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Detection of Extended Spectrum Beta-Lactamase (ESBLs) among Urinary Tract Patients in Khartoum State, Sudan

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ABSTRACT

Background: Many different organisms can cause urinary tract infections, but *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis, and Staphylococcus saprophyticus* are the most prevalent ones. 60% of all antibiotics used to treat infectious diseases around the world are beta-lactam antibiotics, one of the main classes used to fight gram-negative and gram-positive bacteria.

Objective: The purpose of this study is to detect and isolate Extended-Spectrum Beta-Lactamases (ESBLs), which are responsible for urinary tract infections, as well as check for any potential medication resistance.

Materials and Methods: The gram stain technique and biochemical assays were used to identify 100 urine samples from Khartoum state hospitals based on their cultural characteristics and morphological appearance. Using the disk diffusion method, the isolates were tested for antimicrobial susceptibility to third-generation cephalosporins (Cefotaxime, Ceftazidime, and Ceftriaxone). The Combination Disk Technique (clavulanic acid+third-generation cephalosporins) was used to inoculate the bacterial isolates to demonstrate their capacity to create ESBL. In comparison to non-ESBL producers, the ESBL producers were assessed.

Results: *E. coli, Klebsiella, S. aureus, Proteus, and Pseudomonas* were among the microorganisms isolated from UTI patients. 61% of the participants in this study were female, whereas 39% were male. *E. coli* has an increased frequency among isolated bacteria, as it presented in 46% of urine cultures, followed by *pseudomonas* and *Klebsiella,* each with a 22% frequency. Growth of the majority of the bacteria was found among females more frequently than males, and it also seems to be among older age patients than younger. Amoxyl alone and in combination with Clavulanic Acid (AAMC) was the most medicine that bacteria were resistant to (76%), but Ceftriaxone (CTR) has higher sensitivity (45%) and resistance (50%). The growth of the bacteria in the media of antibiotics was sorted into sensitive, intermediate, and resistant.

Conclusions: Tested antibiotic resistance was higher for AAMC than for CTR, which is typically taken without performing urine sample culture and sensitivity testing, which over time leads to increased resistance.

Keywords

Beta-lactam, UTI, E. coli, Proteus, ESBL, Antibiotics

Introduction

Urinary Tract Infections (UTIs) are the most common infections throughout all world regions, leading to significant morbidity and mortality across all age groups [1]. Around 150 million urinary tract infections are recorded each year around the world, and about 10% of people have one at least once in their lives [2]. Six million people worldwide are estimated to seek treatment for UTIs in hospitals each year, with about 300,000 receiving care in the wards [3]. Treatment for UTIs costs the world economy more than $6 billion [4]. Low socioeconomic status, poor hygiene, and hunger are the main risk factors for urinary tract infections [5]. In addition to complicating pregnancy, urinary tract infections can also worsen conditions like diabetes, polycystic kidney disease, sickle cell anemia, and renal transplantation. According to race and age, women have a 3- to 7- fold higher chance of developing UTIs than men [5].

Throughout the first year of life, male kids are more susceptible to UTIs, whilst female babies begin to show a greater tendency to be impacted by UTIs after becoming one year old [6]. In contrast to men, who are more likely to have this infection beyond the age of 50, middle-aged women are more likely to do so. Bacteria such as *Escherichia coli, Klebsiella spp., Pseudomonas erogenous, Proteus spp., Staphylococcus species, Acinetobacter, Enterococcus, Morgnella spp., Citrobacter freundii, and Corynebacterium urealyticum* are responsible for 95% of urinary tract infections [2]. *Escherichia coli,* which causes more than 80% of cases of simple pyelonephritis and 50% of nosocomial infections, is the most common cause of UTI [7]. Males are more likely than females to contract *proteus* infections, which are also linked to kidney stones. Young women

who engage in sexual activity frequently get saprophyticus infections. Patients with diabetes and immunological dysfunction frequently develop a candida urine infection [8]. Extended-spectrum beta-lactams and enzymes are novel chemical derivatives that have been created to combat resistant microorganisms (ESBLs). Germany reported the first hospital epidemic of an ESBL-producing gram-negative bacteria in 1983 [9]. It can be found in various bacterial species and is responsible for many chronic infections of the blood, gastrointestinal tract, reproductive organs, skin, and central nervous system [10]. According to how similar their amino acid sequences are, ESBL is divided into different categories [11].

