



Detection of IS903, IS26 and ISEcp1 Elements in CTX-M-Producing *Klebsiella pneumoniae* and *Escherichia coli* Isolates From Patients with Leukemia in Iran

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Abstract

Background: The ability of extended spectrum beta-lactamases (ESBLs) production is one of the main mechanisms for the emergence of antibiotic resistance in *Escherichia coli* and *Klebsiella pneumoniae*.

Objectives: The aim of this study was to evaluate the occurrence of IS903, IS26, and ISEcp1 insertion elements among the CTX-M-producing *K. pneumoniae* and *E. coli* isolates from patients with leukemia in Tehran.

Methods: Eighty *E. coli* and *K. pneumoniae* isolates were recovered from patients admitted to hospitals of Tehran. Antibiotic susceptibility tests were performed by Kirby-Bauer disc diffusion and broth microdilution methods. Detection of ESBL producers was evaluated by phenotypic confirmatory test. The presence of IS903, IS26, and ISEcp1 insertion elements in CTX-M-positive *E. coli* and *K. pneumoniae* isolates was investigated by PCR-sequencing methods.

Results: The rate of resistance of 80 *E. coli* and *K. pneumoniae* isolates against the nine antibiotics was as follows: 100% to ampicillin, 15% to amikacin, 51% to ciprofloxacin, 30% to gentamicin, 58% to ceftriaxone, 10% to imipenem, 63% to cefotaxime, 51% to levofloxacin, and 55% to ceftazidime. Using phenotypic confirmatory test revealed that 51 (63.75%) isolates were ESBL producers. The prevalence of CTX-M-1, CTX-M-2, CTX-M-9, CTX-M-8, and CTX-M-25 genes was 87.5%, 13.75%, 23.75%, 10%, and 0%, respectively. IS903, IS26, and ISEcp1 elements were detected in 93.75%, 71.25%, and 100% of the isolates, respectively.

Conclusions: This study indicates that the high prevalence of antibiotic resistance, IS and CTX-M-producing *E. coli*, and *K. pneumoniae* isolates could be a major concern and highlights the need for infection control measures.

Keywords: *Escherichia coli*, Extended-Spectrum Beta-Lactamases, Insertion Sequence, *Klebsiella pneumoniae*, Leukemic Patients

1. Background

Cancer has been known as the leading cause of a variety of temporary or permanent physical and psychological changes in patients. Complications of cancer may lead to intolerable pain, hopelessness, fear, and even death. One of the most frequent cancers worldwide is leukemia, which occurred in 12.5 individuals per 100 000 people during 2011 (1). Infection is one of the most serious threats in patients with acute leukemia receiving chemotherapy and radiotherapy (2). Emergence of resistance to β -lactamase antimicrobial drugs began even before the first β -lactam penicillin was developed. The first β -lactamase enzyme was discovered in *Escherichia coli* prior to use of penicillin in clinical practice (3, 4).

The extensive use of β -lactams has led to the emergence of resistant isolates worldwide (5). Resistance to this class of antibiotics is mostly mediated by acquisition of β -lactamase genes located on mobile genetic elements, such as transposons or plasmids. Extended spectrum beta-lactamases (ESBLs) have increased among clinical isolates of members of the *Enterobacteriaceae* family during last four decades. High prevalence of infections caused by ESBL-producing *Enterobacteriaceae* at intensive care units (ICUs) was reported worldwide. Such drug-resistant bacterial infections are top concerns in people admitted to nursing homes, geriatric and oncology patients, transplant recipients, and pediatric populations. Failure to identify β -lactamase-producing isolates by laboratory susceptibility

testing methods may lead to inappropriate empirical therapy and a subsequent increased mortality in these fragile and intolerable people (6).

Extended spectrum β -lactamase-producing *Klebsiella pneumoniae* and *E. coli* are progressively being identified in many parts of the world and are prevalent in countries located in the Asia-Pacific region (7). Over the past 20 years, CTX-M has emerged as a major type of ESBLs, which are now rapidly spreading among members of *Enterobacteriaceae* around the world (8). Since the discovery of the CTX-M-1 from a European patient in the late 1980s, more than 130 allelic variants of CTX-M were characterized (9). Among antimicrobial-resistant Gram-negative pathogens, organisms producing CTX-M-type ESBLs have become a serious threat to public health worldwide (10).

