

# Studying Class 1 & 2 Integrons and Gene Cassettes CS1 & CS2 in Multi-drug Resistant *Klebsiella* Pneumonia Isolates of Clinical Samples Taken from Hospitalized Patients in Tohid Hospital of Sanandaj, in 2013

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**Abstract Literature review and objectives:** *Klebsiella pneumoniae* is one of the most important causes of nosocomial infections and antibiotic resistance. Integron is one of the genetic movable elements that can carry different antibiotic resistance genes. Here, the class 1 integron role in the creation and transfer of antibiotic resistance is very important. The purpose of this study is to isolate *K. pneumoniae* from hospitalized patients of Tohid Hospital in Sanandaj and molecular analysis of class 1 and 2 integrase genes and gene cassettes CS1 and CS2 and also determining susceptibility or resistance to different antibiotics. **Materials and methods:** 120 *Klebsiella* samples were collected from Tohid hospital in Sanandaj. *Klebsiellas* were tested by using standard methods and differential tests and 41 samples, 41 isolates of *Klebsiella pneumoniae* were detected from all the samples. Susceptibility testing was performed by Standard disk diffusion method according to CLSI method. In order to investigate the class 1 & 2 integrons frequency and also, gene cassettes CS1 & CS2, PCR method was applied. **Findings:** Of all the 120 *klebsiella* samples, 41 were *Klebsiella pneumoniae*. The result of antibiotic-sensitivity test showed that 100% of the strains were sensitive to imipenem and meropenem, 83.33% to gentamicin, 100% to ceftriaxone, 75% to amikacin, 100% to cefepime, 83.33% to ciprofloxacin, 100% to ceftazidime, 100% to cefotaxime and 100% were resistant to Ztryvnam. Of the 41 resistant samples, in 12 strains (29.26%) integrase 1 and 5 strains (12.19%) gene cassette CS2 was observed, but in no strain, class 2 integrase and gene cassette CS2 were observed. **Results:** The results of the research indicated that class 1 integrase gene frequency is high in strains which can play an important role in creation and transfer of antibiotic resistance; and this could be due to the indiscriminate use of antibiotics.

**Keywords** *Klebsiella pneumoniae*, Class 1 & 2 integrons, Multi-drug resistant, PCR

## 1. Introduction

*Klebsiella pneumoniae* is an opportunistic pathogen causing urinary tract infection and pneumonia in people with weakened immune systems [1]. *Klebsiella pneumoniae* with 15 to 18 percent, has the second ranking after *Escherichia coli* in creation of septicemia and urinary tract infections as well as hospital Gram-negative infections [1]. Urinary tract infections are the most common infections in all age groups whose lack of diagnosis and treatment results in hypertension, renal disorders, and early pregnancy and even abortion, and urinary tract disorders [2 & 3]. In the past few years throughout the world, resistance to several drugs in

bacteria compared to more than two different class of antibiotics among hospital isolates has been increasing; This is termed Multidrug resistant or MDR, it is said that this phenomenon will unfortunately be worrying [4 & 5]. Studies show that regardless of the pattern of use of antibiotics, antibiotic resistance genes can be transferred between bacterial populations [6]. DNA elements such as transposons and Plasmids which are the location of antibiotic resistance genes have been the cause of transfer of the genes between different species of bacteria; these elements are called the integron.

Integrons have been introduced as a new mechanism for publishing resistance genes among bacteria [7]. Horizontal transfer of integrons is the most successful way in the release of resistance genes and emergence of multi-resistant species. Antibiotic-resistance coding integrons have been divided into four classes; Most of the known integrons belong to class 1 and they contain Sull gene, Class 2 is in the

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transposons of the Tn-7 and class 3 contains Metallo-beta-lactamase [8 & 9 & 10].

Generally based on differences in the integrase gene, integrons are divided into different classes. The four distinct classes of the integrons responsible for multiple resistance are a genetic code of aspecial integrase each. Integrase genes include Int I1, Int I2, Int I3, and Int I4. Most of the isolated samples from patients and hospital environments with MDR conditions belong to class 1 which are located on plasmids or transposons [11].

## 2. Materials and Methods

### Bacterial isolates and data collection

In summer 1392 collected samples that had been frozen were cultured on blood agar and eosin methylene blue prepared by the Merck Company in Germany. Profile of each isolate which include age, sex, history of previous illness, types of samples were recorded in a table. It should be noted that the isolated *Klebsiella pneumoniae* samples were kept in Muller Hinton Broth environment (Merck, Germany) with 18% glycerol at -80°C until usage.