Since there are very few antibiotic choices for treating ESBL-producing organisms, the existence of ESBLs has great therapeutic significance. The primary cause of UTI pathogen resistance to the majority of treatment medicines produced against them in recent years is the abuse of broad-spectrum antibiotics, which altered the intestinal flora and led to bacterial resistance [6,12]. Long-term antibiotic exposure, extended ICU stays, nursing home residence, severe sickness, advanced age, diabetes mellitus catheterization, and recurring UTI cases are the main risk factors connected to ESBL-producing organisms. The prior use of penicillin is one of the other crucial risk factors [13]. According to reports, the prevalence of ESBL is 58%, 44.5%, and 39.5% in India, Iran, and Bangladesh, respectively [14]. In Pakistan, 40–43% of clinical isolates produced gram-negative bacilli that produce ESBL [15]. Due to their weakened immunological responses, children and the elderly produce ESBLs far more frequently. It has been observed that age over 60 is a common risk factor for ESBL infections. Males produced more ESBLs than females, according to studies [16,17]. The knowledge of UTI isolation and identification, antibiotic susceptibility, and ESBL frequency under the geographical conditions of Islamabad must therefore be improved.

Materials and Methods

Study design

This cross-sectional study.

Study population

UTI-infected patients attended different hospitals in Khartoum state.

Study area

Khartoum state's hospitals (Medical force, Rabat, and Omdurman).

Sample size

Includes 100 samples of UTI patients.

Collection of specimen

The mid-stream urine was collected in a sterile wide-neck urine container from each patient, considering labeling and down writing of basic information about treatment, age, and gender.

Isolation of pathogens

Urine samples were inoculated into Citrate Lysine Electrolyte Deficiency (CLED) media and incubated at 37º C overnight. The isolate was cultural characteristics, the gram stain technique, and the biochemical tests reaction each one brought.

Ethical Considerations:

Ethical approval for the study was obtained from the Board of the Faculty of Medical Laboratories Sciences, Shendi University. The written informed consent form was obtained from each guardian of the participant as well as from the subject himself before recruitment into the study. All protocols in this study were done according to the Declaration of Helsinki (1964).

Results

100 UTI-infected patients participated in this cross-sectional study, there were 39 (39%) men and 61 (61%) women among them. According to their age, patients were divided into two groups: those under 40 years old (46%), and those over 40 years old (54%). In Table 1, the presence of microorganisms in urine samples from UTI patients revealed that *E. coli* had a high frequency (46%), followed by *Pseudomonas* and *Klebsiella,* each with 22%, and *Proteus* and *S. aureus,* both with a low frequency. The combination-disk test using Cefotaxime (CTX), Ceftazidime (CAZ), Ceftriaxone (CTR), and Amoxyl alone and in combination with Clavulanic Acid (AAMC) was carried out for the detection of ESBL. The test revealed the effectiveness of the antibiotic in various patterns, including inhibition of growth shown as clear zones (>12 micrometers in diameter) around the drug, indicating the sensitivity of the bacteria, intermediate inhibition of growth Ceftriaxone (CRT) inhibited more germs than Cefotaxime (CTX), Ceftazidime (CAZ), and Amoxyl alone and in combination with Clavulanic Acid (AAMC) in 45%, 40%, and 28% of the isolated bacteria, respectively. Different antibiotics' intermediate inhibition was among the nearly identical frequencies of (5, 3, 5, and 8%)% for CRT, CTX, CAZ, and AAMC, respectively, resistance was present among isolated bacteria more frequently (50, 57, 67, and 74%) for CRT, CTX, CAZ, and AAMC, respectively. *E. coli* was found to have a higher frequency of sensitivity and resistance for the four antibiotics utilized, as shown in Table 2. when the growth pattern against antibiotics was taken into account (Table 3). Females experienced a higher frequency of bacterial development than males. Additionally, bacterial growth was found in all age groups, but it was significantly more prevalent in the group under 40 years old than in the group older than 40 years old, as shown in Table 4.