Insertion sequences (ISs) are the smallest transposable elements (< 2.5 kb) capable of independent transposition in an organism, thereby causing insertion mutations and genome rearrangements (11). Various elements may possibly be involved in the mobilization, spread, and expression of *bla*_{CTX-M} genes, of which *ISEcp1* is often associated with the upstream gene region. In addition, other insertion sequences, such as IS26, IS10, IS5, and IS903 have been detected surrounding the *bla*_{CTX-M} resistance determinant (12).

2. Objectives

This study investigated the genetic environment of *bla*_{CTX-M} genes in terms of the IS903, IS26, and *ISEcp1* insertion sequences in *E. coli* and *K. pneumoniae* isolates recovered from patients with leukaemia in Tehran, capital of Iran.

3. Methods

3.1. Ethics Statement and Patient Consent

The present study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences with reference number IR.SBMU.SM.REC.1394.38. The authors confirm that all patients, and the patients' parents provided their informed consent form to take part in the study.

3.2. Study Patients and Bacterial Isolation

Bacterial isolates included in this study were collected during May 2014 to October 2015 from two medical centers of Tehran, Iran: The Hematology-Oncology Research Center, Dr. Shariati Hospital (with a capacity of 857 bed), and the Mahak Pediatric Oncology Center, a 120-bed oncology hospital with an outpatient clinic. Sixty-one and

nineteen subjects included in this study were patients with acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL), respectively. Bacterial isolates were recovered from various biological material, including blood, urine, wound, sputum, and vaginal swabs. All clinical specimens were immediately sent to the laboratory to be processed for microbial pathogens. Isolates were identified by using standard microbiological and biochemical methods (13). After identification, bacteria were stored in glycerol stock at -70°C before retrieval for testing.

3.3. Antimicrobial Susceptibility Testing

The susceptibility of clinical isolates to imipenem (10 μ g), ceftazidime (30 μ g), ceftriaxone (30 μ g), cefotaxime (30 μ g), ciprofloxacin (5 μ g), levofloxacin (30 μ g), amikacin (30 μ g), ampicillin (30 μ g), and gentamicin (10 μ g) (Mast, UK) was evaluated by the Kirby-Bauer disk diffusion method, based on the Clinical Laboratory Standards Institute (CLSI) guidelines (14). The minimum inhibitory concentration (MIC) of selected antibiotics, including imipenem, ceftazidime, cefotaxime, and ciprofloxacin (Glaxo, UK) was determined by the reference broth microdilution method. All antibiotics were incorporated in Mueller-Hinton broth (Merck, Germany) in serial two-fold concentrations from 0.5 to 256 μ g/mL. Quality control was done using the *E. coli* ATCC 25922 strain.

3.4. Screening Tests for ESBL Production

All isolates were evaluated for production of ESBLs by the phenotypic confirmatory disc diffusion method containing cefotaxime (CTX, 30 μ g) and ceftazidime (CAZ, 30 μ g) with CTX + clavulanic acid (CA, 10 μ g) and CAZ + CA per disc (Mast Group, Merseyside, UK). The zones of inhibition were compared for the CTX and CAZ discs with that of the CAZ + CA and CTX + CA disc. An increase in zone diameter of \geq 5 mm in the presence of CA was interpreted positive for the presence of ESBL in the test organism. *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative and positive controls for ESBL production, respectively (14).

3.5. PCR-Based Detection of *bla*_{CTX-M} Type Genes and Sequencing Analysis

To determine the *bla*_{CTX-M} variants, including *bla*_{CTX-M-1}, *bla*_{CTX-M-2}, *bla*_{CTX-M-8}, *bla*_{CTX-M-9}, and *bla*_{CTX-M-25}, PCR was performed on DNA extracted by a genomic DNA purification kit (Fermentas, Germany), using primers specific to each gene (Bioneer Company, Korea) (Table 1).

