Original article

Extended spectrum β-lactamases in urinary isolates of *Escherichia coli* - prevalence and antibiogram at a tertiary care referral hospital in Khartoum, Sudan

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Abstract

Escherichia coli (*E. coli*) producing extended-spectrum β-lactamase (ESBL) has become prevalent globally, thus severely limiting the therapeutic management. This study was carried out to determine the prevalence and susceptibility pattern of ESBL producing *E. coli* isolates among urinary isolates at Soba University Hospital, Sudan, from March to December 2018. A hundred *E. coli* urinary isolates were obtained; ESBL production and antimicrobial susceptibility were estimated using CLSI guidelines. ESBL phenotype prevalence among the hundred study isolates is, 56%. Most of the ESBL isolates are from inpatient, 64.7% of 79 while 23.8 % of 21 are from outpatient (P = 0.0008), the frequency of ESBLs production is significantly higher in females, 66.7% (42/63) than males 37.8% (14/37) (P = 0.005), however, there is no significant difference among age groups. The result of ESBL producers *E. coli* antibiogram reveals resistant to cefotaxime, 100%, ceftriaxone,100%, ceftazidime, 98.2% and norfloxacine, 89.3%, while the resistance of non ESBL-producers isolates are to co-trimoxazol, 54.5%, nor floxacine, 52.3% and tetracycline, 43.2%. However, the maximum sensitivity of both ESBL-producers and non-producers isolates are seen for imipenem, 100% vs 100 percentage, followed by piperacillin/tazobactum, 80.4% vs 97.7% and amikacin, 42.9% vs 88.6%. In conclusion, results reveal the prevalence of ESBLs among uropathogenic *E. coli* is high in Sudan, thus, further consideration should be needed for control.

Keywords: *E. coli*, ESBL**,** multi-drug resistance.

Introduction

Beta-lactam (β-lactam) antibiotics are a wide range group of antibiotics that commonly used to treat infectious diseases [1]. They consist of the antibiotic agents that enclosed in their molecular structure a β-lactam ring; many examples can be encountered such as carbapenems, monobactams, cephalosporins and penicillins. Many strains of bacteria developed resistance against β-lactam antibiotics via the production of a β-lactamase enzyme that break down the β-lactam ring [2]. Resistance in gram-negative bacteria was increasing, mainly due to the spread of strains producing extended-spectrum β-Lactamases (ESBLs). Options in the treatment of ESBL-producing organism infections were extremely limited; carbapenems were the treatment of choice for serious infections due to such organisms. To overcome resistance, scientists developed a new class of antibiotics. Which were β-

lactamase inhibitors, such as clavamox which was a combination of amoxicillin and clavulanic acid [3]. ESBL producer *E. coli* resisted many of the antimicrobial agents usually recommended for the treatment of its infection, such as penicillin, sulfamethoxazole, trimethoprim and cephalothin, in a frequency of 100%, 30.89%, 16.26% and 20.32%, respectively [4]. The frequency of UTI varies with age and sex, and *E. coli* were responsible for 80% of community-acquired and 40% of hospital associated infection [5].

The study is performed to determine the frequency of (ESBL) and antibiogram for *Escherichia coli* isolates causing urinary tract infection in Soba University Hospital, Khartoum State, Sudan, to guide the suitable treatment strategies for this infection.

Materials and methods

Study design

This is a cross sectional study, conducted during the period from March to December 2018. Samples of *E.coli* isolates obtained from urine collected randomly from various hospital units within Soba University Hospital, which were tested for phenotypic production of ESBLs. The hospital units included medical, surgical and referral clinics units.

The study was conducted at the Microbiology Laboratory Department of National Health Laboratory (ASTAK), Khartoum, Sudan. It serves as the main National Referral Laboratory for Sudan.

Sample size and data collection

Random selection of 100 *E. coli* isolates was taken from urinary samples which isolated during the study period. Data of the isolates were collected from the laboratory records.

Isolates identity confirmation, purity and preservation

Isolates were transported to the National Laboratory, Khartoum, where all phenotypic tests had been done. Single colonies were selected and confirmed by standard bacteriological technique [6].

Colonies inoculated by streaking on MacConkey agar media for the identity confirmation of *E. coli* and after 24 hour incubation, isolates were further characterized using conventional/standard microbiology techniques (colonial morphology, Gram-staining, catalase, indole, citrate utilization, Voges-Proskauer, methyl red and urease tests).

Susceptibility test

A modified Kirby- Bauer susceptibility testing method was used to assess the sensitivity and resistance patterns of the isolates. On Mueller Hinton agar (HiMedia, India), a suspension of tested isolate which was compared with 0.5% Macfarland standard was seeded. A set of antibiotics discs were applied which included imepenem10µg, ciprofloxacin 30µg, co-

trimoxazole 30µg, ceftazidime 30µg, cefotaxime 30µg, ceftriaxone 30µg, amikacin 30µg, norfloxacine, tetracycline and pipercilline/tazopactam (HiMedia, India). Plates were incubated aerobically overnight at 37˚C. Zones of inhibition were measured in mm and compared to a standard interpretation chart [6].

A double disc synergy test (DDST) was used to detect ESBLs. All *E. coli* isolates which showed a diameter of or less than 17 mm for Ceftazidime and or less than 22 mm for Cefotaxime were selected for checking the ESBLs production, the production of ESBL was tested by using a disc of Amoxicillin/Clavulanic acid (20/10µgHiMedia, India) along with two third generation Cephalosporins; Ceftazidime (30µg) and Cefotaxime (30µg) discs, on Mueller Hinton agar plates lawn of tested strains and *E.coli* ATCC 25922 (negative control) were used.

