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Phenotypic detection of Extended Spectrum βeta-Lactamase producing Escherichia coli isolated from urinary tract infected Patients in Khartoum, Sudan

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Abstract:

The aim of this study was to determine the frequency of extended-spectrum β-lactamases (ESBLs) in uropathogenic E. coli isolates. A total of 100 E. coli isolates were obtained, among which 43(43%) and 57(57%) were collected from males and females, respectively. Also the distribution of these samples among age group were children 14 (14%), adult 41 (41%) and geriatric 45(45%). All isolates were screened and confirmed for the presence of ESBL phenotypically with Double Disk Synergy Test, and tested for their susceptibility to non Beta-lactam antibiotics. Among the 100 E. coli isolates, only 35% were judged as ESBLs-positive strains. The frequency of ESBLs production was not significantly different between gender as their frequency were 30%(13/43) in males and 38%(22/57) in females; and among age group as their frequency were 29%(4/14)

in children, 34%(14/41) in adult and 37% (17/45) in geriatric. ESBLS producer E. coli were significantly more resistant to Ciprofloxacin, Nitrofurontoin, Gentamycin and Tetracycline. Also most ESBLs producing E. coli isolates were still susceptible to Impenem, Nitrofurontion, Gentamycin and Amikacin. Our results suggest that the frequency of ESBLs among uropathogenic E. coli is currently in progress in Sudan, and there for further studies is needing.

Key words: ESBL, Urinary tract Infections, *E. coli*, antimicrobial resistance, Sudan

INTRODUCTION:

Urinary tract infections (UTIs) is infection of any part of the urinary system, it is the most common infection reported in hospitals and other health care unit, which mainly cause by *E. coli* worldwide(1,2). Some *E. coli* carry genes that provide resistant to many antibiotics, which make the treatment of their infections is very difficult. One of this genes encode for Extended spectrum B-lactamses (ESBLs), which was emerged within the community, particularly among *E. coli* isolated from urinary tract infections (UTIs) with a widespread prevalence and multidrug resistance in many countries worldwide (1,2). As reported ESBLs were an important cause of transferable multidrug resistance in Gram-negative bacteria throughout the world(3).

ESBLs are a heterogeneous group of enzymes that confer resistant to penicillin; 3 and 4 generation cephalosporins and monobactams (2,4,5). Also ESBLs are undergoing continuous mutation, causing the development of new enzymes that showing expanded substrate profiles, and at present there are more than 300 different ESBL variants(6). ESBLs encoded by genes located on large plasmids, which also carry genes for resistant to other antimicrobial agents such as aminoglycosides,

trimethoprim, sulfonamide, tetracycline, and chloramphenicol (7, 8).

The Clinical and Laboratory Standards Institute (CLSI) was recommend, the detection of ESBL in Gram-negative bacteria including *E. coli* by recognizing their decreased susceptibility to the third generation cephalosporins group such as ceftazidime 30 ug, cefotaxime 30ug and ceftriaxone 30ug (8, 9). Once an ESBL is suspected by this screening, it should be confirmed by standardized methods as double-disc synergy test (9,10).

This study was sought to determine the prevalence of ESBL-producing *E. coli* strains among urinary tract infected individuals.

MATERIALS AND METHODS:

This study is a cross sectional, hospital and laboratory base study conducted in Khartoum state, during the period of March to May 2016. A total of one hindered *E. coli* isolates were collected from UTI infected patients in three different hospitals (Soba Universal Hospital; and Dar Elelag private and Royal Care International Hospital). All isolates were identified base on culture characters, Gram stain and standard biochemical tests. Each isolate was tested for its susceptibility to routine antibiotics then screened and confirmed phenotypically for the presence of ESBLs production by DDST.