Figure 1: Distribution of study group according to age.

Age	Frequency	Percentage (%)
Less than 40 Years	46	46
More than 40 Years	54	54
Total	100	100

Discussion

The purpose of this study was to determine how frequently beta lactamase-resistant bacteria caused Urinary Tract Infections (UTIs) in several hospitals in Khartoum State between May and August 2018. Urine samples were collected and processed in the usual manner of isolation, identification of the *bacteria (*E. coli, pseudomonas, Klebsiella, proteus, and S. aureus), and then sensitivity testing using antibiotics from the third generation of Cephalosporins, including cef (AAMC). According to a similar study that found UTI to be the most prevalent extra intestinal infectious disease entity in women worldwide, with frequent

Figure 1: Distribution of study group according to age.

Bacteria	Growth pattern	Total	CRT	CTX	CAZ	AAMC
E. coli	Sensitive		20	16	1	11
	Intermediate	46	3	2	3	3
	Resistant		23	28	30	33
Pseudomonas	Sensitive		13	9	2	4
	Intermediate	20	-	1	6	1
	Resistant		7	12	14	17
Klebsiella	Sensitive		7	10	7	3
	Intermediate	22	2	-	-	2
	Resistant		13	12	15	17
	Sensitive		2	2	-	-
S. aureus	Intermediate	3	-	-	-	1
	Resistant		1	1	3	2
	Sensitive		3	3	2	-
Proteus	Intermediate	7	-	-	-	1
	Resistant		4	4	5	6

Table 3: Frequency of bacterial growth types among genders.

Bacteria	Gender		Total
	Male	Female	
Pseudomonas	10 (25.6%)	12 (19.7%)	22 (22.0%)
Klebsiella	8 (20.5%)	14 (23.0%)	22 (22.0%)
E.coli	19 (48.7%)	27 (44.3%)	46 (46.0%)
S. aureus	0 (0.0%)	3 (4.9%)	3 (3.0%)
Proteus	2 (5.1%)	5 (8.2%)	7 (7.0%)
Total	39 (100.0%)	61 (100.0%)	100 (100.0%)

Table 3: Frequency of bacterial growth types among age.

Bacteria	Age		Total
	<40 Years	>40 Years	
Pseudomonas	10 (21.7%)	12 (22.2%)	22 (22.0%)
Klebsiella	12 (26.1%)	10 (18.5%)	22 (22.0%)
E.coli	19 (41.3%)	27 (50.0%)	46 (46.0%)
S. aureus	1 (2.2%)	2 (3.7%)	3 (3.0%)
Proteus	4 (8.7%)	3 (5.6%)	7 (7.0%)
Total	46 (100.0%)	54 (100.0%)	100 (100.0%)

recurrence and a wide range of associated morbidities, more women than men had UTIs [18]. In addition, grouping the study's infected participants by age—more than 40 years and less than 40 years showed that the infection frequency was higher among the older group of patients. This finding is partially consistent with the study's conclusion, which stated that adults are more likely than children to experience UTIs and that this could be because of asymptomatic infection and antibiotic resistance. *E. coli* was found to be more common than other bacteria in this study, as evidenced by the fact that it was isolated from 46 (46% of the patients), *Pseudomonas* and *Klebsiella* were each isolated from 22 (22%), *Proteus* and *S. aureus* were isolated from 7 (7%), and 3 (3%) patients, respectively. When all isolated bacteria were tested for sensitivity to cephalosporin medicines (CTR) for Ceftriaxone, 45% of them showed sensitivity or bacterial growth inhibition, 5% showed intermediate inhibition, and 50% showed resistance. About CTX for Cefotaxime, it had an impact and reduced growth by 40%, intermediate inhibition by 3%, and resistance by 57%. 28% of isolated bacteria had their growth suppressed by CAZ for Ceftazidime, whereas 5% showed intermediate suppression and 67% displayed resistance.

