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Prevalence and Antimicrobial Resistance of ESBL-Producing Gram - Negative Bacilli in Urinary Tract Infections at Red Sea Teaching Hospitals, Sudan

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Abstract: ***Urinary tract infections (UTIs) remain among the most prevalent bacterial infections worldwide, particularly in resource-limited settings where empirical treatment is commonly practiced. This study aimed to determine the prevalence and antimicrobial susceptibility of extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacilli among symptomatic UTI patients at Port Sudan Teaching Hospitals. A cross-sectional study was conducted between January 2023 and January 2024, involving 150 patients with UTI symptoms. Midstream urine samples were cultured and tested for antimicrobial resistance using the Kirby-Bauer disk diffusion method. Of the 150 samples, 71 (47.3%) were culture-positive, with Escherichia coli as the most common isolate (20%). High resistance was noted against Ceftazidime (70%) and Nalidixic acid (60%), while Amikacin (64.7%) and Nitrofurantoin (56.9%) exhibited higher sensitivity. ESBL activity was phenotypically confirmed in 8 out of 34 resistant isolates. These findings underscore the urgent need for regular surveillance and informed antibiotic prescribing to curb resistance in the region.***

Keywords: Urinary tract infection, antimicrobial resistance, ESBL, Gram-negative bacilli, Sudan

1. Introduction

- Urinary tract infections (UTIs) rank among the most prevalent bacterial infections that impact people of all ages globally. These infections occur when microbes invade the urinary system, which includes the urethra, bladder, ureters, or kidneys, with the lower urinary tract (cystitis) being the most commonly affected area[1]. Urinary tract infections (UTIs) constitute a substantial portion of both outpatient and inpatient medical consultations worldwide. This is particularly evident among women, for whom the lifetime risk of developing a UTI is estimated to exceed 50%[2].
- In clinical practice, urinary tract infections (UTIs) may manifest with symptoms such as dysuria, urinary urgency, frequency, suprapubic discomfort, and, in more severe instances, fever or flank pain[3]. Identified risk factors encompass female gender, engagement in sexual activity, inadequate hygiene practices, diabetes mellitus, immunosuppression, and the utilization of indwelling urinary catheters[4]. The majority of UTIs are caused by uropathogenic *Escherichia coli* (UPEC), followed by *Klebsiella pneumoniae.*, *Proteus mirabilis.*, and *Pseudomonas aeruginosa* [5]. Timely and accurate diagnosis, in conjunction with appropriate antibiotic therapy, is essential to prevent complications such as pyelonephritis, urosepsis, and antimicrobial resistance.
- In Sudan, like many low-resource settings, empirical treatment of UTIs is common due to limited access to routine microbiological diagnostics. This approach often leads to the misuse of antibiotics and contributes to the growing burden of antimicrobial resistance[6], [7]. Port Sudan, the capital of Red Sea State, serves as a crucial referral center characterized by a substantial patient volume and a heterogeneous population. However, there is a paucity of local data regarding the prevalence, etiological agents, and resistance patterns of urinary tract infections (UTIs).
- In light of the clinical and public health significance of urinary tract infections (UTIs), this study was undertaken to ascertain the prevalence of UTIs among symptomatic patients at Port Sudan Teaching Hospital in 2024. Furthermore, the study sought to delineate the etiological profile of uropathogens and evaluate their antimicrobial susceptibility patterns. The results are intended to inform local treatment guidelines and enhance the broader understanding of UTI epidemiology in Sudan and comparable contexts.

2. Methods

2.1 Study Design, Setting, and Population

A prospective, descriptive cross-sectional study was conducted at Port Sudan Teaching Hospital, located in Red Sea State, Sudan, from January 2023 to January 2024. As the main referral center in the region, the hospital receives patients from various health facilities across the state. The study population included patients of all age groups presenting with clinical symptoms suggestive of urinary tract infection (UTI). Ethical approval for the study was obtained from the administration of Port Sudan Teaching Hospital. Informed consent was obtained from all participants, and strict confidentiality of patient data was

maintained throughout the study.