Reactions were performed in a DNA thermal cycler (Eppendorf, Mastercycler gradient) in a 25- μ L mixture containing 1 U of *Taq* polymerase (Bioneer Company, Korea)

Table 1. List of Primers and Product Size

Primer	Sequence (5'→3')	Amplicon Size (bp)	Reference
CTX-M-1		781	(15)
F	CGTCACGCTGTTGTTAGGAA		
R	ACGGCTTCTGCTTAGGTT		
CTX-M-2		865	(16)
F	ATGATGACTCAGAGCATTCCG		
R	TGGTTACGATTTTCGCCGC		
CTX-M-8		871	(17)
F	TGATGAGACATCGCGTTAAG		
R	TAACCGTCGGTGACGATTTT		
CTX-M-9		870	(16)
F	ATGTGACAAAGAGAGTGCA		
R	CCCTTCGGCGATGATTCTC		
CTX-M-25		924	(18)
F	CACACGAATTGAATGTTACG		
R	TCACTCCACATGGTGAGT		
ISEcp1		526	(19)
F	GCAGGTCITTTTCTGCTCC		
R	TTTCCGCAGCACACCGTTTG		
IS903		588	(20)
F	CGTAGCGGGTTGTGTTTCA		
R	GGTAATTGATTCACCGGGC		
IS26		134	(21)
F	AGCGGTAATCGTGGAGTGA		
R	AGGCCGGCATTTCAGCGTG		

and 1× buffer consisting of 1.5 mM MgCl₂, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, each dNTPs at a concentration of 250 μM, and primer at a concentration of 10 pmol. Thirty-six cycles were performed for each reaction, with the following temperature protocol for each cycle: 94°C for 60 seconds, 57°C for 60 seconds, and 72°C for 60 seconds. Sequencing of amplicons in both directions was done by the Bioneer Company (Daejeon, South Korea). The BLAST program from the national centre for biotechnology information (NCBI) website (<http://www.ncbi.nlm.nih.gov/BLAST>) was used to analyse the nucleotide sequences.

3.6. PCR Assay for Insertion Sequences

All isolates were evaluated for the presence of three insertion elements *ISEcp1*, *IS26*, and *IS903*. The oligonucleotide primer sets specific for each IS are shown in **Table 1**. Sequencing was the same as described previously for CTX-M type genes.

3.7. Genetic Association of the CTX-M gene with *ISEcp1*, *IS26*, and *IS903*

The association of *bla*_{CTX-M} to insertion sequences, such as *ISEcp1*, *IS26*, and *IS903*, was performed by PCR and sequencing. The reaction mixture and running protocols were the same as the above-described approach (12, 22).

4. Results

4.1. Isolation and Identification

A total of 56 *E. coli* and 24 *K. pneumoniae* isolates were recovered from urine (62.5%), blood (26.25%), sputum (8.75%), wound (1.25%), and vagina (1.25%). In total, 35% and 65% of the isolates were obtained from males and females, respectively. The distribution of patients' age groups was as follows: ≤ 1 year, 3.75%; 2 to 17 years, 30%; 18 to 45 years, 40%; 46 to 64 years, 22.5%; and ≥ 65 years, 3.75%.

4.2. Antimicrobial Susceptibility Test and Phenotypic Confirmatory Disc

Both clinical isolates of *E. coli* and *K. pneumoniae* showed resistance towards ampicillin, while all *K. pneumoniae* isolates were susceptible to imipenem. Resistance rate of *E. coli* versus *K. pneumoniae* isolates to nine examined antibiotics was as follows: 44.64% versus 12.5% to ciprofloxacin, 7.14% versus 0% to imipenem, 30.35% versus 12.5% to gentamycin, 53.57% versus 16.66% to ceftazidime, 57.14% versus 20.83% to cefotaxime, 16.07 versus 8.33% to amikacin, 100% versus 100% to ampicillin, 62.5% versus 25% to levofloxacin, and 58.92% versus 20.83% to ceftriaxone (**Table 2**). Thirty-eight (67.85%) isolates of *E. coli* were capable of producing ESBL, with a variable range of resistance to all antibiotics tested from 13.15% against imipenem to 100% against ampicillin. In addition, 13 of 24 (54.16%) *K. pneumoniae* isolates exhibited ESBL-positivity, of which 100% and 100% were susceptible and resistant to imipenem and ampicillin, respectively. The MIC range, MIC₅₀, and MIC₉₀ determinations of four selected antibiotics against *E. coli* and *K. pneumoniae* along with the rate of resistant isolates are shown in **Table 2**. Of tested antibiotics, imipenem showed the lowest determinations of MIC₅₀, and MIC₉₀ (≤ 1 μg/mL) in both *E. coli* (MIC₅₀ ≤ 1 μg/mL, MIC₉₀ ≤ 1 μg/mL) and *K. pneumoniae* (MIC₅₀ ≤ 1 μg/mL, MIC₉₀ = 2 μg/mL) isolates (**Table 2**).

