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Extended-Spectrum β-lactamases and AmpC Production among Uropathogenic Isolates of *Escherichia coli* and Antibiogram Pattern

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***Abstract*—Emergence of drug resistance in *Escherichia coli* due to various mechanisms makes the treatment choices very limited. The objective of this research was to investigate extended-spectrum beta-lactamases (ESBLs) and AmpC lactamases in *E. coli* isolates from urinary tract infections (UTIs) and to assess their antibacterial susceptibility patterns in a health-care context. A total of 70 *E. coli* isolates from clinically assumed cases of UTI patients during the 9 months period. The isolates with bacteriuria (10^5 CFU/ml) were identified. ESBL and AmpC were detected phenotypically. Out of the 70 isolates of uropathogenic *E. coli*, ESBL production was detected in 34 (48.6%) isolates and AmpC producer in 27 (38.6%) of isolates in which 14 (20%) of them showed coexistence phenotype of both ESBLs and AmpC and 23 (32.9%) *E. coli* isolates were both ESBL and AmpC non-producer. The findings donated information regarding drug resistance. The level of resistance recorded in ESBL- and AmpC-producing uropathogenic *E. coli* of this study was raising; therefore, it is crucial to have a strict infection control measures and routine monitoring of ESBL- and AmpC-producing bacteria in clinical laboratory.**

***Index Terms*—AmpC, ESBL, *Escherichia coli,* Urinary tract infections.**

I. Introduction

Increasing resistance to antimicrobials among pathogens that cause common infections is a problem of global proportions given the paucity of novel antibiotics in development (Osthoff, et al., 2015). *Escherichia coli* has been described to be one of the most predominant pathogens for urinary tract infections (UTIs) (Arsalane, et al., 2015; Laxman and Ashok, 2016). Treatment of these infections is often difficult because of the rising bacterial resistance mediated by varying degrees of newly acquired antibiotic resistance of beta-lactamases which is either plasmid or chromosomally mediated (Sheemar, et al., 2016; Alyamani, et al., 2017). Extended-spectrum β-lactamases (ESBLs) and AmpC have become increasingly common globally and have appeared as a major source of multidrug resistance in clinically important *E. coli* (Alqasim, Abu Jaffal and Alyousef, 2018). The hydrolysis of β-lactamases is the most common and efficient process by which microorganisms can become resistant to this category of antibacterial drugs (Rani, et al., 2016). They are typically plasmid-mediated enzymes that are capable of hydrolyzing a wide variety of penicillin and cephalosporin antibiotics such as cefotaxime, ceftriaxone, cefepime, ceftazidime, and monobactam antibiotics, among other things (Thenmozhi, et al., 2014). However, the ESBL-producing bacteria remain susceptible to commercially available β-lactamase inhibitors such as clavulanic acid and sulbactam (Sheemar, et al., 2016). AmpC β-lactamases belong to the molecular Class C as classified by Ambler under a classification scheme of Bush, Jacoby and Medeiros, 1995. The presence of AmpC class β-lactamase demonstrated to be plasmid encoded or chromosomally mediated differentiated from ESBLs by their resistant to cephamycins (e.g. cefoxitin and cefotetan), as well as β-lactam plus β-lactamase inhibitor combination (Kaur, Gupta and Chhina, 2016), cloxacillin and phenylboronic acid, on the other hand, inhibit their activity (Rodríguez-Guerrero, et al., 2022). Furthermore, clinical isolates *E. coli* possessing plasmid encoding AmpC enzymes often are expressed high levels of resistant further constricting the treatment options (Coudron, Moland and Thomson, 2000). Cross-transmission of ESBL- and AmpC-producing bacteria in hospital milieu has been concerned for nosocomial infections globally making the detection of β-lactamases which is extremely important both for epidemiological purposes and for the prevention and control of infection (Madhumati, et al., 2015; Bandekar, et al., 2011). In the developing countries, molecular detection of these enzymes is still costly to be used, hence in the absence of molecular techniques in many clinical laboratories performing specific and sensitive phenotypic tests that provide an effective and reliable alternative to detect ESBL and AmpC beta-lactamase-producing microorganisms (Barua, Shariff and Thukral, 2013), as the genotypic method will confirm the phenotypic method results (Kazemian, et al., 2019). Therefore, the present study was conducted to demonstrate the burden of ESBL and AmpC production in *E. coli* isolated from urine

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specimens taken from patients diagnosed with UTI and their coexistence using phenotypic methods. A comparative analysis of resistant patterns was also done to compare antimicrobial susceptibility among these isolates.

