



Detection of Extended-spectrum β -lactamases' (ESBLs) Resistance among Urinary Tract Pathogens in Khartoum State

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Authors' contributions

This work was carried out in collaboration between both authors. Author EFFE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed literature searches. Authors EFFE and WIE managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study is to isolate and identify the extended spectrum beta lactamase (ESBLs), the causative agents of urinary tract infection and detection of their resistance against β lactam drugs.

Study Design: Descriptive cross sectional studies in which 100 patients with UTI.

Place and Duration of Study: This study was conducted at the Department of Microbiology, Faculty of Medical Laboratories Science, Al-Neelain University, Khartoum – Sudan from “1st September to 31th December 2014.

Methodology: One hundred urine samples were collected from Khartoum state Hospitals and identified on the basis of their culture characteristics and morphological appearance using Gram stain technique and biochemical tests. The isolates were subjected to antimicrobial susceptibility against the third generation cephalosporins (Cefotaxime, Ceftazidime and Ceftriaxone) using Disk-

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Diffusion method. The bacterial isolates were inoculated to show their ability to produce ESBL using Combination Disk Method (Calvulanic acid + Third generation cephalosporins). The ESBL producers were evaluated among non-ESBL producers.

Results: The isolates were identified and enumerated as *E. coli* (49), *Proteus* species (15), *Klebsiella pneumoniae* (18), *Pseudomonas aeruginosa* (9), *Enterococcus faecalis* (7) and 2 Gram positive bacteria (*Staphylococcus* species). The isolates subjected were found to show their antibacterial susceptibility against third generation cephalosporins (Cefotaxime, Ceftazidime, and Ceftriaxone) and the results observed were that sixty out of one hundred isolates depicted resistance to third generation cephalosporins and it included *E. coli* (29), *Klebsiella pneumoniae* (18), *Proteus* species (7), *Pseudomonas aeruginosa* (4) and *Enterococcus faecalis* (2). Among sixty bacterial species that showed resistance to the third generation cephalosporins, *E. coli* 20(68%), *Klebsiella pneumoniae* 7(38%), *Proteus* species 3(42%) and *Enterococcus faecalis* 1(50%) respectively were found to be ESBL producer. *Pseudomonas aeruginosa* showed non-ESBL producers.

Conclusion: We conclude that the ESBL producers were found in large proportion which may be due to the misuse of antibiotics or inadequate treatment.

Keywords: *Extended-Spectrum- β lactamases (ESBL); gram negative bacteria; combination disk method, disk diffusion method; cephalosporins; urinary tract pathogens.*

1. INTRODUCTION

The urinary tract is a system in the body for removing waste and extra water. It consists of bladder, kidneys, ureters, and urethra. The kidneys filter the blood and remove waste and extra water which becomes components of urine. Urinary tract infections are the second most common infection in the body. Over 50% of the women develop UTI. The majority of UTIs is caused by *E. coli*, the common symptoms include frequent urination & painful burning sensation while urination [1].

UTIs diagnosis usually is made by observing symptoms and examining urine samples of infected patients. UTI infections are commonly treated with antibiotics e.g. Gentamicine, ciprofloxacin, ceftriaxone, cefixime, cefuroxime, nalidixic acid and amoxicillin-clavulanate, but observed that bacterial causing UTI exhibits resistance against wide range of beta lactam antibiotics mentioned above. Beta-lactamase is enzymes produced by bacteria that inactivate either penicillin or cephalosporine, but some can inactivate both classes of drugs. Most Gram positive bacteria secrete beta-lactamases so that beta-lactam drugs are inactivated extracellularly, i.e., in the surrounding medium. By contrast, the beta-lactamase of Gram negative remains inside the cell and inactivate beta lactam drugs in periplasmic space, i.e., the space between the outer membrane and cytoplasmic membrane [2].

The term ESBLs is used to mean acquired class A β -lactamases that hydrolyse and confer

resistance to oxyimino- '2nd - and 3rd -generation' cephalosporins, eg cefuroxime, cefotaxime, ceftazidime and ceftriaxone [2].

