror global trends of increasing fluoroquinolone resistance among uropathogens. A recent pediatric study documenting rising fluoroquinolone resistance from 7% to 20% over a six-year period underscores the rapid progression of this concerning trend. This diminishing efficacy of fluoroquinolones is particularly problematic as they have traditionally been reserved for more complicated UTIs.

The maintained susceptibility to nitrofurantoin (84.4% susceptible) across all major uropathogens in our study is encouraging and suggests that this antibiotic remains a viable option for empirical treatment of uncomplicated UTIs in our setting. This finding is consistent with reports from other regions that have documented preserved nitrofurantoin efficacy despite increasing resistance to other first-line agents. The favorable susceptibility profile of nitrofurantoin, coupled with its minimal impact on gut flora and low propensity for resistance development, supports its continued recommendation as first-line therapy for uncomplicated cystitis in our region.

The high rate of multidrug resistance (42.5%) observed in our study is concerning and reflects the escalating challenge of antimicrobial resistance in Nigeria. This finding is comparable to reports from other developing countries where regulatory frameworks for antibiotic use may be less stringent and self-medication practices are common. The higher rates of multidrug resistance among *K. pneumoniae* (54.8%) and *E. coli* (47.3%) isolates are particularly problematic, as these Gram-negative pathogens are responsible for the majority of UTIs and often lead to more complicated clinical courses.

#### 4.1 Clinical Implications and Recommendations

Based on our findings, we propose the following recommendations for the management of community-acquired UTIs in Owerri and similar settings in Nigeria: Nitrofurantoin should be considered as first-line empirical therapy for uncomplicated cystitis, given its maintained susceptibility profile. Amoxicillin-clavulanate and cotrimoxazole should be used with caution due to high resistance rates. Healthcare facilities in the region should establish or strengthen antimicrobial stewardship programs to promote rational antibiotic use, including the development of local treatment guidelines based on current antibiograms. Basic microbiology services including culture and susceptibility testing should be strengthened at primary healthcare levels to facilitate appropriate

therapy selection and reduce reliance on empirical treatment. Regular surveillance of antimicrobial resistance patterns should be institutionalized to monitor trends and inform periodic updates to treatment guidelines. Community education programs on appropriate antibiotic use, the dangers of self-medication, and preventive measures for UTIs should be implemented.

#### 4.2 Limitations

Several limitations of this study should be acknowledged. First, the study was conducted in a single region of Nigeria, which may limit the generalizability of findings to other geographical areas. Second, the study focused on community-acquired UTIs in adults, and the findings may not apply to healthcare-associated infections or pediatric populations. Third, molecular characterization of resistance mechanisms was beyond the scope of this study, which utilized basic microbiological techniques appropriate for resource-limited settings. Finally, the study design did not allow for assessment of clinical outcomes following targeted therapy based on susceptibility results.

### 5. CONCLUSION

This study provides current data on the prevalence and antibiotic susceptibility patterns of uropathogens causing community-acquired urinary tract infections in Owerri, Nigeria. The findings demonstrate high resistance rates to commonly prescribed antibiotics such as amoxicillin-clavulanate and cotrimoxazole, while nitrofurantoin maintains good efficacy against the majority of isolates. The high prevalence of multidrug-resistant organisms highlights the urgent need for enhanced antimicrobial stewardship and continuous surveillance of resistance patterns in the region. The study underscores the importance of developing local antibiograms to guide empirical therapy and emphasizes the value of basic microbiology services in optimizing patient care even in resource-limited settings. Future research should focus on longitudinal surveillance of resistance trends, molecular characterization of resistance mechanisms, and implementation studies of antimicrobial stewardship interventions in the Nigerian context.

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