The next step was AAMC for Amoxyl alone and in combination with Clavulanic acid, which decreased growth by 18%, intermediate inhibition by 8%, and resistance by 74%. *E. coli* was the most common pathogen (60.4%), according to a few studies, the first of which was a Brazilian study that examined the frequency and antimicrobial susceptibility of uropathogens isolated from community-acquired urinary tract infections in

the city of Natal, the state capital of Rio Grande do Norte, in northeastern Brazil, from 2007 to 2010. Enterobacteria were resistant to ciprofloxacin and sulfamethoxazole-trimethoprim by 24.4% and 50.6%, respectively, in terms of uropathogenic susceptibility rates. Nitrofurantoin, aminoglycosides, and third-generation cephalosporins were all susceptible to over 90% of the population. The use of these medications as empirical therapies, particularly in patients with pyelonephritis, is called attention to by the high rates of uropathogenic resistance to quinolones and sulfamethoxazole-trimethoprim. Local knowledge of uropathogenic susceptibility rates is crucial for treatment decision-making for patients with urinary tract infections due to the growing resistance of community bacteria to antimicrobials [19].

The other study looked at how well third-generation Cephalosporins worked against distinct clinical isolates from different clinical labs in Karachi, Pakistan. Methodology: 100 clinical isolates of *E. coli, Enterococci, K. pneumoniae, P. vulgaris, P. aeruginosa,* and *S. aureus* were obtained from patients between December 2013 and May 2014 based on practical sampling. The sensitivity profiles of the clinical isolates were evaluated using Mueller-Hinton broth and agar. Cefotaxime, Ceftizoxime, and Ceftriaxone were the third-generation cephalosporins that were examined against the clinical isolates. Ceftizoxime was shown to be 79% resistant to *E. coli*, whilst Cefotaxime and Ceftriaxone were 67% resistant. Enterococcus was completely resistant to the medicines. Against *Klebsiella spp.*, resistance to Cefotaxime, Ceftriaxone, and Ceftizoxime was 47%, 73%, and respectively. Resistance to Cefotaxime, Ceftriaxone, and Ceftizoxime in *Proteus spp.* was 60% for each drug. Ceftriaxone 77%, Ceftriaxime 58%, and Cefotaxime 50% were found to be resistant to *S. aureus*, whilst Ceftizoxime 91%, Cefotaxime 73%, and Ceftriaxone 64% were found to be resistant to *P. aeruginosa* [20], Additionally, this study's analysis of the antibiotic resistance patterns of isolated Gram (-ve) bacteria against third-generation Cephalosporins brought about the sensitivity and resistance in *E. coli* plates against the 4 antibiotics used, which is consistent with a retrospective cross-sectional study that examined antibiotic resistance patterns in urinary tract infections and the changing ratio of antibiotic resistance by years.

Between 2001 and 2003, 465 uncomplicated Gram (-ve) urinary tract infections obtained in the community were examined, and 400 between 2011 and 2014. The majority of patients (61%) were female (1.5 female : 1 male). 60% of the bacteria isolated during study period 1 and 73% of the bacteria isolated during study period 2 were *Escherichia coli.* Between 2011 and 2014, bacteria other than *E. coli* showed higher levels of resistance to all antimicrobials than *E. coli* bacteria, except for trimethoprim-sulfamethoxazole. Between the two periods, our investigation discovered rising trends in urinary pathogen resistance to Cefixime (from 1% to 15%, p 0.05), Amikacin (from 0% to 4%, p 0.05), and ciprofloxacin (from 0% to 3%, p 0.05). Nitrofurantoin decreased urinary pathogens (from 17% to 7%, p=0.0001) in a trend. Amoxicillin-Clavulanate (from 44% to 43%), Cefazolin (from 39% to 32%), trimethoprim-sulfamethoxazole (from 32% to 31%), Cefuroxime (from 21% to 18%), and Ceftriaxone (from 10% to 14%) between the two periods did not show any discernible changes (p>0.05) [21].

Conclusion

The effects of antibiotics showed response and resistance (more than good response) and in-between patterns, leading to the theory that many prescribed drugs have been used and the severity of the disease moves aside as they gain resistance from UTI causative agents. Patients with urinary tract infections were attacked by multiple bacteria, which caused their illness.

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Conflict of Interest

The author has affirmed that there are no conflicting interests.

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