4.3. Detection of genes by PCR and Sequencing

Molecular characterization of isolates revealed the presence of 89.28% (50/56) *bla*_{CTX-M-1}, 14.28% (8/56) *bla*_{CTX-M-2}, 7.14% (4/56) *bla*_{CTX-M-8}, and 25% (14/56) *bla*_{CTX-M-9} in *E. coli*, while *K. pneumoniae* isolates were found to have 83.33% (20/24) *bla*_{CTX-M-1}, 12.5% (3/24) *bla*_{CTX-M-2}, 16.66% (4/24)

Table 2. Antimicrobial Susceptibility Patterns of Selected Antibiotics for *Escherichia coli* and *Klebsiella pneumoniae* Isolates From Two Oncology Centers

Antimicrobial Agents	Bacterial Isolates					
	MIC Range ($\mu\text{g/mL}$)	MIC ₅₀	MIC ₉₀	Antibiotic Susceptibility (%)		
				R	I	S
<i>E. coli</i> (n = 56)						
Ciprofloxacin	≤ 2-256	4	8	44.64	8.92	46.44
Cefotaxime	≤ 2-256	16	64	57.14	3.57	39.29
Ceftazidime	≤ 2-256	16	64	53.57	5.35	41.09
Imipenem	≤ 1-8	≤ 1	2	7.14	-	92.86
<i>K. pneumoniae</i> (n = 24)						
Ciprofloxacin	2-256	2	4	12.5	-	87.5
Cefotaxime	≤ 1-32	≤ 1	4	20.83	-	79.17
Ceftazidime	≤ 2-256	≤ 2	16	16.66	4.17	79.17
Imipenem	-	≤ 1	≤ 1	0	-	100

*bla*_{CTX-M-8}, and 20.83% (5/24) *bla*_{CTX-M-9}. Prevalence of CTX-M variants among ESBL and non-ESBL-producing isolates and co-existence of resistance determinants is shown in Table 3. In addition, PCR identified the insertion sequences IS903 and *ISEcp1* in 69.64% (39/56) and 96.42% (54/56) of *E. coli* isolates, respectively. Overall, 87.5% (21/24) and 75% (18/24) of *K. pneumoniae* were also positive for *ISEcp1* and IS903 elements, respectively. The IS26 element was detected in all *E. coli* and *K. pneumoniae* isolates. Distribution of CTX-M variants and insertion elements among the isolates and co-existence of IS elements with CTX-M types is illustrated in Figure 1. Moreover, distribution of the insertion elements among the *bla*_{CTX-M}-harboring ESBL and non-ESBL isolates is shown in Table 4. Sequencing analysis of the *bla*_{CTX-M-1} products revealed the presence of *bla*_{CTX-M-15} and *bla*_{CTX-M-55} variants among all clinical isolates.

5. Discussion

Leukemia makes up about one-third of all malignancies in the 0- to 14-year-old age group. The most common subtype, acute lymphocytic leukemia (ALL), represents about 80% of these cases (22, 23). A total of 6000 new cases (3400 males and 2600 females) of ALL are diagnosed annually in the US. It occurs mainly in children and around 60% of cases are children and adolescents aged less than 20 years. Up to 90% of children affected by ALL survive, although it is necessary to improve the treatment of infants and adults (24). Severe, life-threatening infections continue to be a major cause of morbidity and mortality in patients with acute leukemia undergoing intensive chemotherapy (25). It has been reported that several risk factors, including neutropenia, decreased immunity

due to underlying diseases, hemorrhagic diathesis of skin and mucosal tissue, severe mucositis, as well as central venous catheters (CVCs) increase the susceptibility to infections in leukemic patients (20, 26).

Specifically, multidrug-resistant organisms and even those previously considered harmless have currently emerged as a therapeutic challenge and can be catastrophic in this cohort of patients. Furthermore, ESBL-producing bacteria, such as *Klebsiella* spp. and *E. coli*, have become resistant to cephalosporins, fluoroquinolones, and penicillin classes of antibiotics, posing a threat to public health (27). This study focused on the detection of *bla*_{CTX-M} genes, as an important mechanism of resistance to oxyimino cephalosporins (cefotaxime and ceftriaxone) in *K. pneumoniae* and *E. coli*, and analysis of the genetic organization of these determinants in terms of different insertion elements involved in their mobilization (7).