Antimicrobial susceptibility testing:

The susceptibility of *E. coli* isolates to antibiotics (Bioanalyse antibiotics) was examined by modified Kirby-Bauer disk diffusion technique according to Clinical Laboratory Standards Institute (CLSI) 2011 guidelines (11). The antibiotics which were tested included Amikacin (AK 30µg), Gentamicin (GN 10µg), Nalidixic acid (NA 30µg), Ciprofloxacin (CIP 5µg),

Nitrofurantoin(F 300µg), Imipenem (IMP 10ug), Trimethoprim-sulfamethoxazole (TSZ 23.75/1.25µg), and Tetracycline (TE 30µg). Standardized inoculums of bacterial suspension equivalent to 0.5 McFarland standard turbidity of each isolate was inoculated on Muller-Hinton agar plate (Himedia) by using a sterile cotton swab then with sterile forceps the disk of each antibiotic was placed on a plate. All plates were incubated at 37 ºC for 18 hours aerobically, then the inhibition zone was interpreted according to CLSI 2011 guidelines (11). *E. coli* ATCC 25922 was used as Control strain.

Phenotypic detection of Extended-spectrum β-lactamase: ESBLS Screening:

This test was done along with susceptibility testing of each isolate. All *E. coli* isolates were screened for ESBL production by using cefotaxime (CTX 30ug), ceftazidime (CAZ 30µg), and ceftriaxone (CRO 30µg). Each *E. coli* isolate showed resistant to one or more of these antibiotics were confirmed for ESBL production by double disk synergy test (DDST) as recommended by the CLSI 2011 guidelines (11).

ESBLS confirmation by DDST:

Standardized inoculums of bacterial suspension equivalent to 0.5 McFarland standard turbidity of each isolate was inoculated on Mueller-Hinton agar plate (Himedia) by using a sterile cotton swab, then with sterile forceps the disk of amoxicillin-clavulanic acid (MAC 30ug) was placed at centre of plate and the disks of cefotaxime (30ug), ceftazidime (30µg), and ceftriaxone (30µg) were placed (centre to centre) at 20 mm distance from MAC 30ug disk. After incubation at 37 ºC for 18hours aerobically, a clear extension of the edge of the inhibition zone of cephalosporin towards MAC 30ug disk was interpreted as positive for ESBL production(12). *E. coli* strain ATCC 25922 was used as negative controls and anther *E. coli*

strain known as ESBLs positive by phenotypic and genotypic method (PCR and DNA sequencing) was used as a positive control (13).

RESULTS

A total of 100 *E. coli* isolates were recovered from UTI patients from different hospital in Khartoum State-Sudan. Out of 100 *E. coli* isolates tested, only 35(35%) were found to be ESBL-producers by phenotypic methods. The proportion of ESBL-producing *E. coli* did not significantly differ between males and females as it is 30% and 38% respectively, as shown in Table (1). Also the proportion of ESBL-producing *E. coli* did not significantly differ among children, adult and geriatric, as it is 29%, 34% and 37% respectively, as shown in Table (2). Among the ESBL-producing *E. coli*, the highest resistance rates were observed for (Naldixic acid 88%, Tetracycline 85%, trimethoprim-sulfamethoxazole 83%, and ciprofloxacin 80%). And the highest antimicrobial activity against *E. coli isolates* were observed to Impenem (100%), Nitrofurontoin, (85%) Gentamycin(71%) and amikacin (63%), as seen in Table (3).

ESBL-producing *E. coli* isolates were significantly more resistant to gentamicin ($p < 0.05$) as shown in table(3).

Table (1): Show the frequency and percentage of ESBLS producer and non-ESBLS producer *E. coli* isolates among gender.

Gender	ESBLs		Total
	Positive	Negative	
Male	13(30%)	30 (70%)	43(43%)
Female	22(38%)	35(62%)	57(57%)
Total	35(35%)	65(65%)	100(100%)

Table (2): Show the frequency and percentage of ESBLS producer and non-ESBLS producer *E. coli* isolates among age group.

Age group	ESBLs		Total
	Positive	Negative	
Children	4 (29%)	10 (71)	14(100%)

Adult	14 (34%)	27 (66%)	41(100%)
Geriatric	17 (37%)	28 (63%)	45(100%)
Total	35(35%)	65(65%)	100(100%)

Table (3): Show the susceptibility of ESBLS producer and non-ESBLS producer *E. coli* isolates to antibiotics.

