International Journal of Chemical and Biomolecular Science
Vol. 2, No. 2, 2016, pp. 51-55
http://www.aiscience.org/journal/ijcbs
ISSN: 2381-7372 (Print); ISSN: 2381-7380 (Online)

Molecular Characterization of Extended Spectrum B-lactamase Producing *Escherichia coli* Isolated from Urine in Kurdistan Region-Iraq

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Abstract

Molecular characterization of ESBL-related *bla* genes including *bla*TEM, *bla*SHV, and *bla*CTX-M has been performed for *Escherichia coli* isolated from urine and collected from three cities in Kurdistan/region/Iraq (Erbil, Sulymani and Duhok). One hundred sixty nine isolates of *E. coli* have been identified and their production of ESBLs enzymes have been determined using phenotypic methods. All these isolates were successfully amplified producing a single band of the *uidA* locus in all strains with a molecular weight of about 670bp in order to confirm at molecular level that all these isolates were *E. coli*. One hundred sixty ESBL *E. coli* isolates out of 169 appeared to have one or more ESBLs genes accounting for 94.7%. CTX-M constituted the high prevalent type of ESBLs genes compared to the others represented by 94.1% of all isolates in all the three cities of Kurdistan region followed by TEM and SHV in a percentages of 43.8% and 2.5%, respectively. In Duhok, TEM showed the higher prevalence (60.8%) in comparison to the other two cities in percentages of 36.2% for Sulaimania while Erbil represented by 25%. Furthermore, it was clear that SHV type of ESBLs had the lower prevalence of all types and there were only four isolates out of 160 appeared to carry this type of gene representing 2.5%. The presence and/or absence of the three genes in all isolates were also investigated and it was shown that 86/160 isolates (53.75%) had the CTX-M gene only while the rest of genes were lacking. Moreover, 69/160 isolates had both CTXM and TEM. Interestingly, 3/160 harbored all three involved genes. The isolates characterized by the presence only TEM gene and those that had both CTX-M and SHV, shared the same percentage (0.6%). after taking sequencing of the PCR product of studied genes for 12 *E coli* isolates into consideration, it was obvious that all the PCR products of CTX-M were belonged to type CTXM-15; while TEM-1 type appeared predominant among all sequences PCR product for TEM gene.. Finally, from the three isolates which revealed positive PCR amplification for the SHV gene, two isolates showed 100% similarity to the SHV-12 genome type while the rest single isolate was similar (99%) to SHV11.

Keywords

PCR Assay, ESBLs, *Escherichia coli*

Received: September 5, 2015 / Accepted: March 16, 2016 / Published online: April 5, 2016

1. Introduction

Beta-lactam antibiotics are the most common treatment for bacterial infections. Production of β- lactamase is considered as a main mechanism of bacterial resistance to these classes of antibiotics (Thenmozhi, *et al*., 2014). Extended spectrum β-lactamase enzymes (ESBLs) have the ability to hydrolyze broad spectrum of β lactams (third generation

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cephalosporins) and monobactams, but do not affect cephamycins or carbapenems (Pfaller and Segreti, 2006). The location of genes responsible for producing ESBLs is on a plasmids facilitates the transfer of these genes among different isolates (Rupp and Fey, 2003). The resistance of ESBL-producing strains to a wide variety of commonly used antimicrobials made their proliferation and distribution serious global health concern and complicated treatment strategies for the growing of nosocomial infections (Pfaller and Segreti, 2006). The major types of ESBLs are TEM, SHV and CTX-M (Harada *et al.,* 2013) and most of these enzymes evolved by point mutations around the active site of native β-lactamases, particularly TEM-1, TEM-2, and SHV-1 (Pfaller and Segreti, 2006; Pitout, 2009). The CTX-M types, now exceeding 50 different types, have been classified into five groups depending on the identity of their amino acids: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 (Bonnet, 2004). CTX-M-15 b-lactamase an enzyme that has been associated with epidemic and mosaic plasmids, belongs to the CTX-M-1 group, was first described in India during 2001, but is now has been emerging worldwide (Nazik, *et al.,* 2011). In the last decade, *Escherichia coli* producing CTX-M (especially CTX-M-15) has distributed worldwide as an important cause of community acquired urinary tract infections (Pitout, 2009).

Although the Phenotypic tests for detection of ESBLs have been used for a long time, they have some limitations including their inability to detect the subtypes of ESBLs, the prerequisite to be evaluated from time to time and their changing performance while introducing new enzyme (Shiri *et al.*, 2003; Sharma *et al.,* 2010). Therefore, the precise detection of ESBL producing microorganisms requires not only phenotypic tests, but also the application of genotypic tests for detecting all genes responsible for beta-lactamase production (Kaftandzieva *et al.,* 2011). Polymerase chain reaction-based methods are considered the most reliable molecular techniques for the identification and confirmation of the presence of ESBLs (Grover *et al.* 2006) and these methods gained a significant importance in clinical microbiology laboratories (Natarajan and Singaram, 2013).