II. Material and Methods

A. Identification of Bacterial Isolates

A total of 70 *E. coli* clinical isolates were acquired from patients who appeared with symptoms of UTI and were admitted to Rizgary Teaching Hospital in Erbil city. Isolated microorganisms were identified at the species level using standard microbiological methods and confirmed using the Vitek II. These isolates were grown in trypticase soy broth containing (TSB) and stored at −70°C after the addition of 15% glycerol (Šišková, et al., 2015).

B. The Antimicrobial Susceptibility Testing

The antimicrobial susceptibility patterns of the bacterial isolates were examined by the disk diffusion method as recommended by the Clinical Laboratory Standard Institute (CLSI) (Humphries, et al., 2018). Each of the stains was standardized to 0.5 McFarland equivalent and aseptically inoculated on Mueller-Hinton agar (MHA). The inoculated plates were allowed to stand for 10 min. Antibiotic disks, namely, ampicillin (10 μg), ampicillin/sulbactam (20 μg), amoxicillin/clavulanate (20/10 μg), imipenem (10 μg), ertapenem (10 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefepime (30 μg), cefazolin (30 μg), gentamicin (10 μg), levofloxacin (5 μg), nitrofurantoin (300 μg), piperacillin/tazobactam (110 μg), ciprofloxacin (5 μg), and trimethoprim/sulfamethoxazole (1.25/23.75 μg), (Oxoid, UK) were placed on the inoculated plates. Incubation at 37°C for an overnight period was followed by measurement and interpretation of the zones of inhibition in accordance with guidelines (Moroh, et al., 2014).

C. Screening of ESBL-producing Isolates

Isolated bacteria were examined for their susceptibility to third-generation cephalosporins. After overnight incubation at 37°C, any enhancement of the zone of inhibition of the isolate was indicative of the presence of an ESBL as proposed by CLSI guideline and further tested by confirmatory procedures was performed. Bacteria that produce ESBLs were spotted using the Phenotypic Confirmatory Disk Diffusion Test (PCDDT). Concisely, the 0.5 McFarland standard concentration of bacterial suspension was used to inoculate microorganisms onto the MHA surface. Then, ceftazidime (30 μg) disk alone and the combination of clavulanic acid (30 μg/10 μg), as well as cefotaxime (30 μg) alone, combined with clavulanic acid (10 μg) disks were placed 25 mm apart from each other. The plates were incubated at 37°C for 24 h. Isolate that showed an enhancement in the zone of inhibition and an increase of ≥5 mm diameter of the combination disks in comparison to that of the antibiotic disk alone was interpreted as ESBL producer. *E. coli* ATCC 25922 was used as positive controls (Iroha, et al., 2017).

D. Screening for AmpC β-lactamase Production

AmpC disk test was used to identify AmpC-producing isolates. The AmpC disks were formed by applying 20 mL of a 1:1 combination of normal saline and ×100 Tris-EDTA to each of the sterile blank paper disks. The disks were stored at refrigerator 4°C. A lawn culture of the a cefoxitin (FOX)-susceptible strain (*E. coli* ATCC 25922) was inoculated onto the surface of MHA plate. A 30 μg cefoxitin disk was placed on the bacterial lawn. The disks of an AmpC were hydrated, and some colonies of the tested bacteria were spread throughout it. After that, with its inoculation surface close to and touching the agar surface, an AmpC disk was placed into an inoculated agar plate. After overnight incubation at 37°C, the results were interpreted. When there is an indentation or destruction in the zone of inhibition, of cefoxitin disk indicated an enzymatic deactivation of the antimicrobial agent (positive result), and is measured as an AmpC producer, in contrast, A lack of distortion, indicating no inactivation of the cefoxitin disk, was interpreted as evidence of a non-AmpC producer. Out of 70 E. coli strains, 34 (48.6%) were ESBL producer, 27 (38.6%) were AmpC producer in which 14 (20%) isolates showed coexistence phenotype of both ESBLs and AmpC and 23(32.9%) E. coli strains were negative, which were both ESBL and AmpC non-producer, as shown in Table II.

III. Results

The mid-stream urine samples were collected from patients having symptoms, a total of 70 isolates were with significant growth $\geq 10^5$ CFU/ml of *E. coli*. Among these isolates, 20 (28.6%) were male patients whereas 50 (71.4%) belonged to female patients. Through regard to the age groups of patients, maximum proportion 45 (64.3%) were obtained from adults' population, 20 (28.6%) isolates were the elderly, and 5 (7.1%) were belonged to children. Highest percentage were from females 71.4% (n = 50) and 28.6% (n = 20) were obtained from males. Demographic characteristics of patients are presented in Table I.