Amoxicillin/Clavulanic acid (20/10µg) disc was placed in the center of the plate and Ceftazidime center to center to Amoxicillin /Clavulanic acid and incubated for 18-24 hours at 37˚C. Any increase in the zone towards the disc of Amoxicillin /Clavulanic acid was considered as a positive result for the ESBL production [7].

Control strains for all the phenotypic testing were *E. coli* ATCC 25922 (negative control) and another *E. coli* strain obtained the Central Heath Laboratory, Sudan, identified by phenotype and genotype, used as positive control.

Ethical consideration

Approval was taken from Shendi University and Soba University Hospital.

Data analysis

Data were analyzed using the Statistical Package for Social Sciences version 20.0 Software (SPSS). A Chi-square test was employed to compare the association of categorical variables. *P*-value <0.05 was considered as statistically significant.

Results and discussion

This study was conducted to detect the extended spectrum β-Lactamases (ESBL) in *E. coli* causing urinary tract infections, whereas a total of 100 *E. coli* isolates were obtained. The female patients isolate were 63 and males were 37. Patient's ages ranged from 8 to 62 years, with mean 35 years.

Dissemination of ESBL-producing bacteria could be attributed to the presence of multiple risk factors such as inappropriate use of broad-spectrum antimicrobials, inappropriate prescription, long duration hospital stay and transfer of ESBL genes [8]. This study shows out of 100 samples isolate of *E. coli,* 56% (56) are ESBL positive. This result is higher than studies carried out in different regions of the world such in Saudi Arabia [9], which reported the ESBL positivity was 35.8%. Studies in Sudan revealed a frequency of 30.2% and 35%

respectively [10,11]. Moreover, this study reveals ESBL positivity lower than that in Uganda, 62% [12] and Egypt, 87% [13]. But the study is consisting to previous reports that ESBL positivity was 55.5% and 57.2% [12,14]. These reports indicated the prevalence of *E. coli*-ESBL producer was variable worldwide and these differences could be attributed to, time at which the study done and the health authority law regulation of antimicrobial regimens in different geographical regions.

The study shows the frequency of *E. coli* ESBL producer in females were 75% (42/56) compared to 25% (14/56) for males, P = 0.005. This agreed with a previous study that ESBLs producers were higher in females, 72.8% than males, 27.2% [15]. The study reveals no significant differences between the age group in ESBL producing *E. coli* isolates. But the most infected age group with ESBL-producing *E. coli* is ≥ 46 years age group, 53.8% (21/39) followed by ≤ 16 years age group, 62.5% (20/32) and 17-45 years 48.3% (14/29). This consistent with a result that no significant differences between ESBL-producing isolates of adults, 31% and 27% for children [10]. The study shows significant differences in ESBLs producing *E. coli* isolates between inpatient departments, 89.3% and outpatient departments, 10.7%, with P = 0.05. This study shows that all ESBL-producing *E. coli* isolates are significantly resistant to ceftazidime, cefotaxime, ceftriaxone, piperacillin/tazobactum, tetracycline, co-trimixazol, ciprofloxacine norfloxacine and amikacin compared to non-ESBL producing isolates with $P < 0.05$ as shown in Table (1). This agreed with a previous report except for amikacin [10]. The high resistances of ESBL-producers isolates are to cefotaxime, 100%, ceftriaxone, 100%, ceftazidime, 98.2% and norfloxacine, 89.3%, while the high resistances of non ESBL-producers isolates are to co-trimixazol, 54.5%, norfloxacine, 52.3% and tetracycline, 43.2%. However the maximum sensitivity of both ESBL-producers and non-producers isolates are seen for imipenem, 100% vs.100%, followed by piperacillin/tazobactum, 80.4%-97.7% and amikacin, 42.9%-88.6%. This is similar to a done study that the resistances of ESBL-producing isolates were to cefotaxime, 98.7%, ceftriaxone, 97.3%, ceftazidime, 81.3% [14]. The lowest rates of resistance in ESBL-producing isolates were observed for imipenem, 2.7% and amikacin, 14.7%. Overall, the study results show an alarm for clinicians that blinded, and no health policy regulation for consumption and prescription of antibiotics must be changed.

In conclusion, this study reveals a high frequency of ESBLs-producing *E. coli* infection among UTI patients, and the multi-drug resistance is more frequent in females and hospital acquired infections. This attributed to the extensive inappropriate used

of antimicrobial therapy. The imipenem remains the most effective treatment for urogenic *E. coli* ESBL producer caused urinary tract infection.

Further phenotypic surveillance of multi-drug-resistant and sensitivity for uropathogenic *E. coli* are needed, in combination with molecular studies of ESBLs producer, which are essential to guide the suitable treatment strategies for this infection in Sudan.

Table 1. Frequency of antimicrobial susceptibility of *E. coli* ESBL producer and non-producer

Antibiotic	Sensitive (%)	Intermediate (%)	Resistance			*P*-value
			ESBL(n=56) No. (%)	Non ESBL(n=44) No. (%)	Total resistance	
IPM	100	00	00	00	00	00
CAZ	23	05	55 (98.2)	17 (38.6)	72	0.001
CTX	22	07	56 (100)	15 (34.1)	71	0.000
CRO	14	18	56 (100)	12 (27.3)	68	0.000
TZP	88	04	07 (12.5)	01 (2.3)	08	0.027
TE	18	23	40 (71.4)	19 (43.2)	59	0.014
SXT	20	12	44 (78.6)	24 (54.5)	68	0.007
CIP	36	02	48 (85.7)	14 (31.8)	62	0.005
NOR	16	11	50 (89.3)	23 (52.3)	73	0.000
AK	63	05	27 (48.2)	05 (11.4)	32	0.000

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