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Full Length Research Paper

Detection of integron elements and gene groups encoding ESBLs and their prevalence in *Escherichia coli* and *Klebsiella* isolated from urine samples by PCR method

Abdollah Karimi[1], Mohammad Rahbar[2,3], Fatemeh Fallah[1], Masoumeh Navidinia[1] and Mohammad Ali Malekan[1]*

[1]Pediatric Infections Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2]Department of Microbiology, Iranian Reference Health Laboratory, Ministry of Health and Medical Education, Tehran Iran.
[3]Antimicrobial Resistance Center, Tehran University of Medical Sciences, Tehran, Iran.

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Integrons, which are short sequences of deoxyribonucleic acid (DNA) like transposes have the capability of transferring genes of antibiotic resistance to other bacteria. Studies have revealed that these elements could bear the extended spectrum beta lactamase (ESBL), genes that are transmitted to many strains of bacteria especially Enterobacteriaceae members. The aim of this study was to determine prevalence of integron elements and gene groups encoding ESBLs in *Escherichia coli,* and *Klebsiella pneumoniae* isolated from urine specimens. In total 5000 specimen were collected from children with urinary tract infection (UTI) referred to Mofid children hospital in Tehran, during the 6 month study period by consecutive sampling. The 100 confirmed isolates of *E. coli* and 50 *K. pneumoniae* were tested for susceptibility to different antimicrobial drugs like cefotaxime, ceftriaxone, cefpodoxime, aztreonam, ceftazidime, cefpodoxime plus clavulanate antibiotic susceptibility testing was performed by disk diffusion method and E-test method were used for detection MIC. ESBL positive bacteria were preserved in -70°C freezers and finally polymerase chain reaction (PCR) performed on them. The gene groups studied for presences of ESBLs were CTX, TEM, SHV and Int. Thirty isolates of *E. coli* were positive for ESBL; the frequency of gene groups was as follows: CTX (24), TEM (26), SHV (15), and Int (12). In the 19 isolated stains of *K. pneumonia* positive for ESBL, the frequency of gene groups was: CTX (18), TEM (18), SHV (15) and Int (10). The prevalence of class 1 Integrons was 52% in non-outbreak *E. coli* isolates and 48% in *K. pneumoniae* isolates from patients in our study. Less than half of the selected isolates from patients' samples that we tested were positive for class 1 Integron. 48% of *K. pneumoniae* and 54% of *E. coli* strains possessed Integron and ESBL genes concomitantly.

Key words: Integron, extended –spectrum β-lactmase (ESBLs), PCR, *Escherichia coli*, *Klebsiella Pneumoniae.*

INTRODUCTION

Extended –spectrum β-lactmase (ESBLs) has been emerged as an important drug resistance among Gram-negative bacilli especially *Escherichia coli* and *Klebsiella pneumoniae* (Mehrgan et al., 2010; Ghafourian et al., 2011). The need for systematic epidemiologic studies of the role of Integrons in antimicrobial drug resistance in bacteria has recently been emphasized (Norrby, 2005). The prevalence of integrons is high among Gram-negative isolates from patients in Europe (Sallen et al.,

*Corresponding author. E-mail: alborzpersia@yahoo.com. Tel: 00989127681016.

1995; Martinez-Freijo et al., 1998). Reports from Asian countries also have noted a high prevalence of class I Integrons in Gram-negative clinical isolates (Yu et al., 2003). Most of the resistant integrons found to date in clinical isolates of Enterobacteriaceae are class 1 integrons, which bear the extended spectrum beta lactamase, (ESBL), genes that are transmitted to many strains of bacteria especially Enterobacteriaceae members. ESBLs are highly associated with resistance to antimicrobial agents (Sallen et al., 1995; Machado et al., 2007). Members of the family Enterobacteriaceae commonly express plasmid-encoded β-lactamases (e.g., TEM-1, TEM-2, and SHV-1) which confer resistance to penicillins but not to broad-spectrum cephalosporins (Knothe et al., 1983). These cephalosporins include Cefotaxime, Ceftriaxon, and Ceftazidime, as well as the oxyimino-monobactam Aztreonam (Emery and Weymouth, 1997). Typically, the ESBL derive from genes for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these β-lactamases. TEM-1 is the most commonly-encountered beta-lactamase in Gram-negative bacteria (McGowan et al., 2002). Up to 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1 (Paterson et al., 2003; Bradford, 2001; George and Luisa, 2005). The SHV-1 beta-lactamase is most commonly found in *K. pneumoniae* and is responsible for up to 20% of the plasmid-mediated ampicillin resistance in this specison et al., 2003). Another class of enzymes CTX-M beta-lactamases (class A), were named for their greater activity against Cefotaxim than other oxyimino-beta-lactam substrates (e.g., Ceftazidime, Ceftriaxone, or cefepime) (Lau et al., 2008). They have mainly been found in strains of Salmonella enterica serovar Typhimurium and *E. coli*. OXA beta-lactamases were long recognized as a less common but also plasmid-mediated beta-lactamase variety that could hydrolyze oxacillin and related anti-staphylococcal penicillins. Integrons are genetic elements, located on the bacterial chromosome or a plasmid, that often carry genetic determinants for antimicrobial drug resistance (Leverstein-van et al., 2003). Data suggest that integrons are relatively common, especially among the Enterobacteriaceae; however, very few studies from the Islamic Republic of Iran have assessed the association between integron carriage and antimicrobial susceptibility patterns. This study analyzes the association between class 1 integrons and resistance to selected antimicrobial agents in a convenience sample of *E. coli* and *Klebsiella* isolates in an Iranian children hospital.