Out of 70 *E. coli* strains, 34 (48.6%) were ESBL producer, 27 (38.6%) were AmpC producer in which 14 (20%) isolates showed coexistence phenotype of both ESBLs and AmpC and 23(32.9%) *E. coli* strains were negative, which were both ESBL and AmpC non-producer.

Tables III and IV show the comparison of antimicrobial resistance between ESBL and non-ESBL, AmpC and non-AmpC uropathogenic isolates.

Table III reveals the antimicrobial resistance profile of 34 ESBL expressing isolates that are phenotypically positive and 36 non-ESBL producer isolates. The rate of resistance to

Table I
Distribution of Isolates According to Age and Gender

Age category	Gender		Total
	Female	Male	
Children*	4 (5.7%)	1 (1.4%)	5 (7.1%)
Adults**	34 (48.6%)	11 (15.7%)	45 (64.3%)
Elderly***	12 (17.1%)	8 (11.5%)	20 (28.6%)
Total	50 (71.4%)	20 (28.6%)	70 (100%)

*Children: 0–18 years, **adults: 19–64 years, ***elderly: ≥65 years

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all isolates was: In ESBL producer, all isolates were totally resistant for ampicillin, ceftriaxone, and cefazolin. Overall, the highest resistance rate was detected for ceftazidime 94.1%, cefepime 88.2%, and ciprofloxacin 79.4% and the lowest resistant observed for nitrofurantoin 2.9% followed by imipenem and ertapenem 29.4%, while in non-ESBL producer were expressively more susceptible than ESBL producer to most antimicrobial agents.

Of the 70 *E. coli* isolates, 27 (38.6%) were AmpC producers. Antibiotic susceptibility pattern of AmpC-positive isolates disclosed 77.8% to ampicillin, 70.4% ceftriaxone, 66.7% to ciprofloxacin, and 63% to ampicillin/sulbactam, ceftazidime, and trimethoprim/sulfamethoxazole (Table IV), of the 43 (61.4%) non-AmpC producers, the greatest degree of resistance was detected for ampicillin 79.1%, and the lowest rate for nitrofurantoin 2.3% followed by imipenem 14%. A striking resemblance existed between the antibiotic resistance profiles of AmpC producers and non-AmpC-producing isolates.

Table II
Detection of ESBL, AmpC b-lactamase, and ESBL+AmpC among Uropathogenic Isolates

Microorganism	Phenotypic detection of AmpC- and ESBL-producing *E. coli* isolates			
	Screening positive ESBL, *n* (%)	Screening positive AmpC, *n* (%)	Both (ESBL+AmpC), *n* (%)	ESBL+AmpC negative, *n* (%)
E. coli (*n*=70)	34 (48.6%)	27 (38.6%)	14 (20%)	23 (32.9%)

E. coli: *Escherichia coli*

Table III
Comparison of Antibiotic Resistance Pattern of ESBL- and Non-ESBL-Producing *Escherichia coli*

Antibiotic	No. (%) of resistant strains					
	ESBLs producers (*n*=34)			Non-ESBLs producers (*n*=36)		
	Resistant, *n* (%)	Intermediate, *n* (%)	Sensitive, *n* (%)	Resistant, *n* (%)	Intermediate, *n* (%)	Sensitive, *n* (%)
Ampicillin	34 (100)	0	0	23 (63.9)	0	13 (36.1)
Ampicillin/sulbactam	24 (70.6)	3 (8.8)	7 (20.6)	14 (38.9)	2 (5.6)	20 (55.5)
Amoxicillin/clavulanate	17 (50)	8 (23.5)	9 (26.5)	10 (27.8)	6 (16.7)	20 (55.5)
Ceftazidime	32 (94.1)	0	2 (5.9)	10 (27.8)	0	26 (72.2)
Ceftriaxone	34 (100)	0	0	11 (30.5)	1 (2.8)	24 (66.7)
Cefepime	30 (88.2)	0	4 (11.8)	4 (11.1)	0	32 (88.9)
Cefazolin	34 (100)	0	0	8 (22.2)	2 (5.6)	26 (72.2)
Ciprofloxacin	27 (79.4)	1 (2.9)	6 (17.7)	14 (38.9)	2 (5.6)	20 (55.5)
Ertapenem	10 (29.4)	0	24 (70.6)	4 (11.1)	0	32 (88.9)
Gentamicin	18 (52.9)	2 (5.9)	14 (41.2)	7 (19.4)	0	29 (80.6)
Imipenem	10 (29.4)	0	24 (70.6)	2 (5.6)	0	34 (94.4)
Levofloxacin	18 (52.9)	0	16 (47.1)	10 (27.8)	0	26 (72.2)
Nitrofurantoin	1 (2.9)	6 (17.7)	27 (79.4)	2 (5.6)	3 (8.3)	31 (86.1)
Piperacillin/tazobactam	17 (50)	4 (11.8)	13 (38.2)	6 (16.7)	1 (2.7)	29 (80.6)
Trimethoprim/sulfamethoxazole	23 (67.6)	0	11 (32.4)	12 (33.3)	0	24 (66.7)