2.2 Data Collection

Demographic and clinical data—including age, gender, hospital unit, and site of infection—were collected using structured questionnaires and patient laboratory records.

2.3 Specimen Collection and Processing

Midstream urine specimens were collected aseptically in sterile containers. Samples were transported promptly to the microbiology laboratory using appropriate transport media and were processed immediately upon arrival. Urine samples were cultured on Cysteine Lactose Electrolyte Deficient (CLED) agar, MacConkey agar, and Blood agar. All plates were incubated aerobically at 37°C for 24 hours.

2.4 Bacterial Identification

Preliminary identification of isolates was performed based on colony morphology, Gram staining, and oxidase testing. Confirmatory identification of Gram-negative uropathogens was conducted using the Analytical Profile Index 20E (API 20E) system (bioMérieux, France) following the manufacturer's instructions.

2.5 Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was carried out using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested included amikacin, Amoxicillin, Amoxicillin-Clavulanic acid, Ceftazidime, Ceftriaxone, Cefuroxime, Chloramphenicol, Ciprofloxacin, Gentamicin, Nalidixic acid, Nitrofurantoin, Tetracycline, Tobramycin, and Trimethoprim-sulfamethoxazole. Bacterial inoculation were adjusted to match the 0.5 McFarland turbidity standard. *Escherichia coli* ATCC 25922 was used as the quality control strain.

2.6 Phenotypic detection of ESBL

The double disc synergy test (DDST) was carried out on Muller-Hinton agar plate seeded by bacterial suspension. A disc containing the amoxiclav (amoxicillin 20 µg plus clavulanic acid 10 µg) was placed at the center of the Muller-Hinton agar plate. Four discs (cefepime(30µg), ceftazidime (30µg), cefotaxime (30 µg), and ceftriaxone (30 µg) were around amoxiclav disc at a distance of 25mm center to center (Clinical and Laboratory Standards Institute). After overnight incubation. there was an extension of the inhibition zone towards the disc containing the amoxiclav (Augmentin), which indicated an ESBL activity.

2.7 Statistical Analysis

Data were entered and analyzed using SPSS version 20 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize demographic and clinical variables. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Continuous variables were analyzed using independent sample t-tests or one-way analysis of variance (ANOVA). A p-value of < 0.05 was considered statistically significant.

3. Results

A total of 150 patients presenting with clinical features suggestive of urinary tract infection (UTI) were enrolled in the study. Urine samples were collected and processed for culture and antimicrobial susceptibility testing. Confirmed UTIs, based on significant bacterial growth, were identified in 71 cases, yielding a frequency of 47.3% (Figure 1). The remaining 79 samples (52.7%) showed no bacterial growth. The mean age of the participants was 35.71 ± 18.2 years, and the majority were female, accounting for 53.3% (80/150) of the study population (Table 1). *E. coli* remained the dominant uropathogen, followed by *Pseudomonas spp.*, *Klebsiella spp.*, and *Proteus spp.*, respectively (Figure 2).

3.1 Distribution of UTI Pathogens by Gender

Among the positive cultures, the most frequently isolated uropathogen was *Escherichia coli*, detected in 30 cases (20.0%), more common in females (25.0%) than males (14.3%). (Table 1). *Pseudomonas spp.* were the second most common, accounting for 16 cases (10.7%), slightly more in males (9 cases, 12.9%) than in females (7 cases, 8.8%). *Klebsiella spp.* and *Proteus spp.* were isolated in 14 (9.3%) and 11 (7.3%) cases, respectively, with comparable distributions across genders. No statistically significant differences were observed in organism distribution between male and female patients.

3.2 Antimicrobial Susceptibility Patterns:

Antimicrobial susceptibility testing was performed on all 71 culture-positive isolates. High levels of resistance were observed against Ceftazidime (70%) and Nalidixic acid (60%). In contrast, the highest sensitivity rates were recorded for Amikacin (64.7%), followed by Nitrofurantoin (56.9%) (Table 2). These findings suggest limited efficacy of some commonly used antibiotics and highlight the need for regular susceptibility surveillance.

