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RESEARCH ARTICLE

Prevalence and molecular characterization of extended spectrum beta-Lactamases-producing uropathogenic *Escherichia coli* isolated in Zakho, Iraq

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ABSTRACT

Objectives: Uropathogenic *Escherichia coli* are the most important group of microorganisms responsible for urinary tract infection. A high percentage of uropathogenic *E. coli* over the world are detected to be ESBLs producers, which is now a problem that limits therapeutic options. The aim of this study is to determine the prevalence of ESBLs *E. coli* and study the prevalence of different ESBLs genotype patterns among the ESBLs producing isolates.

Methods: One hundred and six uropathogenic *E. coli* isolates were analyzed for their ESBL production by molecular methods.

Results: 55 (52%) isolates were detected as extended spectrum β-lactamases (ESBLs) producers. Based on the PCR results, all *E. coli* isolates possessed one or more ESBL gene. CTX-M type ESBL was the most dominant ESBL (87.2%) among the isolates. While those for TEM-type and SHV-type were 54.5% and 21.8% respectively. Six genotype patterns were detected (TEM, CTX-M, TEM+SHV, TEM+CTX-M, SHV+CTX-M and TEM+SHV+CTX-M). The genotype CTX-M was the most prevalent genotype (40%) followed by the genotype TEM+CTX-M combination (30.9%). The occurrences of the genotypes (TEM, TEM+SHV, SHV+CTX-M and TEM+SHV+CTX-M) were 7.3%, 5.5%, 5.4%, 10.9%, respectively.

Conclusions: Control measures and education programs are necessary to avoid the uncontrollable use of β-lactam and cephalosporins in order to minimize the emergence of ESBLs producing *E. coli. J Microbiol Infect Dis 2016;6(4): 163-167*

Key words: ESBL, Uropathogenic *E. coli*, SHV, TEM, CTX-M, Iraq

INTRODUCTION

Antimicrobial resistance in uropathogenic *Escherichia coli* is of major concern worldwide [1]. β-lactam antibiotics are the most commonly used antibacterial agents, but there are high prevalence of multi drug resistant *E. coli* in urinary tract infections (UTIs) because of their ability to produce extended spectrum β-lactamases (ESBLs) [2]. ESBLs are enzymes that are capable of hydrolyzing the β-lactam ring of penicillins, cephalosporins, cephamycins, carbapenems (ertapenems) and monobactams [3]. *E. coli* may acquire other drug resistance traits from surroundings or environmental bacteria and conversely it can spread its resistance genes to potential pathogens in different habitats. In Kurdistan Region, Iraq, little information is available regarding to ESBLs producing uropathogenic *E. coli*. Therefore, this study was carried out to determine the prevalence of ESBLs *E. coli* using molecular detection of three β-lactamase genes (TEM, SHV, CTX-M) and study the prevalence of different ESBLs genotype patterns among the ESBLs producing isolates.

METHODS

Antibiotic sensitivity test

One hundred and six uropathogenic *E. coli* isolates from people referring to Zakho emergency hospital and private laboratories in Zakho city/Kurdistan Region, Iraq [4] were tested toward different antimicrobial drugs (Table 1). Drugs were supplied by Bioanalyse (Turkey), using disk diffusion assay according to Bauer *et al*. [5]. The inhibition zones were measured in comparison to the Clinical Laboratory Standards institute CLSI [6].

Preparation of genomic DNA

Chromosomal DNA was purified from *E. coli* isolates according to the manufacturer's instructions using the Accuvisbio kit (Canada). The concentration and purity of the DNA was determined using NanoDrop-Spectrophotometer (Thermo scientific, USA).