Disk diffusion testing: Ten antibiotic disks produced by (MAST Company-UK), as well as a number of other antibiotics produced by the company (Exir -Iran) were tested for all the samples.

### 2.1. Antibiotic Sensitivity Follows

Imipenem (10 micrograms), gentamicin (10 mg), ceftriaxone (30 micrograms), amikacin (30 mg), cefepime (30 micrograms), ciprofloxacin (5 micrograms), ceftazidime (30 micrograms), cefotaxime (30 micrograms), meropenem (10 micrograms) and aztreonam (30 mcg). *Klebsiella pneumoniae* bacterium was cultured in Broth *vitro* (Barin Heart Infusion BHI) and incubated at 37°C for 24 hours. After bacterial growth and creation of turbidity according to semi McFarland standard, they were cultured using steel swab on Mueller Hinton Agar environment. After the disks, the plates were placed in temperature of 37°C for 24 hours. Susceptibility or resistance to antibiotics were reported based on NCCLS. Then the samples that were resistant to at least three classes of antibiotics were considered as examples of multiple resistance (multidrug resistance).

### 2.2. DNA Extraction, Performing PCR

At this level phenol-chloroform method was used to extract DNA. After DNA extraction, each sample was maintained until PCR performance at a temperature of 20°C. To perform PCR primers sequences (Table 1) for a plurality of sequences of genes *int I* & *int II*, and also, the gene cassettes CS1 and CS2 were designed. PCR reactions were performed in a volume of 50 microliter which contained 10 microliters of extracted DNA (1 microgram), 5 pmol of each primer per liter, 1.5 mmol per liter  $\text{MgCl}_2$ , 0.2mM per liter dNTPs and 1.5 unit of Taq enzyme. Proliferation of the intended pieces in the thermo cycler device is as follows:

**Table 1.** Sequences and temperatures of primers used in the PCR

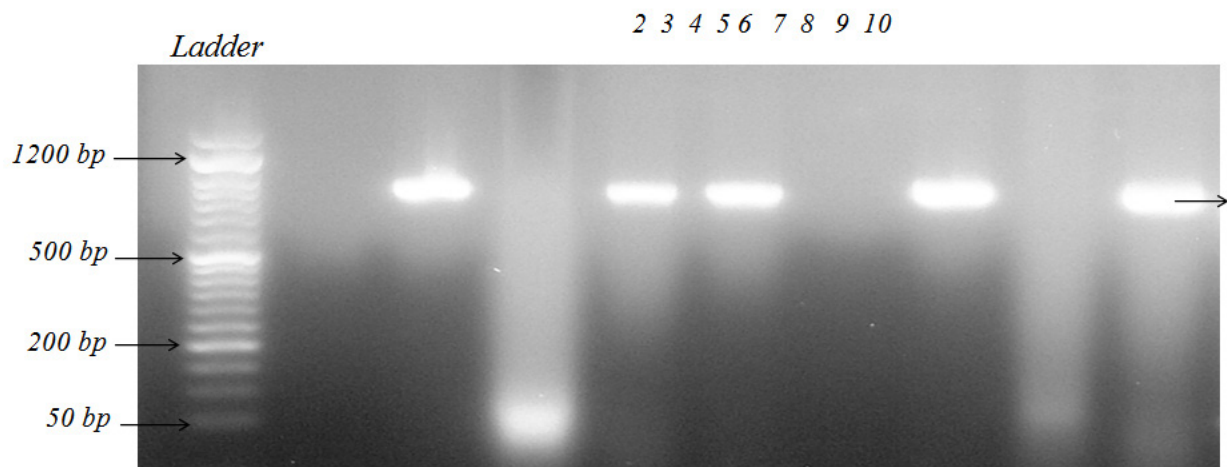
Primer	Nucleotide Sequence (5-3)	Denaturation	Annealing	Cycle
Int I F Int I R	ATCATCGTCGTAGAGACGTCGG GTCAAGGTTCTGGACCAGTTGC	94	55	72 30
Int II F Int II R	CACGGATATGCGACAAAAAGGT GTAGCAAACGAGTGACGAAATG	94	60	72
CS I F CS I R	GGCATCCAAGCAGCAAG AAGCAGACTTGACCTGA	94	57	72
CS II F CS II R	ACCTTTTGTGCGCATATCCGTG TACCTGTTCTGCCGATATCT	94	60	72

Electrophoresis of PCR products was done in Agarose gel of 1.5% and the voltage of 80 V for an hour. The gel was examined under UV light. The achieved bands compared to DNA size Marker of a thousand base pairs bp (Fermentas) were considered as the intended fragment of the genes.