The high occurrence of ESBL-producing *E. coli* and *K. pneumoniae* isolates recovered from leukaemia patients admitted to therapeutic centres is alarming (28). Recently, Liu et al. reported even a higher prevalence (68.2%) of these isolates that were mainly positive for the *bla*_{CTX-M-15} (29). In a study from India, 48.27% of isolates were found to be ESBL-producers. Of these, 55.69% and 44.31% were identified as *E. coli* and *K. pneumoniae*, respectively (7). Yousefichaijan et al. showed that 53% of *E. coli* strains, isolated from infected children, were ESBL producing (30). In another study, Samet et al. showed that 0.41% of *E. coli* isolates from the leukemia patients were ESBL-producers (31). In the present study, *bla*_{CTX-M} genes were detected in both clinical isolates of ESBL- and non-ESBL-producing *E. coli* and *K. pneumoniae*. CTX-M-type ESBL was first reported among *E. coli* isolates from Iran, during year 2008 (32). The percentage

Table 3. Number of Strains That Produce CTX-M-Type β -Lactamases as Detected by PCR

CTX-M Group	Bacterial Species, No. (%)					
	<i>Escherichia coli</i>			<i>Klebsiella pneumoniae</i>		
	ESBL-Positive (N = 38)	Non-ESBL (N = 18)	Total (N = 56)	ESBL-Positive (N = 13)	Non-ESBL (N = 11)	Total (N = 24)
<i>bla</i> _{CTX-M-1}	34 (89.47)	16 (88.88)	50 (89.28)	11 (84.61)	7 (63.63)	20 (83.33)
<i>bla</i> _{CTX-M-1} + <i>bla</i> _{CTX-M-2}	7 (18.42)	1 (5.55)	8 (14.28)	1 (7.69)	2 (18.18)	3 (12.5)
<i>bla</i> _{CTX-M-1} + <i>bla</i> _{CTX-M-8}	2 (5.26)	1 (5.55)	3 (5.35)	1 (7.69)	2 (18.18)	3 (12.5)
<i>bla</i> _{CTX-M-1} + <i>bla</i> _{CTX-M-9}	11 (28.94)	3 (16.66)	14 (25)	1 (7.69)	2 (18.18)	4 (16.66)
<i>bla</i> _{CTX-M-1} + <i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> _{CTX-M-2}	7 (18.42)	1 (5.55)	8 (14.28)	1 (7.69)	2 (18.18)	3 (12.5)
<i>bla</i> _{CTX-M-2} + <i>bla</i> _{CTX-M-8}	1 (2.63)	5.55 (1)	1 (1.78)	0 (0)	1 (9.09)	1 (4.16)
<i>bla</i> _{CTX-M-2} + <i>bla</i> _{CTX-M-9}	4 (10.52)	5.55 (1)	5 (8.92)	0 (0)	2 (18.18)	2 (8.33)
<i>bla</i> _{CTX-M-2} + <i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> _{CTX-M-8}	2 (5.26)	2 (11.11)	4 (7.14)	1 (7.69)	0 (0)	4 (16.66)
<i>bla</i> _{CTX-M-8} + <i>bla</i> _{CTX-M-9}	1 (2.63)	0 (0)	1 (1.78)	0 (0)	1 (9.09)	1 (4.16)
<i>bla</i> _{CTX-M-8} + <i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> _{CTX-M-9}	14 (36.84)	0 (0)	14 (25)	0 (0)	3 (27.27)	5 (20.83)
<i>bla</i> _{CTX-M-9} + <i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> _{CTX-M-1} + <i>bla</i> _{CTX-M-2} + <i>bla</i> _{CTX-M-8}	1 (2.63)	0 (0)	1 (1.78)	0 (0)	1 (9.09)	1 (4.16)
<i>bla</i> _{CTX-M-1} + <i>bla</i> _{CTX-M-2} + <i>bla</i> _{CTX-M-9}	4 (10.52)	1 (5.55)	5 (8.92)	0 (0)	2 (18.18)	1 (4.16)
<i>bla</i> _{CTX-M-1} + <i>bla</i> _{CTX-M-2} + <i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> _{CTX-M-1} + <i>bla</i> _{CTX-M-8} + <i>bla</i> _{CTX-M-9}	1 (2.63)	0 (0)	1 (1.78)	0 (0)	1 (9.09)	1 (4.16)
<i>bla</i> _{CTX-M-1} + <i>bla</i> _{CTX-M-9} + <i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> _{CTX-M-2} + <i>bla</i> _{CTX-M-8} + <i>bla</i> _{CTX-M-9}	2 (5.26)	1 (5.55)	1 (1.78)	0 (0)	1 (9.09)	1 (4.16)
<i>bla</i> _{CTX-M-2} + <i>bla</i> _{CTX-M-8} + <i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> _{CTX-M-2} + <i>bla</i> _{CTX-M-9} + <i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> _{CTX-M-8} + <i>bla</i> _{CTX-M-9} + <i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> _{CTX-M-1} + <i>bla</i> _{CTX-M-2} + <i>bla</i> _{CTX-M-8} + <i>bla</i> _{CTX-M-9}	1 (2.63)	0 (0)	1 (1.78)	0 (0)	1 (9.09)	1 (4.16)
<i>bla</i> _{CTX-M-2} + <i>bla</i> _{CTX-M-8} + <i>bla</i> _{CTX-M-9} + <i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> _{CTX-M-1} + <i>bla</i> _{CTX-M-8} + <i>bla</i> _{CTX-M-9} + <i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> _{CTX-M-1} + <i>bla</i> _{CTX-M-2} + <i>bla</i> _{CTX-M-8} + <i>bla</i> _{CTX-M-9} + <i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