MATERIALS AND METHODS

In total 5000 urine samples were collected from children with urinary tract infection (UTI) referred to Mofid children hospital in Tehran, during the 6 month study period by consecutive sampling. These samples were inoculated on blood agar, EMB agar; after overnight growth, diagnostic biochemical tests were performed to identify different types of bacteria. The 100 confirmed isolates of *E. coli* and 50 *K. pneumoniae* were subject of our study. Susceptibility testing was done by disk diffusion method. ESBLs production was performed by using the double disk diffusion synergy test as recommended by Clinical Laboratory and Standards Institute (CLSI). EBLS presence was determined using following antibiotics: Cefotaxime (30 µg), Cefotaxime/clavulanic acid (30/10 µg), ceftazidim (30 µg) ceftazidime / clavulanic acid (30/10 µg), cefopodexim (30 µg), cefopodexim// clavulanic acid (30/10 µg), E-test were used for detection MIC. Positive isolates for presence of ESBLs were stored at –70°C and were subcultured to trypticase soy agar plates containing 5% defibrinated sheep blood (BD Biosciences. Isolates were analyzed by polymerase chain reaction (PCR) amplification techniques to determine whether a class 1 integron was present. Integrons were detected by PCR amplification of a class 1 integrase–specific fragment of the IntI1 gene previously described (Table 1).

Sequences of the primers used to detect bla and Int genes and their adjacent regions were:

Amplifications were performed in 10 µL of Taq PCR Master Mix (Qiagen, Valencia, CA, USA), 1.5 mmol/L $MgCl_2$, 5 pmol/L each primer, and 1 µg template Deoxyribonucleic acid (DNA). DNA was extracted with Qiagen extraction kit. Amplification specifications were as follows: 5 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 30 s at 72°C (Rao et al., 2006). PCR products were analyzed by gel electrophoresis with 1% agarose (Fermentas, Spain). All PCRs included positive and negative controls. A statistical comparison of the frequencies of integron presence in *E. coli* and Klebsiella spp. was conducted by using odds ratios and 95% confidence intervals. Intermediate and resistant isolates were pooled as nonsusceptible for analysis. Multivariate logistic regression analysis was conducted with one representative from each major class of drugs tested. The studied gene groups for ESBL were CTX, TEM, SHV and Int.

RESULTS

Thirty isolates of *E. coli* were positive for ESBL; the frequency of gene groups was as follows: CTX (24), TEM (26), SHV (15), and Int (12). 19 isolated stains of *K. pneumoniae* were positive for ESBL the frequency of gene groups being: CTX (18), TEM (18), SHV (15) and Int (10). The prevalence of class 1 Integrons was 52% in non-outbreak *E. coli* isolates and 48% in *Klebsiella* isolates from patients in our study. Less than half of the selected isolates from patients' samples that we tested were positive for class 1 Integron. Among these strains 48 to 54% ESBL genes that found in *K. pneumoniae* and *E. coli,* respectively were present concomitantly with Integrons.

DISCUSSION

E. coli and *K. pneumoniae* are the most important causative agents of nosocomial infections. In our study resistance to third generation of cephalosporins among isolates of these microorganisms was prevalent and imipenem was the most effective antibiotics against above mentioned organisms. Our study revealed that the prevalence ESBLs production is higher in our country in compassion with developing countries (Behroozi et al.,

Table 1. The typical agarose gel documented for presence of ESBLs genes and integron elements.