Table IV
Comparison of Antibiotic-Resistant Pattern of AmpC- and Non-AmpC-Producing *Escherichia coli*

Antibiotic	No. (%) of resistant strains					
	AmpC producers (*n*=27)			Non-AmpC producers (*n*=43)		
	Resistant, *n* (%)	Intermediate, *n* (%)	Sensitive, *n* (%)	Resistant, *n* (%)	Intermediate, *n* (%)	Sensitive, *n* (%)
Ampicillin	21 (77.8)	0	6 (22.2)	34 (79.1)	0	9 (20.9)
Ampicillin/sulbactam	17 (63)	2 (7.4)	8 (29.6)	19 (44.2)	2 (4.7)	22 (51.1)
Amoxicillin/clavulanate	12 (44.4)	6 (22.2)	9 (33.4)	15 (34.9)	8 (18.6)	20 (46.5)
Ceftazidime	17 (63)	0	10 (37)	25 (58.1)	0	18 (41.9)
Ceftriaxone	19 (70.4)	0	8 (29.6)	26 (60.5)	1 (2.3)	16 (37.2)
Cefepime	14 (51.9)	0	13 (48.1)	14 (32.6)	0	29 (67.4)
Cefazolin	15 (55.6)	1 (3.7)	11 (40.7)	15 (34.9)	1 (2.3)	27 (62.8)
Ciprofloxacin	18 (66.7)	3 (11.1)	6 (22.2)	23 (53.5)	0	20 (46.5)
Ertapenem	7 (25.9)	0	20 (74.1)	7 (16.3)	0	36 (83.7)
Gentamicin	9 (33.3)	0	18 (66.7)	16 (37.2)	1 (2.3)	26 (60.5)
Imipenem	6 (22.2)	0	21 (77.8)	6 (14)	0	37 (86)
Levofloxacin	14 (51.9)	0	13 (48.1)	14 (32.6)	0	29 (67.4)
Nitrofurantoin	3 (11.1)	3 (11.1)	21 (77.8)	1 (2.3)	5 (11.6)	37 (86.1)
Piperacillin/tazobactam	9 (33.3)	1 (3.7)	17 (63)	14 (32.6)	4 (9.3)	25 (58.1)
Trimethoprim/sulfamethoxazole	17 (63)	0	10 (37)	19 (44.2)	0	24 (55.8)