of *E. coli* and *K. pneumoniae* isolates carrying the *bla*_{CTX-M} has increased significantly since then. In the previous studies during 2012 to 2013, *bla*_{CTX-M} determinants was detected among 74% of ESBL-producing *E. coli* and the 58.33% of *K. pneumoniae* isolates (33, 34). To date, CTX-M-producing *E. coli* and *K. pneumoniae* were recognized in other parts of the country, including northern Iran (*Enterobacteriaceae*, 2014 to 2015, n = 8) (35), Kashan (*Klebsiella* spp, 2012 to 2013, n = 9) (36), Shiraz (2010, n = 2) (37), and Tehran (*K. pneumo-*

niae, 2015 to 2016, n = 40) (38).

The results revealed that the CTX-M-1 group, mainly CTX-M-15, was the most prevalent genotype, followed by CTX-M-9. The prevalence of CTX-M-15 in the current study was similar to that of previous reports. These findings also agree with recent studies from China and other countries, in which CTX-M-15 was reported as the dominant ESBL genotype (29). In the study by Chen et al., CTX-M-2 and CTX-M-8 groups of ESBLs were identified only in one isolate of *K.*

Table 4. Distribution of Insertion Elements Among the *bla*_{CTX-M} Harboring Clinical Isolates

CTX-M Group	Bacterial Isolates, No. (%)							
	<i>Escherichia coli</i> (N = 56)				<i>Klebsiella pneumoniae</i> (n = 24)			
	ISEcp1	IS26	IS903	CTX-M + IS ^a	ISEcp1	IS26	IS903	CTX-M + IS ^a
<i>bla</i> _{CTX-M-1}	50 (89.28)	50 (89.28)	37 (66.07)	36.56 (64.28)	14.24 (58.33)	20 (83.33)	14 (58.33)	13 (45.16)
<i>bla</i> _{CTX-M-2}	10 (17.85)	8 (14.28)	6 (10.71)	5 (8.92)	3 (12.5)	3 (12.5)	2 (8.33)	2 (8.33)
<i>bla</i> _{CTX-M-8}	3 (5.35)	4 (7.14)	4 (7.14)	3 (5.35)	4 (16.66)	4 (16.66)	3 (12.5)	3 (12.5)
<i>bla</i> _{CTX-M-9}	14 (25)	14 (25)	9 (16.07)	9 (16.07)	5 (20.83)	5 (20.83)	4 (20.83)	4 (20.83)
<i>bla</i> _{CTX-M-25}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

^a CTX-M + IS, coexistence of IS types with CTX-M types.