PCR target	Primer sequence
CTX-M internal	SCSATGTGCAGYACCAGT[a] CCGCRATATGRTTGGTGGTG[a]
SHV consensus	GGGAAACGGAACTGAATGAG ATCGTCCACCATCCACTGCA
CTX-M-2	ATGATGACTCAGAGCATTCG TGGGTTACGATTTTCGCCGC
CTX downstream CTX upstream	CACCACCAACGATATCGCGG GCCGCCATCACCTTACTGGT
IntI1	AGGAGATCCGAAGACCTC TCTCGGGTAACATCAAGG
TEM full	AGCCCCATACCTACAAAGCC GGAAGAGTATGAGTATTCAA TTACCAATGCTTAATCAGTG
TEM upstream TEM downstream	TGCAACAGTGCCTCTCGATA CTCGTGCACCCAACTGATCT

2010). The results for integron prevalence in this study are different from other results obtained in some documented articles around the world. For instance in one of the studies performed in Richmond, Virginia, in 1997 reports that approximately 1.5% of isolates of the family Enterobacteriaceae which were isolated from 1.2% of patients (23 of 1,844), were found to express ESBLs (Emery and Weymouth, 1997) In another study from the United States done during the same period occurrence of ESBL production in *Enterobacteriaceae* ranged from 0 to 25% (Emery et al., 1997).

Since the most frequent Enterobacteriaceae strains which are usually isolated from the patients in Mofid Children's Hospital are *E. coli* and *K. pneumonia*, we selected these two types of bacteria for our study. In previous studies about ESBLs in Mofid Children's Hospital, the researchers have studied the prevalence of ESBLs between these two types of bacteria for their susceptibility testing using standard techniques and also molecular detection for ESBLs genes by PCR method. In the present study we searched the likely presence of Integron elements in the strains which contained ESBLs (Figure 1). In a study performed in Israel in 2004, Integron-mediated ESBL resistance in serotypes of *E. coli* causing infections in an elderly population of Israel was researched and results of this study indicate that none of the serotypes belonged to non-ESBL strains; however in other study only a few of the isolated strains were in the non-ESBL group (Sompolinsky et al., 2005).

In a study which carried out in Tehran (Feizabadi et al., 2010) ESBL production was detected in 72.1% (n = 75) of isolates of *K. pneumoniae.* The prevalence of blaSHV, blaTEM and blaCTX-M genes among the isolates was 55.7% (n = 58), 30.7% (n = 32) and 45.2% (n = 47), respectively. The sequencing revealed the amplicons corresponding to bla (TEM-1, TEM-79, SHV-1, SHV-12, SHV-31, CTX-M-15) genes. While the blaCTX-M-15 was the dominant gene among the Iranian isolates. Other studies in region and neighboring countries showed a high rate of ESBLS producing *K. pneumoniae* which varied from 85% in Russia, 66% in India 54.7 and 61% in Turkey, 41% in United Arab Emirates and 31.7% in Kuwaiti. (Feizabadi et al., 2010). The rate of ESBls among isolates of *E. coli* in our country is also very high. In a study which carried out by Mehrgan and Rahbar (2008), they found a high rate of (67.2%) ESBLs producing in *E. coli* in a 1000-bed tertiary care hospital in Tehran.

In conclusion our study showed a high rate of ESBLS among of *E. coli* and *K. pneumonia* isolates. In our study imipenem following amikacin were the most effective antibiotic against ESBLs isolates. However emerging resistance to these antibiotics in future may limit their prescription.

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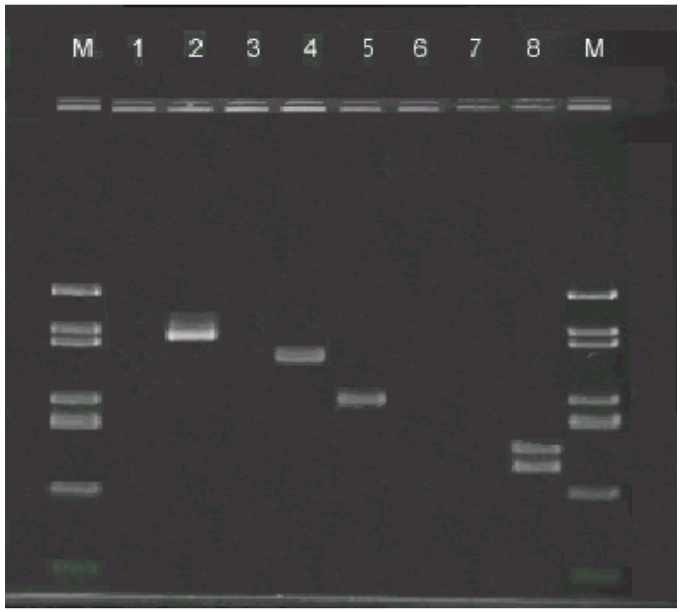

Figure 1. Demonstrating one of PCR results that was performed for the detection of Int, SHV, TEM and CTX genes in prominent isolated strains which contained ESBLs after confirmation by susceptibility testing. Left and right bands are for ladder DNA strands (M). Lane 2 Int (900 bp), lane 4 SHV (870 bp), lane 5 indicate TEM (717 bp) lane 8 shows CTX (450 to 500 bp).

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