2. Materials and Methods

2.1. Sampling

One hundred sixty nine isolates of *E. coli* were obtained from urine sample of patients suffering from UTI. Samples were collected from patients who attended Rezgari Teaching Hospital in Erbil and Azadi General Hospitals in Sulaimania and Duhok/Kurdistan region/IRAQ. All obtained isolates were identified and determined *E. coli* ESBLs producing enzymes using BD^{TM}Phoenix system (Becton-Dickinson Diagnostic System, Sparks, MD). This work has been done in Azadi General Hospital/Duhok City.

2.2. Genomic DNA Extraction

Genomic DNA of the collected isolates were obtained by applying the method described Al-Mizory, 2007. The concentration and the purity of the extracted DNA were determined by Nano drop system.

2.3. Detection of *E. coli* Producing ESBLs Genes by PCR-assay

Internal fragment of uidA gene was amplified using uidA as species specific primer for *E. coli* detection. Three genes coding for ESBLs have been chosen for PCR amplification, namely the CTX, TEM, and SHV. PCR assay for each gene was performed in 25µl mix containing 12.5µl master mix (aidgen/Korea), 10pmol each primer and 2.0µl template. Primer sequences and amplification conditions were performed as described by Adamus-Bialek *et al.,* 2009, Yun-Tae *et al.,* 2006. The presence of the PCR product was confirmed electrophoretically using 1.5% (w/v) of agarose in TBE Buffer. Molecular marker (100-1500bp) was used to determine the molecular weight of PCR product.

2.4. Sequencing Analysis

The PCR products of ESBLs genes including CTX-M, TEM, and SHV were partially sequenced and applied on 12 selected isolates of *E. coli*. This work has been done in Scientific Research Center-Faculty of Science/ University of Dohuk using a capillary electrophoresis sequencer (ABI 3130 DNA sequencer, Singapore). Raw sequences were reviewed by visual inspection with Chromas3.5V software to form contig of each target gene using forward and reverse sequences. The sequences of each fragment were trimmed to a uniform length that corresponded with the region used to identify the target gene using Bioedit softwareV7.1.11.

3. Results and Discussion

All 169 collected samples from UTI suffered patients were identified as ESBL producing *E. coli* isolates using BD^{TM}Phoenix system, This system has ability for detection of 20 different antibiotics and several types of biochemical tests, it is highly specific because the confidence value of all *E. coli* isolates was between 95-99%. However, the sensitivity of this system was low because sometimes it failed to give accurate results regarding to some antibiotics.

All *E. coli* isolates were successfully amplified a single band of the *uidA* as the species specific locus in all strains with a molecular weight of about 670 bp. The prevalence and molecular characterization of ESBLs genes; including *bla*

CTXM, *bla* TEM, and *bla* SHV were studied by using three primers including CTXM, TEM and SHV specify for these genes. General results for three cities revealed that 160/169 isolates of *E. coli* harbored one or more of these genes accounting (94.7%), while only 10 isolates lack these genes accounting 5.9%. It has been found that (159/169) isolates carried CTX-M type enzymes accounting (94.1%), (74/169) isolates produced TEM-type enzymes accounting (43.8%) and (4/169) isolates harbored SHV-type enzymes accounting (2.4%). Since this study included the three cities in Kurdistan region/Iraq, the results of these experiments are described separately for each city in Table (1).

Table 1. The prevalence of CTX-M, TEM and SHV genes with their percentages among *E. coli* isolates producing ESBLs enzymes in Erbil, Sulymani and Dohuk cities.

City	ESBLs		
	CTXM (%)	TEM (%)	SHV (%)
Erbil	47/48(97.9)	12/48(25)	2/48(4.16)
Sulymani	44/47(93.6)	17/47(36.2)	1/47(2.1)
Dohuk	68/74(91.9)	45/74(60.8)	1/74(1.35)
Total	159/169(94)	74/169(43.8)	4/169(2.4)

It's obvious from the results high prevalence rate of CTX-M among *E. coli* isolates in all cities, while TEM gene is found more prevalence among isolates collected from Dohuk city with rate (60.8%) compared to Erbil and Sulymani cities (25, 36.2%) respectively. SHV genes was found with low rate among all isolates from the three cities (Erbil, Sulymani and Dohuk) with rate (4.16, 2 and 1.35%) respectively.

Genotyping of ESBLs *E. coli* isolates based on the presence (+) and/or absent (-) of the three ESBLs genes, namely *bla*-CTX-M and *bla*-TEM and *bla*-SHV have been performed. It has been found that 86/160 (53.75%) of the strains characterized by the presence of only *bla*CTX-M gene with profile (CTXM+/TEM-/SHV-) represented the most prevalent genotype among all others followed by genotype ($CTXM^+/TEM^+/SHV^-$) 69/160(43.12%) characterized by the presence of *bla*CTX-M and TEM genes, 3/160 (1.9%) of these isolates harboring the three ESBLs genes with genetic profile ($CTXM^+/TEM^+/SHV^+$), the isolates that characterized by presence of TEM gene ($CTXM^-/TEM^+/SHV^-$) and isolates which characterized by the presence of CTX-M and SHV ($CTXM^+/TEM^-/SHV^+$) have the same percentage 1/160 (0.6%) as shown in Table (2).