ESBLs are not the only β -lactamases to confer resistance to 2nd and 3rd generation cephalosporins while sparing carbapenems, but are the most important. Moreover, as plasmid-mediated enzymes, they have great potential to spread. They occur mostly in Enterobacteriaceae (eg *E. coli*, *Klebsiella* species and *Enterococcus* species) and rarely in non-fermenters (eg. *P. aeruginosa*). They should be distinguished from other important modes of resistance to 2nd and 3rd generation cephalosporins [3].

Between February 2008 and January 2009 in India, a total of 213 isolates were tested for ESBLs production by using both the double disk approximation and the combination disk methods [4].

Among the *E. coli* (132), *Klebsiella* specie (54), *Pseudomonas* specie (27) isolates were tested, 81%, 74%, and 14%, respectively were found to be ESBLs producers [4].

In 2011, study was done in a Chennai suburban tertiary care hospital (India) and its antibiogram pattern a total of 131 urine sample were collected from out patients clinically suspected to have UTIs Out of 131 urine samples obtained, 126 samples were considered positive for UTI; the positive samples included *E. coli* (58), *Klebsiella* species (39), *Proteus mirabilis* (13), *Pseudomonas areuginosa* (9), *Staphylococcus* species (6) and *Citrobacter* species (1). Only *E.*

coli and *Klebsiella* species were included to detect ESBLs production, and the result was positive for both [5].

The resistance against β - lactam has become one of the major problems in therapy of urinary tract infections in our country as well as world. [6]

The aim of this study is to isolate and identify the extended spectrum beta lactamase (ESBLs), the causative agents of urinary tract infection and detection of their resistance against β lactam drugs.

2. MATERIALS AND METHODS

2.1 Experimental Work

This descriptive cross sectional studies included 100 individual of UTI induced. Approval was obtained from Al-Neelain Research Ethical Board.

2.2 Collection of Specimen

The mid-stream urine was collected in clean, dry, wide neck, screw capped container [7].

2.3 Isolation of Pathogens

Urine sample was inoculated into Citrate Lysine Electrolyte Deficiency (CLED) media and incubated at 37°C over night. The isolates were identified base on of their cultural characteristics, Gram stain technique and their reaction of biochemical tests [7].

2.4 Antibiotic Susceptibility Test

An overnight culture suspension of the test isolates which was adjusted to 0.5 McFarland's standard was inoculated by using a sterile cotton swab on the surface of a Mueller Hinton Agar plate. Susceptibility of different isolates against 30- μ g disc of Cefotaxime, Ceftazidime, and

Ceftriaxone was performed using Kirby-Bauer disc diffusion technique placed 15 mm apart. After incubating overnight at 37°C, the presence of zones of inhibition was interpreted as positive for 3rd-generation cephalosporins susceptibility [2].

2.5. Detection of Extended-Spectrum β -Lactamases' (Esbls) Resistance for UTI Resistant Isolates

2.5.1. Combination disk method

The combination-disk test using Cefotaxime, Ceftazidime, Ceftriaxone, and Amoxyl alone and in combination with clavulanic acid, were performed for the detection of ESBL according to the Clinical Laboratory Standards Institute (CLSI) guidelines [8]. In this test, an overnight culture suspension of the test isolates which was adjusted to 0.5 McFarland's standard was inoculated by using a sterile cotton swab on the surface of a Mueller-Hinton Agar plate. The Cefotaxime (30 μ g) and cefotaxime-clavulanic acid (30 μ g / 10 μ g) disks were placed 20 mm apart on the agar. Similarly, the ceftazidime (30 μ g) and ceftazidime-clavulanic acid (30 μ g / 10 μ g) disks were placed 20 mm apart. This step was repeated with ceftriaxone, ceftriaxone-clavulanic acid and amoxyl-calvulanic acid. After incubating overnight at 37°C, a diameter \geq 5-mm increase in the zone diameter for either antimicrobial agent which was tested in combination with clavulanic acid vs. its zone when tested alone was interpreted as positive for ESBL production [8].

3. RESULTS AND DISCUSSION

As can be seen in Table 1, total of 100 urine samples were collected from patients of UTI from hospitals of Khartoum State – Sudan during September-December 2014.