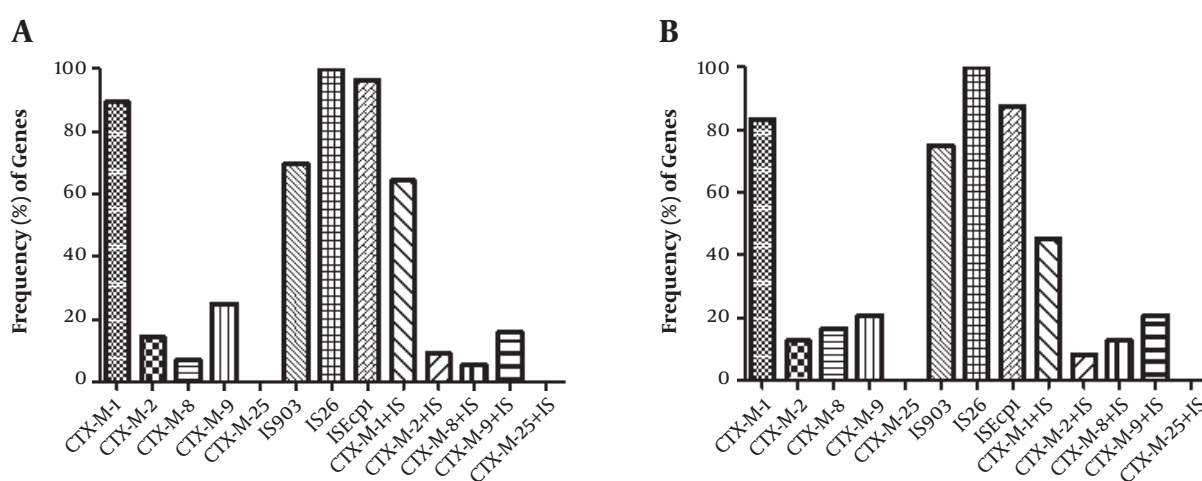


Figure 1. Distribution of CTX-M variants and insertion elements among the 56 *Escherichia coli* (A) and 24 *Klebsiella pneumoniae* (B) isolates from leukaemia patients. CTX-M + IS, coexistence of IS types with CTX-M types.

pneumoniae (3). Xia and coworkers reported that 96.2% (1, 124/1, 168) of the isolates harboured *bla*_{CTX-M} determinants, with a 40.7% (457/1, 124) and 48.7% (547/1, 124) *bla*_{CTX-M-1} and *bla*_{CTX-M-9}-positive subgroups, respectively (39). Mirkalan-tari et al. showed that imipenem, with 84.7% susceptibility, was the most effective antibiotic against *K. pneumoniae*. Seventy (63.63%) isolates had ESBL-positive results and 42 (60%) of them were positive for the CTX-M-1 gene (40).

Findings from previous studies suggest that more than one mobile element may be associated with different members of the CTX-M group (41). In the present study, ISEcp1, IS26, and IS903 elements were found to be associated with *bla*_{CTX-M-1}, *bla*_{CTX-M-2}, *bla*_{CTX-M-8}, and *bla*_{CTX-M-9} in a significant percentage of studied isolates ($P \leq 0.05$). In addition, a high presence of IS26 and IS903 elements was detected in CTX-M-1-producing isolates. It is interesting to note that the presence of IS26 flanking ISEcp1 may affect the impact of this insertion sequence on the mobilization

and expression of the *bla*_{CTX-M-15} gene. These elements could play an important role in the spread of such ESBLs. Thus, IS26, IS903, and ISEcp1 may be efficient tools for mobilization and expression of β -lactamase genes (42).

5.1. Conclusions

Multidrug-resistant *Enterobacteriaceae* that produce ESBLs, such as the CTX-M enzymes, have emerged both within the community and hospital settings as a serious issue. The rapid diagnosis of ESBL-producing isolates is helpful in selection of appropriate antimicrobials for therapy and prevention of the spread of these strains. Clinical microbiology laboratories should consider detection of ESBL producing *K. pneumoniae* and *E. coli* isolates important. It is recommended to routinely check all antibiotic resistant *K. pneumoniae* and *E. coli* isolates for ESBL production. This study indicates that the high prevalence of antibiotic resistance, IS and CTX-M-producing *E. coli* and *K. pneumoniae* iso-

lates could be a major concern and highlights the need of infection control measures.

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Footnotes

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