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PCR for β-lactamase encoding genes

PCR analysis for β-lactamase genes of the family SHV, TEM and CTX-M was carried out. Primers obtained from Accuvisbio (Canada). The oligonucleotide primer sets (Table 2) specific for the *bla*SHV, *bla*TEM and *bla*CTX-M genes and the cycling conditions used in the PCR assays have been describe previously [7]. The PCR was performed in a PCR Thermocycler (Applied biosystem, Singapore) with initial cycle of heat denaturation of 95°C for 2 min and followed by 30 cycles of heat denaturation at 94ºC for 60 s, primer annealing at 50-65ºC for 30 s, and DNA extension at 72ºC for 60 s and final cycle of exertion at 72°C for 5 min. PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide (1 µg/ml), and the gels were imaged under UV light.

Table 1. Antibiotic disks used in this study

Class	Antimicrobial	Symbol	Concentration µg/ml
β-lactams			
Penicillins	Penicillin	P	30
Carbapenems	Imipenem	IPM	10
	Meropenem	MEM	10
Monobactams	Aztreonam	ATM	10
Aminopenicillin	Ampicillin	AM	25
Cephalosporins	Cefazolin (1G)	CZ	30
	Cefuroxime(2G)	CXM	30
	Ceftazidime (3G)	CAZ	30
	Cefotaxime (3G)	CTX	30
	Ceftriaxone (3G)	CRO	30
	Cefepime (4G)	FEP	30
β-lactamase Inhibitors			
	Amoxicillin-clavulanic	AMC	20/10
	Ampicillin-Sulbactam	SAM	25/5
	Piperacillin-Tazobactam	TZP	20/10

Table 2. PCR primers for ESBLs se gene

Target	Primer name	Primer sequences (5'-3')	Product size (bp)	Ref.
*bla*TEM	TEM-F	ATAAAATTCTTGAAGAAGACGAAA	1100	7
	TEM-R	GACAGTTACCAATGCTTAATC		
*bla*SHV	SHV-F	TCGTTATGCGTTATATTCGCC	868	7
	SHV-R	GGTTAGCGTTGCCAGTGCT		
*bla*CTX-M	CTX-M-F	CGCTTTGCGATGTGCAG	550	7
	CTX-M-R	ACCGCGATATCGTTGGT		

Table 3. Antimicrobial susceptibility among uropathogenic *E. coli*

Antibiotics		No. of resistant isolates (%)	No. of sensitive isolates (%)
	P	106 (100)	0 (0.0)
	AM	106 (100)	0 (0.0)
β-lactam	ATM	106 (100)	0 (0.0)
	IPM	0 (0.0)	106 (100)
	MEM	0 (0.0)	106 (100)
	CZ	55 (52)	51 (48.1)
	CXM	55 (52)	51 (48.1)
Cephalosporins	CAZ	55 (52)	51 (48.1)
	CTX	55 (52)	51 (48.1)
	CRO	55 (52)	51 (48.1)
	FEP	55 (52)	51 (48.1)
	AMC	18 (17)	88 (83)
β-lactamase inhibitors	SAM	73 (68.9)	33 (31.1)
	TZP	44 (41.5)	62 (58.5)

Table 4. ESBLs genotype patterns among *E. coli* isolates

ESBLs genotype	No. of isolates	Percentage (%)
TEM only	4	7.3
SHV only	0	0
CTX-M only	22	40
TEM+SHV	3	5.5
TEM+CTX-M	17	30.9
SHV+CTX-M	3	5.4
TEM+SHV+CTX-M	6	10.9
Total	**55**	**100**

RESULTS

Antibiotic susceptibility test

The 106 *E. coli* isolates from previous study [4] were tested for their antibiotic susceptibility pattern against 14 antibiotics of different β-lactam and cephalosporin groups. Results showed that *E. coli* isolates were 100% resistant to β-lactam antibiotics Penicillin, Ampicillin and Aztreonam, whereas, all isolates were 100% susceptible to β- lactam antibiotics Imipenem and Meropenem (Table 3). Only 55 (52%) *E. coli* isolates were resistant for all four cephalosporin generations Cefazolin, Cefuroxime, Ceftazidime, Cefotaxime, Ceftriaxone and Cefepime. These results indicate that these isolates are ESBLs producers. Antibiotics containing β-lactamase inhibitors showed variable susceptibility rates.