Table 1. The percentage of resistance for 3rd-generation cephalosporins and their rate to producing ESBL

Isolated Microorganisms	No. of isolates	Urine samples from Khartoum State Hospitals		
		Resistance to 3 rd -generation cephalosporins	No. of ESBL producers	Percentage of ESBL producers
<i>E. coli</i>	29	(48.33%)	20	(68.00%)
<i>K. pneumoniae</i>	18	(30.00%)	7	(38.00%)
<i>Proteus species</i>	7	(11.67%)	3	(42.00%)
<i>Enterococcus faecalis</i>	2	(3.33%)	1	(50.00%)
<i>Pseudomonas aeruginosa</i>	4	(6.67%)	0	(0.00%)
Total	60		31	

Five bacterial isolates, namely, *E. coli* (49), *K. pneumoniae* (18), *Proteus* species (15), *Enterococcus faecalis* (7), *Pseudomonas aeruginosa* (9) and (2) Gram positive bacteria (*Staphylococcus* species) were isolated from these specimens but only 60 (60%) microorganisms showed resistance against the third-generation cephalosporins and they were tested for the ESBL production. These findings were in agreement with Yuksel et al., [9] who reported that the UTI most causative agent was *E. coli* (87% of cases) followed by *Klebsiella pneumoniae* (10%).

These tested microorganisms exhibit resistance to the third-generation cephalosporins as *E. coli* 29 (48.33%), *K. pneumoniae* 18 (30.00%), *Proteus spp* 7 (11.67%), *Enterococcus faecalis* 2 (3.33%), and *Pseudomonas aeruginosa* 4 (6.67%). The overall of ESBL producer is 31(51.67%); among these isolate, *E. coli* 20(68%), *K. pneumoniae* 7(38.00%), *Proteus* species 3(42.00%), *Enterobacter faecalis* 1(50.00%) were detected to be ESBL producers. *Pseudomonas aeruginosa* did not exhibit any observation of ESBL production Fig. 1.

The findings of this study are supported by Umadevi et al. [4] who reported that the overall ESBL producers were 74.32% and the overall isolates which exhibit to 3rd-generation

cephalosporin were 67% which is disagree with our findings which appeared as 60% for 3rd-generation cephalosporins resistance and 51.67% for ESBL production.

Bradford [10] states that the resistance to extended spectrum cephalosporins is mainly mediated by the production of ESBLs and it is also agreed with this study.

In this study we found that the common ESBL producer is *E. coli* (68.00%), followed by *K. pneumoniae* (38.00%) and this is in disagreement with the findings of Jarlier et al. [11], Walsh et al. [12], and Nayal et al. [13] who stated that *K.pneumoniae* was observed high resistance to ESBL more than *E. coli*.

Also Walsh et al. [12], Nayal et al. [13], and Singhai et al. [14] reported *Pseudomonas* specie ESBL production is less as compared to *Enterococcus faecalis*, because their resistance is mediated by various other mechanisms such as the production of metallo-beta-lactamases, lack of drug penetration due to mutations in the porins and the loss of certain outer membrane proteins and efflux pumps which is in agreement with the present findings. Also the present study results reveal that none of *Pseudomonas* was ESBL producer.



Fig. 1. ESBL production by *E. coli* to Ceftriaxone-clavulanic acid (CTC), Ceftazidime-clavulanic acid (CZC), and Cefotaxime-clavulanic acid (CFC); Amoxyl-clavulanic acid (AMC) as control to β -lactam antibiotics

Babypadmini and Appalaraju [15] stated a report from Coimbatore (India) that ESBL production was 41% in *E. coli* and 40% in *K. pneumoniae* which is almost near to our observation in this study as 38.00% in *K. pneumoniae*.

In a similar study by Babypadmini and Appalaraju [14], 62% of the *E. coli* and 73% of the *K. pneumoniae* isolates were reported to be ESBL producers and less than our study 68.00% in *E. coli*.

4. CONCLUSION

In conclusion, the ESBL-producing organisms were multidrug-resistant pathogens that are increasing rapidly and have become a major problem in the area of infectious diseases. It is essential to report ESBL production along with the routine sensitivity reporting, which will help the clinicians in prescribing proper antibiotics. Further researches relying on molecular techniques are very important to confirm these results.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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