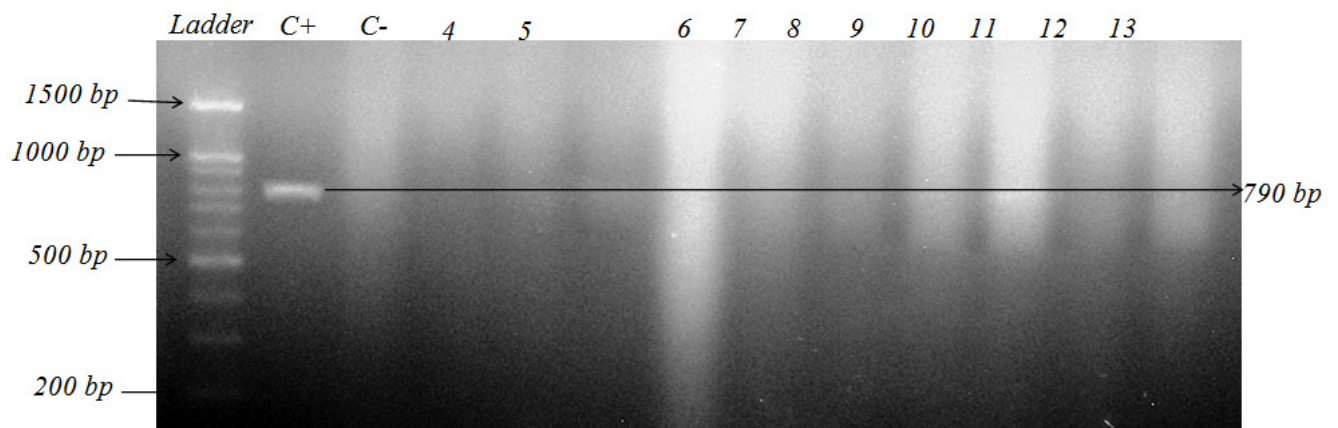
## 3. Findings

Of all the 120 *Klebsiella* samples, 41 were *Klebsiella pneumoniae*. The result of antibiotic-sensitivity test showed that 100% of the strains were sensitive to imipenem and meropenem, 83.33 % to gentamicin, 100% to ceftriaxone, 75% to amikacin, 100% to cefepime, 83.33 % to ciprofloxacin, 100% to ceftazidime, 100 % to cefotaxime and 100% were resistant to aztreonam. Of the 41 resistant samples, in 12 strains (29.26%) integrase 1 and 5 strains (12.19%) gene cassette CS2 was observed, but in any strain, class 2 integrase and gene cassette CS2 were not observed. In this study, 41 isolates of *Klebsiella*

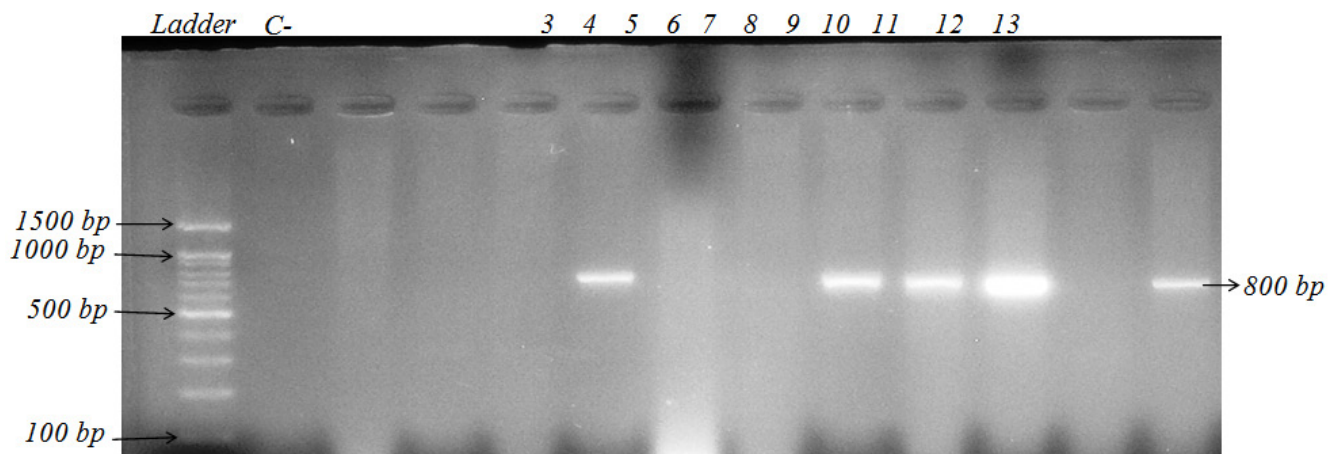
pneumoniae from Tohid Hospital in Sanandaj, were taken out. After performing PCR with specific primers, 29.26 % of samples (12 samples) possessed gene int I and 12. 19 % of the samples (5 samples) possessed gene cassette CS1; and Gene int II and gene cassette CS2 were not found.



