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Original Article

Prevalence of Class 1 Integron in *Klebsiella Pneumoniae* Isolates from Hospitals of Sanandaj, Iran

Pegah Shakib[1*] Ph.D.

[1]Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran.

ABSTRACT

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Key words
Class 1 Integron
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Background and Aims: Integrons as mobile genetic elements are located on the chromosome or on a plasmid in bacteria. Integrons play a main role in the dissemination of antibiotic resistance genes among different families of bacteria. The aim of this study was to identify the prevalence of class 1 integron in *Klebsiella pneumoniae* isolates from hospitals of Sanandaj, Kurdistan province, Iran.

Materials and Methods: Seventy *Klebsiella pneumoniae* isolates were collected from Hospitals of Sanandaj. Antibiotic susceptibility pattern was performed by disc diffusion method. Class 1 integrons gene was screened by polymerase chain reaction assay. Data were analyzed by Fisher tests with STATA software program.

Results: The highest and lowest rates of resistance were related to cefotaxime and imipenem, respectively. Thirteen (18.5%) out of 70 *Klebsiella pneumoniae* isolates caring class 1 integron gene. Out of 28 multidrug resistant isolates, 11 isolates were identified to be positive for the existence of class 1 integrons.

Conclusions: class 1 integron positive isolates, compared to class 1 integron negative isolates, reveals resistance to more antibiotics.

***Corresponding Author:** Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran. **Tel:** +989166613806, **Email:** Shakib.pegah@yahoo.com

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Introduction

Klebsiella pneumoniae (K. pneumoniae) is one of the gram negative rods from *Enterobacteriacea* family. *K. pneumoniae* is the major cause of nosocomial infection, septicemia, tract respiratory infection, meningitis, and urinary tract infection [1-3]. Recently, family members of *Entero-bacteriaceae* especially *Escherichia coli (E. coli)* and *K. pneumoniae* are found to be resistant to most antibiotics used for treatment. Resistance to antimicrobial agent such as beta-lactam antibiotics, fluoroquinolones, and aminoglycosides in these bacteria is induced by various mechanisms [4-6].

The rapid emergence of multidrug resistant *Klebsiella pneumoniae* (MDRKP), becoming resistant to different categories of generally used antibiotics, is a major global medical challenge [7, 8]. Integrons as mobile genetic elements are located on the chromosome or on a plasmid in bacteria [9]. Integrons play a main role in the dissemination of antibiotic resistance genes among different family of bacteria [10]. Classes 1, 2, and 3 integrons are most commonly identified in Gram-negative bacteria [11].

Class 1 integrons are predominantly integron-type among the antibiotic resistance clinical isolates of *Enterobacteriaceae* family, including *K. pneumoniae* [12]. Class 1 integrons consist of two conserved regions, a 3′-conserved segment (3′-CS) and a 5′-conserved segment (5′-CS), which have a physical connection to Tn402-like transposons, and an internal variable region (VR) made up of gene cassettes in tandem that encode antimicrobial resistance determinants [13, 14].

In this study, we report the prevalence of class 1 integron in *K. pneumoniae* isolates from hospitals of Sanandaj in Kurdistan province.

Materials and Methods

Bacterial isolates and identification

Seventy *K. pneumoniae* isolates were taken from different specimens including blood, urine, wound and tracheal aspirates from October 2015 to July 2016 out of the hospitals of Sanandaj.

All the isolates were identified by Gram stain and biochemical tests such as lactose fermentation, methyl red, voges proskauer, indole, citrate (IMViC), urea hydrolysis, lysine decarboxylase, H2S production, and oxidase tests [15] .

Antibiotic susceptibility pattern of clinical isolates

Antibiotic susceptibility Pattern of antibiotic agents ceftazidime (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), gentamicin (10 µg), kanamycin (10 µg), imipenem (10 µg), and co-trimoxazole (1.25+23.75 µg) (Roscoe, Denmark) were performed according to Clinical and Laboratory Standards Institute's (CLSI) 2016 guidelines by disc diffusion method [16].

Screening for class 1 integron by polymerase chain reaction (PCR)

After DNA extraction by SinaClon Kit, all the

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isolates were screened out of class 1 integron gene by PCR amplification using Corbett thermal cycler and specific primers, as described in table 1. Cycling program was as follows: initial denaturation at 94˚C for 5 min followed by 35 cycles of denaturation at 94˚C for 1 min, annealing at 56˚C for 1 min, elongation at 72˚C for 1 min, and final elongation at 72˚C for 7 min. Then, PCR products and marker-100 bp were evaluated on 1.5% agarose gels [17]. *K. pneumoniae* (ATCC 1029) was used as positive control for detecting class 1 integron through PCR method.