Table 2. Represents the genotypic profile of *E. coli* isolates that harbored ESBLs gene including; CTX-M, TEM and SHV genes collected from Erbil, Sulymani and Dohuk Cities.

Genetic Profile	Erbil	Sulymani	Dohuk	Total
($CTXM^+/TEM^-/SHV^-$)	34/47	28/44	24/69	86/160
($CTXM^+/TEM^+/SHV^-$)	11/47	15/44	43/69	69/160
($CTXM^+/TEM^+/SHV^+$)	1/47	1/47	1/69	3/160
($CTXM^-/TEM^+/SHV^-$)	-----	----	1/47	0.6/160
($CTXM^+/TEM^-/SHV^+$)	1/47	----	----	0.6/160

Indeed, CTX-M type represents the most rapidly enzymes spreading among *Enterobacteriaceae* worldwide and nowadays it is the most prevalent ESBLs in many parts of the world particularly increased in *E. coli* isolated from both community and nosocomial settings compared to TEM and SHV types (Canton and Coque, 2006*;* Vidhya and Sudha, 2013). Two recent reports from Turkey have shown that the CTX-M enzyme is common among ESBL positive isolates accounting (86.8 %) (Aktaş *et al.,* 2009 and (76.5%) (Yumuk *et al.,* 2011). Mahboobeh *et al.,* 2014 in Iran found that CTX-M type β-lactamases are widespread in the studied community (96.3%). In other hand, Moosavian and Deiham, 2012 in Iran found the prevalence of *blaTEM* and *blaSHV* were 65.5 and 15%, respectively and (19%) of ESBL isolates had both *blaTEM* and *blaSHV* genes, *blaCTX-M* was not detected in ESBL-producing strains. In India Natarajan and Singaram, 2013 were found that (90%) of ESBL producing UPEC harbored TEM gene whereas, the prevalence of this gene have also been reported in Iran and Turkey with rate accounting 63% and 20.6% respectively (Jazi *et al.,* 2007; Bali *et al.,* 2010). These reports suggest a high incidence rate of *E. coli* producing TEM gene in the hospitals studied and considered SHV and TEM type ESBL's were highly associated with nosocomial outbreaks during the last twenty years (Natarajan and Singaram, 2013). The movements of patients between the community and the health care system may have an important role in spread and differentiation of *E. coli* in the community and hospitals (Zahar *et al.,* 2009).

In this study, some of the TEM, SHV, and CTX-M PCR products were subsequently sequenced and compared with DNA GenBank sequences using BLAST search. From the results, it has been found that all PCR products of CTX-M were 100% similar to CTXM-15, and all sequenced PCR products of TEM were resemble to TEM-1 type. The sequencing alignment of SHV PCR products with the partial sequences of previously published strains and deposited in GeneBank under accession numbers (KP162337.1|*E. coli* SHV-12 gene) and (GQ389702.1 *Kl pneumoniae* SHV-11) revealed that two of three were 100% similar to SHV12 genotype, whereas other has (99%) similarity to SHV11 genotype.

CTX-M-15 type being the most common ESBL reported in the Middle East area and North Africa and now really the most prevalent ESBL type throughout the world (Paterson & Bonomo 2005; Khalaf *et al* 2009), In a study conducted by Saleh *et al.* 2013 in Jordan University hospital found that 73.2% of *E coli* isolates were CTX-M-15 producer. Study in Turkey reported the dissemination of CTXM-15 genotype among clinical isolates of *E. coli* in a percentage 85% and also demonstrated both CTX-M15 and TEM-1 genes carried on the a conjugative plasmid in *E. coli* (Gonullu *et al.,* 2008). CTX-M-15 type is sometimes associated with other ESBLs encoding genes, such as *bla*TEM and *bla*SHV derivatives and *bla*OXA-1 as well as genes encoding for resistance to other antimicrobial agents (Saleh *et al.*, 2013). This may give a hint that CTXM-15-producing isolates are commonly multidrug resistant, which poses a particular challenge for the treatment of nosocomial infections. The predominant of TEM-1 type was also reported by Kiratisin *et al*., 2008. In a study conducted by Khalid in 2013 in Kurdistan region/ Iraq found that 75% of sequenced *Kl. Pneumonia* isolates harbored genotype TEM-1. This may indicate an endemic genotype TEM-1 among isolates of *Enterbacteriacea* in Kurdistan Region in Iraq. All over these results may give interestingly indication of high rate of ESBL producing *E coli* strains especially which carrying CTXM-15 therefore, determination of ESBL genes by molecular techniques in ESBL producing bacteria and their pattern of antimicrobial resistance can supply useful data about their epidemiology and risk factors associated with these infections (Bali *et al.,* 2010).

4. Conclusion

Molecular analysis revealed the high prevalence of CTX-M gene among ESBL-producing *E. coli* isolates collected from urine with predominant CTXM-15 type, followed by TEM-gene especially type TEM-1 and low incidence rate of SHV gene. To our knowledge, this is the first report in the Kurdistan Region/Iraq of *E. coli* producing CTX-M especially CTXM-15, TEM and SHV genes.

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