**Figure 1.** PCR results of Class I integron gene of *K. pneumoniae* strains isolated from patients hospitalized in Tohid hospital in Sanandaj. (The first plate marker bp 50 to 1500 bp, plates 3, 5, 6, 8 & 10 are positive class I integron gene and plates 2, 4, 7, 9 have reported negative results for class I integron gene)



**Figure 2.** PCR results of Class II integron gene of *K. pneumoniae* strains isolated from patients hospitalized in Tohid hospital in Sanandaj. (The first plate marker 200 bp to 1500 bp, the second plate *Acinetobacter baumannii* strains positive control and in plate three, negative control and class II integron gene cannot be seen therefore there is no CSII gene cassette)



**Figure 3.** PCR results CS1 gene cassette of *K. pneumoniae* strains isolated from patients hospitalized in Tohid hospital in Sanandaj. (Plates 6, 9, 10, 11, 13 contain gene cassette CS I. CS I bands are variable and this indicates the patients in the hospital have been contaminated with a specific clone of bacteria)

For 41 isolates of *Klebsiella pneumoniae*, antibiotic sensitivity testing was performed, and the antibiotic resistance percentage of the isolated samples are as shown below in order:

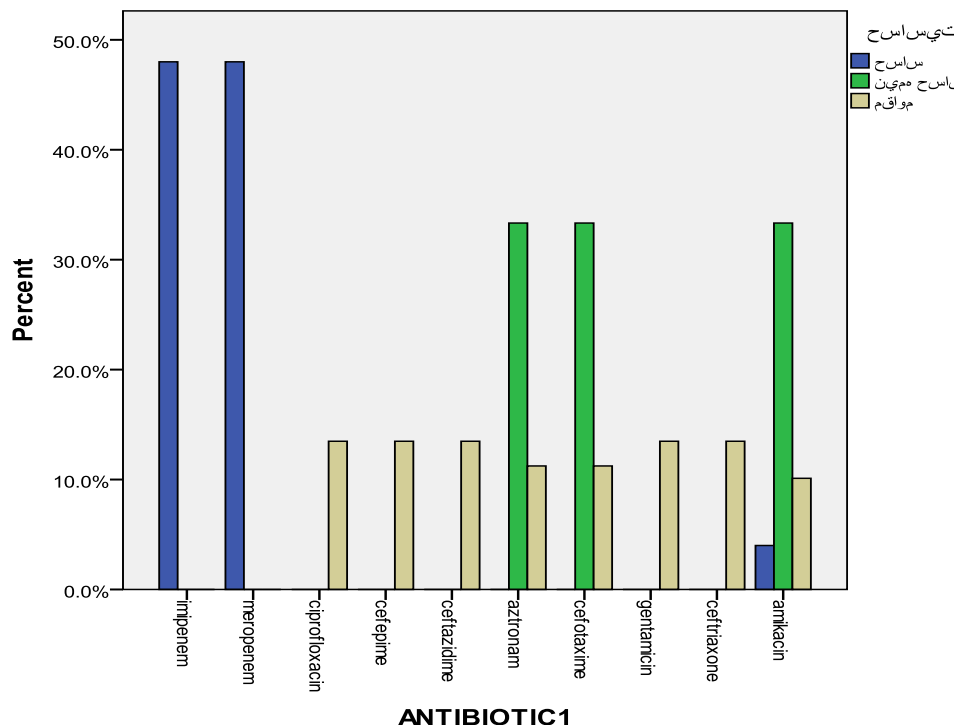


Diagram 1. The frequency of antibiotic sensitivity

## 4. Results and Discussions

Urinary tract infections in humans is considered one of the most important infections whose abundance is in second place after respiratory infection. However, Shah Cheraghi *et.al.* Reported the outbreak of *Klebsiella pneumoniae* isolates from urine samples greater than other urinary pathogens factors. In the present study 41 isolates of *Klebsiella pneumoniae* were taken out of a total of 175 urine samples from patients hospitalized in Tohid Hospital in Sanandaj, of these, 58.54 % of isolates belonged to female patients and 41.46 % of the isolates were males. Khalili, *et.al* and also Ahangarzade Rezaee *et.al* have reported the results of positive urine culture in females 82.08 % and 55.55% and the results related to males 27.72% and 44.44% respectively. This indicates that women more than men are likely to develop urinary tract infections particularly *Klebsiella pneumoniae*. Frequency of class I integron genes in *K. pneumoniae* strains was 29.26 % which is consistent with the results reported by similar studies 34.02 % and 32.09 %. In a study conducted in a hospital in the United States in 2006, the outbreak of class I integron gene was reported 70 %; and in another study carried out in three hospitals in Australia in 2005 on *Klebsiella* producers of broad-spectrum beta-lactamase, the incidence of this gene was reported 73% which was not consistent with those of ours. This difference

