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Original Article

Assessment of the Prevalence of Class I and II Integrons in *Klebsiella pneumoniae* Isolates from Patients Referred to the Hospitals of Semnan, Iran

Shiva Mirkalantari[1], Nastaran Momeni[2], Reza Mirnejad[3], Farahnaz Bineshian[4*]

Abstract

Integrons are mobile genetic elements which carry effective genetic factors in antibiotic resistance. These elements have several classes and play an important role in the development of antibiotic resistance in Gram-negative bacteria. *Klebsiella pneumonia* as Gram-negative bacteria caused a variety infection and increasing the antibiotic resistance of this bacterium leads to a majority of problems in its treatments. The present study was conducted to investigation of class I and II integrons among *Klebsiella pneumoniae* with focus on association with antibiotic resistance. In this cross sectional study, a total of 100 *Klebsiella pneumoniae* isolates were collected from hospitals of Semnan were identified by biochemical tests. Detection of antibiotic susceptibility was performed by disk diffusion method. For detection of class I and II integrons, PCR by integrase genes, *intI* and *intII*, were performed. A p value of <0.05 was considered statistically significant. The highest rate of resistance was observed for trimethoprim/sulfamethoxazole (49%), ceftriaxone (41%) and ceftazidime (40%) while only 10% of isolates showed resistance to imipenem. PCR for *intI* were positive in all resistance isolates (46%) and *intII* was positive in lower rate (40%). Overall a significant association was observed between the prevalence of integrons and resistance to antibiotics (p<0.05). This study demonstrated that integrons are widely prevalent and play an important role in multidrug resistance in *Klebsiella pneumoniae* isolates in this region.

Keywords: *Klebsiella pneumoniae*, Integron, Genetic Element, Microbial Resistance

1. Department of Microbiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Microbiology, Damghan Branch, Islamic Azad University, Damghan, Iran
3. Molecular Biology Research Center, Systems Biology and Poisoning Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
4. Department of Parasitology & Mycology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran.

*** Corresponding Author**
Farahnaz Bineshian
Department of Parasitology & Mycology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran
E-mail: fzbineshian@yahoo.com

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Introduction

Klebsiella pneumoniae is an opportunistic pathogen that causes infections such as pneumonia, ulcer, urinary tract infections, septicemia and meningitis [1-3]. In recent years, the increase of antibiotic resistance in *Klebsiella pneumoniae* has caused major problems in its treatment.

The acquisition of mobile genetic elements including plasmids, transposons, and integrons among Gram-negative bacteria plays an important role in the development of antibiotic resistance [4, 5]. Up to now, more than 9 classes of integrons have been identified based on the differences in the integrase gene in Gram-negative bacteria, but there are only four main classes associated with clinical isolates. Class I and Class II integrons, respectively, are the most common classes among clinical isolates [6]. Integron of Class I carry more than 40 resistance genes related to resistance of aminoglycosides, chloramphenicol, beta-lactams, sulfonamides, macrolides and disinfectants [7]. Integron of Class II has been found in Tn7 transposons and affiliated transposons [8]. Since the integrons have promoter sequences, they can express the genes in the genetic cassettes. Therefore, the integrons act as vector of gene expression and as a natural cloning system [9].

The availability of new information on the pattern of antibiotic resistance in bacteria and the study of causative agents can help in choosing appropriate therapies. The aim of this study was prevalence of class I and II integrons in *Klebsiella pneumoniae* isolated from patients referred to hospitals of Semnan.

Materials and Methods

Bacterial collection and identification of Klebsiella pneumoniae

Between September 2015 and September 2016, a total of 100 clinical isolates of *Klebsiella pneumoniae* were collected from patients who referred to tertiary hospitals in Semnan. Strains of *Klebsiella pneumoniae* were isolated from different clinical specimens including urine, sputum, wound exudates, cerebrospinal fluid. All isolates were cultured on MacConkey's agar (Sigma-Aldrich, USA). These isolates were identified by conventional biochemical tests. All isolates were stored in Brain Heart Infusion broth (BHI) (Sigma-Aldrich, USA) containing 15% (v/v) glycerol at -70ºC until use.