Molecular detection of ESBL genes

All ESBL-producing *E. coli* isolates were subjected to PCR to detect ESBL genes, including *bla*TEM, *bla*SHV, *bla*CTX-M. Amplified PCR-products for *bla*TEM gene using the TEM-F and TEM-R primers gave an expected band of 1100 bp and the molecular weight of the amplified PCR-products for the gene *bla*SHV was about 868 bp using SHV-F and SHV-R primers. The amplified PCR-products for the gene *bla*CTX-M gave a molecular weight about 550 bp using CTX-M-F and CTX-M-R primers.

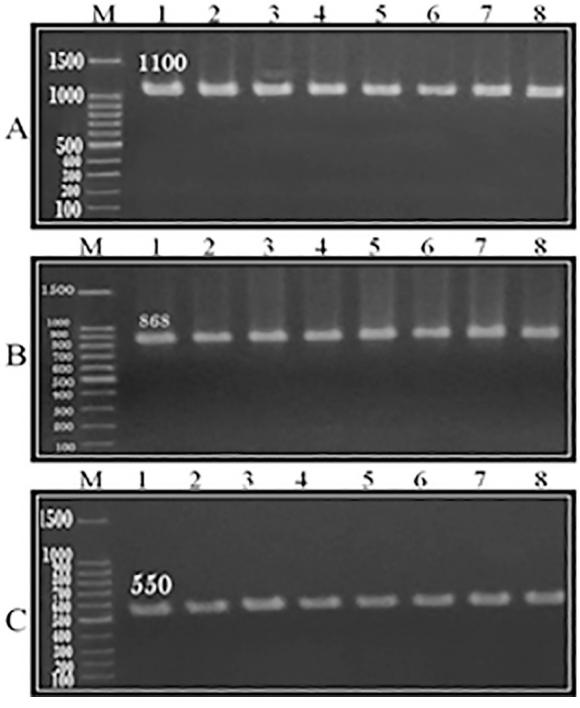

Figure 1. Amplified products of TEM, SHV and CTX-M genes. 1.5% Agarose gel analysis of PCR amplified fragment of (A) *bla*TEM (*ca*. 1100 bp) using TEM-F and TEM-R primers, (B) *bla*SHV (*ca*. 868 bp) using SHV-F and SHV-R primers, (C) CTX-M (*ca*. 550 bp) using CTX-M-F and CTX-M-R Primers. Lanes 1-8 are PCR products of ESBL genes from *E. coli* isolates. DNA markers (1500-100bp) are shown in lane M.

The prevalence of the three different ESBL genes

This study showed that CTX-M gene was the most dominant type (48/55; 87.2%) while TEM and SHV genes were less dominant types (30/55; 54.5% and 12/55; 21.8%, respectively).

Genotype patterns of ESBLs

Analysis of the PCR amplified products of all 55 *E. coli* ESBLs producers using the specific primers

revealed that six genotype patterns were obtained (Table 4). These results also showed that the most prevalent genotype was CTX-M (40%) followed by the genotype combination TEM+CTX-M (30.9%).

DISCUSSION

55 (52%) *E. coli* isolates were identified as ESBLs producers. *E. coli* and other genera of gram negative bacteria possess a naturally occurring, chromosomally mediated β-lactamase and plasmid mediated β-lactamase [8]. The antibiotic misuse, ineffective empiric antibiotic therapy, poor dosing, prolonged antibiotic treatment and spontaneous use of these antibiotics in developing countries are the reason for the development of high resistance rates in bacterial isolates [9-12].

Antibiotics containing β-lactamase inhibitors showed variable susceptibility rates. The efficacy of β-lactam-β-lactamase inhibitor combinations may be reduced for organisms producing multiple ESBLs. The addition of clavulanate significantly expands amoxicillin's spectrum to include penicillinase-producing *E. coli* [13].