Antibiotics	ESBLS	Susceptibility			P.value
		Sensitive	Intermediate	Resistant	
Amikacin 30µg	Positive	22 (63%)	7 (20%)	6 (17%)	0.903
	Negative	42(65%)	12 (18%)	11 (17%)	
Gentamycin 10µg	Positive	25 (71%)	3 (9%)	7 (20%)	0.020
	Negative	31 (48%)	7 (10%)	27 (42%)	
Ciprofloxacin 5µg	Positive	7 (20%)	0 (0%)	28 (80%)	0.244
	Negative	19 (29%)	2 (3%)	44 (68%)	
Naldixic acid 30µg	Positive	2 (6%)	2(6%)	31(88%)	0.051
	Negative	14 (21%)	3(5%)	48(74%)	
Nitrofurontoin 300µg	Positive	30 (85%)	1(3%)	4(12%)	0.071
	Negative	43 (66%)	8(12%)	14(22%)	
Impenem 10µg	Positive	35(100%)	0(0%)	0(0%)	0.060
	Negative	61(94%)	0(0%)	4(6%)	
Co-trimoxazole 100µg	Positive	5(14%)	1(3%)	29(83%)	0.426
	Negative	14(21%)	1(2%)	50(77%)	
Tetracycline 30µg	Positive	4(12%)	1(3%)	30(85%)	0.276
	Negative	13(20%)	2(3%)	50(77%)	

DISCUSSION:

In recent years, the problem of increasing resistance to antibiotics has threatened the entire world. Production of beta-lactamase, which hydrolyses and inactivates beta-lactam antibiotics, has been one of the most important resistance mechanisms of many bacterial species, mainly in the *Enterobacteriaceae* family. Resistance to an extended spectrum beta-lactams among Gram-negative pathogens is increasingly associated with ESBLs. *E. coli* is a one of the most ESBL-producing microorganisms (14, 15, 16, and 17).

In this study the ESBLs producing uropathogenic *E. coli* isolate*s* were 35%. This finding is a little bit higher than those obtained from the studies done by Mutasim 2011(18), Jafar 2012(19) and Pryia 2014(20) who reported ESBLs producer

were 24.5%, 19.02%, and 21.4% respectively. And lower than those obtained from the studies done by Goudarzi M 2014(21), and Aruna K, 2012 (22), who reported the frequency as 55.5%, and 40.6% respectively.

Our study reports ESBLs producers were 30% in males and 38% in females respectively. This finding are lower than result obtained by the study which done by Nwosu 2014(23) that reported ESBLs producers were 41.2% in males, and 52.7% in females. Also lower than other study done by Vidhya 2013(24) who reported ESBLs producers were 47.22% in males, and 52.77% in females.

As reported by our study the frequency of ESBLs among age were 29%in Children, 34% in Adult, and 37% in Geriatric which were lower than the results which obtained by the study did in Pryia 2014(20) that reported ESBLs producers among elderly was 53%.

This study showed that all ESBLs producers were sensitive to impenem (100%); and most were sensitive to Nitrofurontoin (85%), Gentamycin (71%), and amikacin (63%); while are higher resistant to trimethoprim-sulfamethoxazole (83%), tetracycline(85%), Nalidixic acid (88%), and ciprofloxacin (80%) than non-ESBLs producers. This result agree with other study which done by Akram 2010 (25) who reported that all ESBLs producers were sensitive to impenem (100%); and resistant to trimethoprim-sulfamethoxazole (100%), nalidixic acid (100%), and ciprofloxacin(97.96%). And agree with other study which done by Aruna K 2012 (22) who reported the resistant to Nalidixic acid and ciprofloxacin were 90%, and 72.05% respectively.

CONCLUSIONS:

The current situation of Multi-drug-resistant (MDR) bacteria has become a worrisome issue in UTI. MDR ESBL-producing

uropathogenic *E. coli* undoubtedly will limit the clinicians choices to treat their patients with UTIs. There for, there is an urgent need for surveillance studies on antimicrobial resistance and prevalence of ESBLs among uropathogenic *E. coli* isolates to guide the clinical treatment of UTIs in Sudan in the future.

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Authors' contributions

All authors contributed equally to this work.