Statistical analysis

Data were analyzed by Fisher tests with STATA software program.

Results

Antibiotic susceptibility pattern of clinical isolates

Out of 70 *K. pneumoniae,* 47 (67.1%), 40 (57.1%), 24 (34.3%), 27 (38.6%), 21 (30%), 28 (40%), 6 (8.6%), and 40 (57.1%) were resistant to cefotaxime, ceftazidime, ciprofloxacin, gentamycin, kanamycin, amikacin, imipenem, and co-trimoxazole, respectively. Totally, 28 isolates (40%) were detected as multidrug resistant isolates.

Screening for class 1 integron gene

Thirteen (18.5%) out of 70 *K. pneumoniae* isolates caring class 1 integron gene (Fig. 1).

In our study, out of (40%) 28 multidrug resistant 11 were identified to be positive for the existence of class 1 integrons.

Table 1. Sequence of primers used in this study

Gene target	Sequence of primers (5′ to 3′)	Amplicon size (bp)	Reference
intI1	F:5′-CAGTGGACATAAGCCTGTTC-3′ R: 5′-CCCGAGGCATAGACTGTA-3′	160 bp	[17]

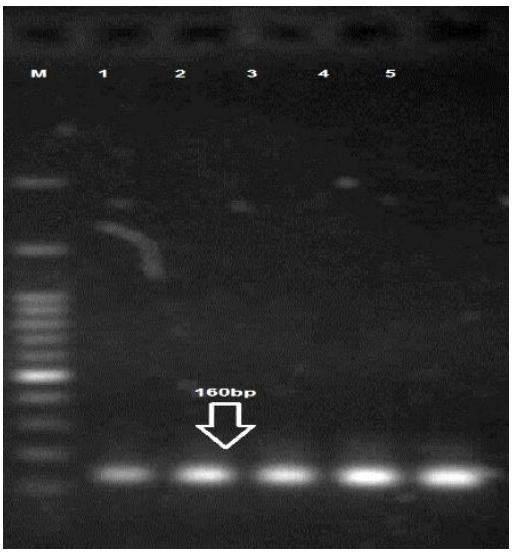

Fig. 1. Gel electrophoresis of PCR products for detection of class 1 integron genes in *K. pneumoniae* isolates.
M= Marker-100 bp; 1-4= Positive isolates for Class 1 Integron; 5= Positive control for Class 1 Integron

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Discussion

K. pneumoniae is one of the major causes of bacterial and nasocomial infections [18, 19]. During the last decades, MDRKP isolates have increased dramatically [20, 21]. multidrug resistant isolates are resistant to at least three antibiotic groups. The emergence of multidrug resistant isolates has been proposed as the most important threat in the management of nosocomial infections [22]. Diverse strategies are involved in the spread of antibiotic resistance in *K. pneumoniae* strains. Among them, Integrons as one of the mobile genetic elements is described as the key factor in the dissemination of these multidrug resistant clinical isolates. An integron including the gene for an integrase site (int) and for an adjacent recombination site (attI), can be situated on the bacterial chromosome or plasmid. Class 1 integrons are the most predominant and have often been described in clinical isolates *K. pneumoniae* [23].

In this study, out of 13 *K. pneumoniae* isolates positive for class 1 integron gene, 11 isolates were multidrug resistant. We found that class 1 integron positive isolates, compared to class 1 integron negative isolates, reveal resistance to more antibiotics. However, we identified that they are not significantly associated with the presence of class 1 integrons and multidrug resistant isolates. These results are consistent with those of Derakhshan's et al. in Tehran hospitals reporting 25.8% Class 1 integron in isolates [24]. In a study in Kashan, 82.9% were identified as multidrug resistant isolates and all multidrug resistant *K. pneumoniae* were positive for class 1 integrons. Moreover, Firoozeh et al. repoted high frequency of integrons among multidrug resistant isolates; this could be due to the fact that integrons bear the advantage of being found in hospitals isolates [25]. In another study, conducted by Li reported 61.4% multidrug-resistant strains and 51.1% Class 1 integron in isolates [26].

Conclusion

Data from this study suggest a high prevalence of class 1 integrons in *K. pneumoniae* strains isolated from Sanandaj, Kurdistan province, Iran. Also, The presence of class I integron genes among MDRKP highlights the need for continued identification and tracking of drug resistance in health centers to decrease the spread of multidrug resistant clinical isolates.

Conflict of Interest

The authors did not declare any conflic of interests.

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