IV. Discussion

UTIs have been established to be the most encountered bacterial infection and leading patients to pursue medical care (Giwa, et al., 2018). *E. coli,* in particular, showing multiple resistance to β-lactam antibiotics, especially penicillin and third generation of cephalosporin. Among these, ESBL- and AmpC-producing strains have been reported to be responsible for serious hospital-acquired infections globally (Lee, et al., 2015), with the spread of ESBL- and AmpC-producing strains all over the world, it is necessary to know the risk of prevalence of these strains in hospitals. In the present study out of 70 isolates, 20 (28.6%) were obtained from male patients and 50 (71.4%) were from female patients. Different studies conducted by other researchers found that 48.01% were male and 51.98% were female, that showed the higher occurrence of UTI in female (Alqasim, Abu Jaffal and Alyousef, 2018). The current results are in agreement with earlier study that only 35.6% of isolates were from men patients (Bakshi, et al., 2019). Other research has been shown that ESBL-producing *E. coli* incidences were significantly higher in females 73.6% than males 26.4% (Senbayrak, et al., 2017). It has been revealed that the high UTI prevalence in female can be attributed to many factors such as anatomical differing that allow quick admission of bacteria to the urinary tract (Rowe and Juthani-Mehta, 2013; Foad, 2016). The findings of this study show that bacterial proliferation was most pronounced in the 19–64 age range and least bacterial growth was detected in children and elderly among the isolates. Similarly, a research conducted in Nepal's South Terai found the identical results (Yadav and Prakash, 2017). The frequency of UTI rises with age and sexual activity, poor hygiene, use of contraceptives, earlier antibiotic usage, and prolonged catheterization duration, all are predisposing factors for the UTI (Kizilay, et al., 2020). Among 70 strains of *E. coli*, the number of ESBLs positive strains was 34 (48.6%) and ESBLs negative strains was 36 (51.4%) by double-disk synergy method. According to an Iranian investigation, ESBL production was identified in 40.8% of all the isolates tested (Seyedjavadi, Goudarzi and Sabzehali, 2016), whereas other study reported 42.5% of the isolates recovered from outpatients (Koshesh, et al., 2016). Another investigation from tertiary care hospital in Istanbul revealed that 44.7% and 22.8% of isolates were ESBL enzyme producer among inpatients and outpatients, respectively (Senbayrak, et al., 2017). Based on the results of this study, the number of isolates that were found to be positive for AmpC screening were 27 (38.6%) which is in accordance to the previous published studies. Numerous studies from different states of the world have conveyed the presence of AmpC producers in isolates of *E. coli,* the percentage of AmpC production in *E. coli* was 57.7%, 40.8%, and 32%, respectively (Fam, et al., 2013, Barua, Shariff and Thukral, 2013; Madhumati, et al., 2015). The present study revealed that 14 (20%) were coproducers of ESBL + AmpC. As many other study had been reported that there is not high incidence of phenotypic coexistence of ESBL/AmpC β-lactamases, 11.5% of coexistence occurred in India (Nayar, et al., 2012). Another study described that the coexistence phenotype of both ESBLs and AmpC was 11.5% of the isolates (Nasir, et al., 2015). In the existing study, ESBL production was found to be higher than AmpC production. Higher prevalence of ESBL-producing *E. coli* was seen possibly because of geographic areas and sample distinction can also be another reason. This study correlates with the former study by Mandal, et al., 2020, that established ESBL much higher in their isolates and the existence of ESBL and AmpC was 30.92% and 18.4%, correspondingly. This study also corroborates the earlier finding that higher ESBLs production was observed in 52.6% of isolates and AmpC production was perceived only in 8% of isolates (Gupta, et al., 2013). Furthermore, the resistance rate to cephalosporin was also high 100% and 82% resistance to cephalexin and ceftriaxone, respectively. This result was predictable because this group of drugs was widely used in both hospital and community settings. This result supported the outcomes realized by other researchers. It has been reported the highest resistance rate to antibiotics was piperacillin 86.6%, ceftriaxone 66.5%, and cefotaxime 66% and the most sensitivity was to the imipenem 90% and amikacin 80% (Hoseini, et al., 2017). Numerous studies have documented a remarkable increase in resistant degree to antibiotics such as penicillin, 2nd and 3rd generation cephalosporin (Bakshi, et al., 2019). These results exhibited a high frequency of resistance among *E. coli* isolates to the common antibiotics which are used routinely in the treatment of UTIs. Few options such as amikacin and carbapenems remain the utmost effective drugs against these resistant pathogens (Al-Zarouni, et al., 2008, Giwa, et al., 2018). It has been realized the clinical outpatient ESBL-producing isolates displayed high resistance to all cephalosporins, ranging from 25% (cefepime) to 100% (cefuroxime) (Ibrahimagić, 2016). In another study, all ESBL-producing isolates were resistant toward β-lactam and cephalosporins (ampicillin, cefotaxime, ceftriaxone, and ceftazidime), most ESBL producers were susceptible against imipenem (89.7%), nitrofurantoin 82.8%, and amikacin 72.4% (Kayastha, et al., 2020). The results of the present study exhibited that the rate of resistance to the β-lactam group (ampicillin and amoxicillin) was high, which might be due to the improper use of this class of antibiotics in the health-care setting. These results are in agreement with another study (Rahamathulla and Harish, 2016; Tillekeratne, et al., 2016). It is noticeable that the ESBL- and AmpC-producing *E. coli* were often resistant to other antibiotics such as aminoglycosides and fluoroquinolones. This could be due to coexistence of genes encoding drug resistance to those classes of antibiotics.

V. Conclusion

The present study highlights the incidence of ESBL and AmpC beta-lactamase-producing uropathogenic *E. coli* which alarming and crucial action needs because therapeutic choices may be limited due to the high percentage of drug-resistant bacteria. There is necessity to assume continued surveillance of the resistant bacteria and their underlying mechanisms so as to control further spread of the infections.

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