3.3 Phenotypic detection of ESBL:

Based on 71 culture- positive isolates, **39** isolates were shows high level of resistance to most antibiotics, out of 34 isolates only **8** isolates were ESBL producers by DDST.

3.4 Figures and Tables

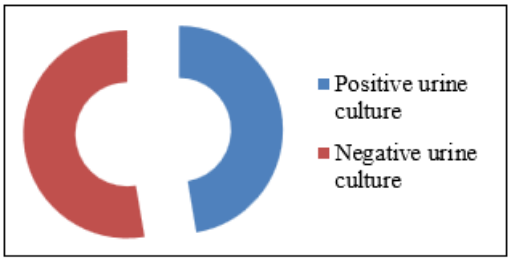

Figure 1: Frequency of urinary tract infection (UTI) among study population. N=150.

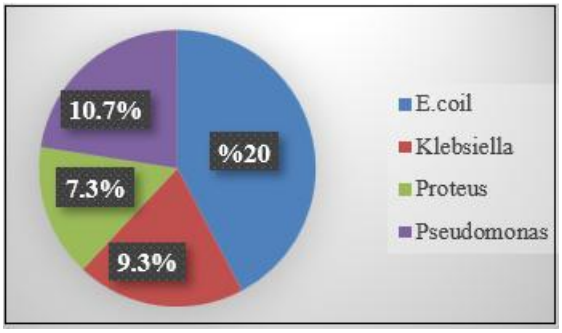

Figure 2: Frequency of Bacterial agents caused UTI among positive culture.

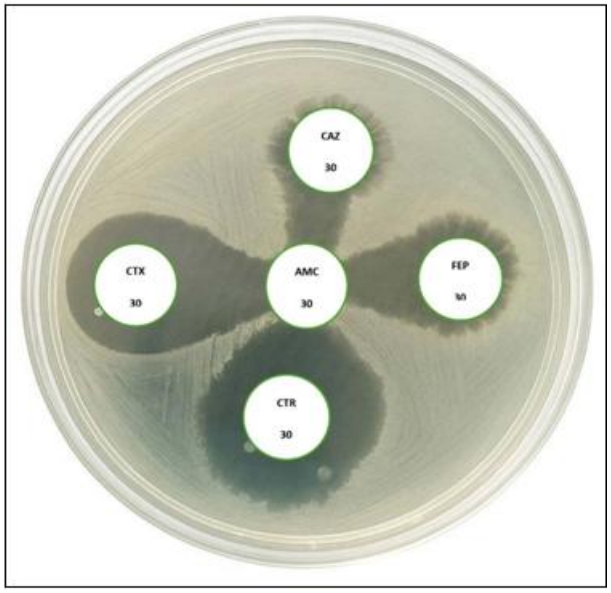

Figure (3): Double disc synergy test (DDST) phe showing clear extension of the edge of the inhibition zone of cephalosporin towards Augmentin disc as positive for ESBL production

3.5 Tables

Table 1: Frequency of UTI causative agents according to gender, N=150

Organism	Gender		Total
	Male	Female	
E.coil	10 (14.3%)	20 (25.0%)	30 (20.0%)
Klebsiella pneumoniae	7 (10.0%)	7 (10.0%)	14 (9.3%)
Proteus mirabilis	5 (7.1%)	6 (7.5%)	11 (7.3%)
Pseudomonas aeruginosa	9 (12.9%)	7 (8.8%)	16 (10.7%)
No. growth	39 (55.7%)	40 (50.0%)	79 (52.7%)
Total	70 (100.0%)	80 (100.0%)	150 (100.0%)

Table 2: Antimicrobial Susceptibility Patterns among UTI patients. N=71

Antibiotics patterns	Antibiotics	Percentages
Resistant	Ceftazidime	70%
	Nalidixic acid	60%
Sensitive	Amikacin	64.70%
	Nitrofurantoin	56.90%