PCR-products for *bla*TEM, *bla*SHV and *bla*CTX-M yielded expected bands of 1100 bp, 868 bp and 550 bp respectively. Amplified products of similar molecular weight were obtained in different locally and worldwide studies using the same primers [14]. Studies performed in Iraq, Turkey and Iran to detect ESBLs genes using different primer sequences gave amplified products with different molecular weights [15]. The variation in the molecular weights of the amplified products could be attributed to the difference in primer sequences or the type of the gene that detect as currently there are more than 70 SHV and 150 TEM types are recognized [16]. CTX-M are divided into five subgroups having more than 80 enzymes. This variety could provide a useful way to follow the spread of individual resistance genes.

Interestingly, 87.2% of ESBLs producer *E. coli* harbored the CTX-M gene. TEM and SHV found in 54.5% and 21.8% of isolates respectively. This is consistent with the present situation in worldwide including Middle East area, where CTX-M-type have replaced TEM and SHV types and became the predominant ESBL among Enterobacteriaceae [17].

Studies in Iraq and neighboring countries have declared that the CTX-M type was the predominant gene type in both *E. coli* and *Klebsiella pneumonia* [18]. However, studies from Turkey and India showed that TEM type was the predominant type [19]. European studies on Enterobacteriaceae have confirmed the persistence of strains producing TEM and SHV and the increasing prevalence of strains harboring CTX-M [20]. Data analysis revealed that there are six genotype patterns of ESBLs exist among the 55 isolates. CTX-M was the most prevalent genotype (40%) followed by the genotype combination TEM+CTX-M (30.9%). This genotype combination has also been reported to be the most dominant genotype in Saudi, India and Japan [21,22]. Another study in Macedonia showed the predominance of the genotype TEM+SHV combination [23]. The presence of more than one genotype in some of the isolates means that the ESBL producing strains may be related to a complex antimicrobial resistance. TEM gene is a broad spectrum β-lactamase that is always combined with CTX-M on the same plasmid. The occurrence of TEM+SHV+CTX-M combination along can cause resistance to carbapenems; this is worrisome and more serious for the community [24].

In conclusion, in this study, 52% of the uropathogenic *E. coli* isolated was detected as ESBLs producers. All isolates were confirmed by PCR to have one or more ESBL gene. CTX-M type was the dominant ESBL in the isolated *E. coli*. The majority of the isolates harbored more than one ESBL gene and the genotype TEM+CTX-M combination was the most dominant one. It is important to highlight that antimicrobial resistance must be viewed as an ecological problem and increase efforts to monitor and control the spread of antimicrobial resistant strains in hospitals and community. The introduction of molecular diagnosis in laboratories is important for the quick detection of ESBLS.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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REFERENCES

1. Moyo SJ, Aboud S, Kasubi M, Lyamuya EF, and Maselle SY. Antimicrobial resistance among producers and non-producers of extended spectrum beta-lactamases in urinary isolates at a tertiary Hospital in Tanzania. BMC Res Notes 2010;3:348.
2. French GL. Clinical impact and relevance of antibiotic resistance. Adv Drug Deliv Rev 2005; 57:1514-1527.
3. Queenan AM and Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev 2007; 20:440-458.

4. Polse RF, Yousif SY, and Assafi MS. Prevalence and antimicrobial susceptibility patterns of uropathogenic *E. coli* among people in Zakho, Iraq. Int J Res Med Sci 2016; 4:1219-1223.

5. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-496.

6. CLSI, Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement. CLSI-NCCLS document M100-S15. Clinical and laboratory standards institute, Wayne, PA. 2004.

7. Yun-Tae K, Kim TU, and Baik HS, Characterization of extended spectrum β-lactamase genotype TEM, SHV, and CTX-M producing *Klebsiella pneumoniae* isolated from clinical specimens in Korea. J Microbiol 2006;16:889-895.