could be due to geographical regions and bacterial strains; thus the frequency percentage of integrons in different bacteria can vary, for example, in a report released in 2006 in China, the outbreak of Class I integron in *Pseudomonas* bacteria was reported 38% and in Amazon region it was 41.15 %. In 2006, in 13 states of the United States of America the outbreak of integron gene among *Escherichia coli* and *Klebsiella* bacteria were studied using PCR; of the 320 bacteria isolates, 57% possessed class I integron, of which 49% *Escherichia coli* bacteria and 70% *Klebsiella pneumoniae* bacteria possessed this gene. In this research, all the *Klebsiella pneumoniae* isolates possessing class I integron were resistant to cefepime, ceftazidime, aztreonam, cefotaxime, and ceftriaxone antibiotics. The reason for this resistance can be the presence of antibiotic resistance gene cassettes in class I integron gene. The antibiotic resistance level among isolates obtained from society and hospitals is increasing and this is a universal problem. Resistance in strains causing hospital infections is increasing which has led to many problems in curing the diseases. Regardless of the antibiotic consumption patterns, their resistant genes can be transferred among the bacterial population, meanwhile, the integron using the new mechanism of spread, transfer the resistance genes among bacteria. Horizontal transfer of integrons is the most successful way to release resistance genes and lead to the emergence of species with multi-drug resistance (MDR).

**Table 2.** Antibiotic susceptibility test of isolates possessing positive integron taken from Tohid Hospital in Sanandaj in 2013

Antibiotic	Sensitive F	Sub-critical F	Resistant F	Sensitive M	Sub-critical M	Resistant M
imipenem	4			8		
meropenem	4			8		
cefepime			4			8
cefotaxime			4			8
aztreonam			4			8
gentamicin			4	1	1	6
ciprofloxacin		1	3		1	7
ceftazidime			4			8
ceftriaxone			4			8
amikacin	1		4	2		6

**Table 3.** Distribution of patients according to gender

	Frequency	Percent	Valid Percent	Cumulative Percent
<b>Valid Man</b>	17	41.5	41.5	41.5
<b>Woman</b>	24	58.5	58.5	100.0
<b>Total</b>	41	100.0	100.0	

(Most patients were female, with a frequency of 58.5percent)

**Table 4.** Distribution of the relationship between Resistance gene and patients' age groups

Age	Gene resistance	Gene resistance		Total
		+	-	
	5-40 Count	6	5	11
	% within gene resistance	50.0%	17.2%	26.8%
	41-60 Count	3	14	17
	% within gene resistance	25.0%	48.5%	41.5%
	61-80 Count	3	10	13
	%within gene resistance	25.0%	34.5%	31.7%
	<b>Total</b> Count	12	29	41
	%within gene resistance	100.0%	100.0%	100.0%

#### Chi-Square Tests

	Value	df	Asymp.Sig. (2-sided)	Exact Sig. (2-sided)
<b>Pearson Chi-Square</b>	4.745	2	.093	.101
<b>Likelihood Ratio</b>	4.525	2	.104	.141
<b>Fisher's Exact Test</b>	4.344			.141
<b>Linear-by-Linear Association</b>	2.534	1	.111	.124
<b>N of Valid Case</b>	41			

According to the Fisher test there is no significant relationship between patients' age the resistance gene ( $X^2=4.34$   $P=0.14$   $df=2$ )

**Table 5.** Comparison of the mean age of patients in the resistant gene with non-resistance genes

P	t	Standard deviation	average	number	Gene resistance
		19.69	39.92	12	+
0.18	2.47	15.37	54.10	29	-

According to t-test, there is a significant difference between the mean age of patients in resistant genes and non-resistant genes. ( $p = .018$ )

**Table 6.** Distribution of the relationship between resistant gene existence and gender

Noresistance gene		resistance gene		Gene resistance
Percent	number	Percent	number	Sex
31	9	66.7	8	Man
69	20	33.3	4	Woman
100	29	100	12	Total

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