Table (3): frequency MDR& DDST in bacterial isolates

		DDST	
Isolates	Full Resist	Positive	Negative
E. coli	14	4	10
Klebsiella.pneumoniae	06	1	05
Proteus mirabilis	06	2	04
Pseudomonas aeruginosa	11	1	10
Total	37	8	29

Table 4: Double disc synergy test

Method	Antibiotic Disc	Confirmation is positive if
Double-Disc Synergy Test (DDST)	Cefotaxime, Ceftriaxone, Ceftazidime, Cefepime CTX 30 µg CRO 30 µg CAZ 30 µg FEP 30 µg	Expansion of indicator cephalosporin inhibition zone towards antibiotic with Clavulanic acid
	Amoxicillin+ Clavulanic acid (AUG 20+10 µg)	

4. Discussion

1) This study investigated the frequency and etiological profile of urinary tract infections (UTIs) among patients presenting with suggestive clinical symptoms at Port Sudan Teaching Hospital. The overall frequency of culture-confirmed UTIs was 47.3%, which is comparable to previous studies conducted in similar settings. For instance, a study in Wad Medani, Sudan, reported a prevalence of 41.8% among symptomatic patients[8], while other study across sub-Saharan Africa have shown UTI prevalence was 32.1%[9].
2) The predominance of females in our study (53.3%) and the higher isolation rate of Escherichia coli among females (25.0%) compared to males (14.3%) aligns with the well-established understanding that females are more susceptible to UTIs due to anatomical and physiological factors[10]. The age distribution (mean age 35.71 ± 18.2 years) suggests that UTIs are common among adults, particularly within the reproductive age group, which has been similarly reported in studies from Ethiopia and Nigeria[11], [12].
3) Among the isolated uropathogens, E. coli was the most prevalent organism (20.0%), followed by Pseudomonas spp. (10.7%), Klebsiella spp. (9.3%), and Proteus spp. (7.3%). The predominance of E. coli is consistent with global and regional findings where it remains the primary uropathogen in both community and hospital-acquired infections[13], [14]. The significant presence of Pseudomonas spp. and Klebsiella spp. both known for multidrug resistance highlights a concerning trend towards more resistant and opportunistic pathogens in UTI cases.
4) Antimicrobial susceptibility testing revealed alarming resistance to commonly prescribed antibiotics such as Ceftazidime (70%) and Nalidixic acid (60%). These findings are in agreement with previous reports from Sudan and other low-resource settings that have documented increasing resistance to beta-lactams and quinolones[15], [16]. On the other hand, Amikacin (64.7%) and Nitrofurantoin (56.9%) retained moderate levels of effectiveness, suggesting they may still be

suitable options for empirical treatment, especially in uncomplicated cases.

5) The high resistance rates observed underscore the importance of continuous antimicrobial surveillance and rational antibiotic prescribing. The local antibiotic policy should be regularly updated based on current susceptibility trends to ensure optimal therapeutic outcomes and minimize the spread of resistant strains.
6) Despite the valuable findings, this study is limited by its single-center design and relatively small sample size. Future studies incorporating molecular methods and broader geographical representation are recommended to further elucidate resistance mechanisms and pathogen diversity.

5. Conclusion

This study demonstrated a high frequency of urinary tract infections (47.3%) among patients with suggestive symptoms at Port Sudan Teaching Hospital in 2024. Escherichia coli was the predominant uropathogen, followed by Pseudomonas spp., Klebsiella spp., and Proteus spp. The antimicrobial susceptibility patterns revealed significant resistance to commonly used antibiotics, particularly Ceftazidime and Nalidixic acid, while Amikacin and Nitrofurantoin showed relatively higher effectiveness.

These findings underscore the urgent need for continuous antimicrobial resistance surveillance, evidence-based empirical treatment guidelines, and strengthened infection prevention and control practices. Implementing routine culture and sensitivity testing before initiating therapy can improve patient outcomes and mitigate the spread of multidrug-resistant organisms.

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