8. Dhillon RH, Clark J. ESBLs: A clear and present danger? Crit Care Res Pract 2012;2012:625170.

9. Rudresh SM, Nagarathnamma T. Extended spectrum beta-lactamase producing Enterobacteriaceae & antibiotic co-resistance. Indian J Med Res 2011;133:116-118.

10. Habeeb A, Hussein NR, Assafi MS, and Al-Dabbagh SA. Methicillin resistant *Staphylococcus aureus* nasal colonization among secondary school students at Duhok City-Iraq. J Microbiol Infect Dis 2014; 4:59-63.

11. Assafi MS, Mohammed RQ, and Hussein NR. Nasal Carriage Rates of *Staphylococcus aureus* and CA-Methicillin Resistant *Staphylococcus aureus* among University Students. J Microbiol Research 2015; 5:123-127.

12. Assafi MS, Ibrahim NMR, Hussein NR, Taha AA, Balatay AA. Urinary Bacterial Profile and Antibiotic Susceptibility Pattern among Patients with Urinary Tract Infection in Duhok City, Kurdistan Region, Iraq. Int J Pure Appl Sci Technol 2015; 30:54-63.

13. Drawz SM and Bonomo RA. Three decades of beta-lactamase inhibitors. Clin Microbiol Rev 2010; 23:160-201.

14. Krishnamurthy V, Vijaykumar GS, Sudeepa Kumar M, Prashanth HV, Prakash R, Nagaraj ER. Phenotypic and genotypic methods for detection of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from ventilator associated pneumonia. J Clin Diagn Res 2013; 7:1975-1978.

15. Goudarzi M, Sabzehali F, Tayebi Z, et al. Prevalence of blaCTX-M gene in multi-resistant *Escherichia coli* Isolated from urinary tract infections, Tehran, Iran. Novel Biomed 2014;2:107-113.

16. Harada S, Ishii Y, and Yamaguchi K, Extended-spectrum beta-lactamases: implications for the clinical laboratory and therapy. Korean J Lab Med 2008; 28:401-412.

17. Livermore DM. Current epidemiology and growing resistance of gram-negative pathogens. Korean J Intern Med 2012;27: 128-142.

18. Barguigua A, El Otmani F, Talmi M, et al., Characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from the community in Morocco. J Med Microbiol 2011; 60:1344-1352.

19. Bali EB, Açık L, Sultan N. Phenotypic and molecular characterization of SHV, TEM, CTX-M and extended-spectrum β-lactamase produced by *Escherichia coli*, Acinobacter baumannii and Klebsiella isolates in a Turkish hospital. Afr J Microbiol Res 2010;4:650-654.

20. Canton R, Novais A, Valverde A, et al. Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. Clin Microbiol Infect 2008; 14 Suppl 1:144-153.

21. Bindayna K, Khanfar HS, Senok AC, Botta GA. Predominance of CTX-M genotype among extended spectrum beta lactamase isolates in a tertiary hospital in Saudi Arabia. Saudi Med J 2010;31: 859-863.

22. Harada Y, Morinaga Y, Yamada K, Migiyama Y, Nagaoka K. Clinical and molecular epidemiology of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* in a Japanese tertiary hospital. J Med Microb Diagn 2013; 2:2161-0703.

23. Kaftandzieva A, Trajkovska-Dokic E, Panovski N. Prevalence and molecular characterization of extended spectrum beta-Lactamases (ESBLs) producing *Escherichia coli* and *Klebsiella pneumoniae*. Prilozi 2011;32:129-141.

24. Manoharan A, Premalatha K, Chatterjee S, Mathai D. Correlation of TEM, SHV and CTX-M extended-spectrum beta lactamases among Enterobacteriaceae with their *in vitro* antimicrobial susceptibility. Indian J Med Microbiol 2011; 29:161-164.