Antimicrobial susceptibility testing

The antibiotic susceptibility of *Klebsiella pneumoniae* to 11 antibiotics was determined using Kirby Bauer disk diffusion method according to CLSI [10].

Antibiotics were used including amikacin (30 µg/disc), ceftazidime (30 µg/disc), ciprofloxacin (5µg/disc), imipenem (10 µg/disc), gentamicin (10 µg/disc), tobramycin (10 µg/disc), cefepime (30 µg/disc), amoxicillin-clavulanate (20/10 µg/disc), tetracycline (5 µg/disc), and sulfamethoxazole/trimethoprim (12.5/23.75 µg/disc).

These antibiotics were purchased as from Sigma-Aldrich (USA)

Molecular detection of class I and II integrons

All isolates were investigated for two classes of integron genes, *intI and intII*, by polymerase chain reaction (PCR) using specific oligonucleotide primers (Table 1). DNA was extracted by boiling methods as previously described by Yu *et al.*, (11). The PCR reaction was performed at a final reaction volume of 25 µl. The reaction mixture contained 1 µl of DNA template, 12.5 µl of 2X master mix, 0.5 µl of forward and reverse primers (50 pM/µl) and 11.5 µl of distilled water. Amplification was performed by thermal cycler (Eppendorf, Germany) in the following conditions: one cycle of 5 min at 95ºC, 30 cycles of 1 min at 95ºC, 1 min at 65ºC, 1 min at 72ºC and one cycle of 10 min at 72ºC. PCR products were electrophorezed on 1.5% (w/v) agarose gel and stained using ethidium bromide (0.5 mg/ml) and visualized under ultraviolet light.

Table 1. Primer sequences used in the PCR assay.

Gene	Primer	Sequence (5'→3')	Amplicon Size	Ref
intI	F	CAG TGG ACA TAGCC TGTTC	223	12
	R	CCC GAG GCA TAG ACT GTA		
intII	F	TTA TTG GTG GGA TTA GGC	160	13
	R	ACG GCT ACC CTC GTG TATC		

Results

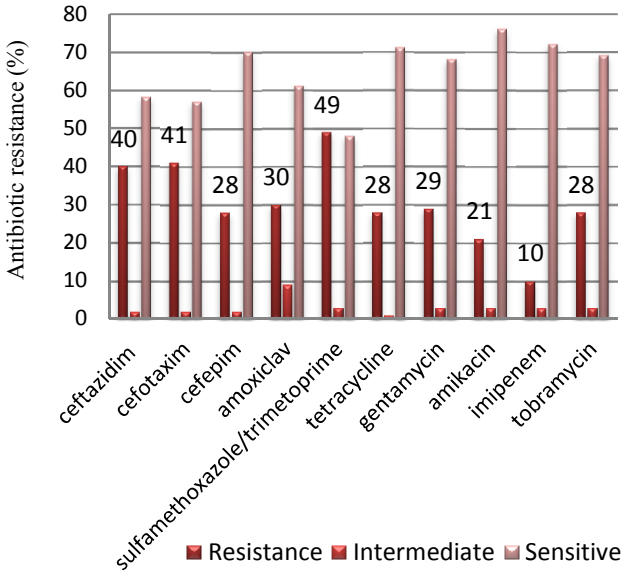
Bacterial collection and identification of Klebsiella pneumoniae

During the study period, a total of one hundred *Klebsiella pneumoniae* clinical isolates were confirmed by biochemical tests. Each isolates was belonged to separate patient. Of the one hundred *Klebsiella pneumoniae* isolates, the most were obtained from female (71%) and the lowest (29%) was isolated from male. The mean age of the patients was 39 years with a range from 1 to 90 years. Sixty percent isolates were obtained from hospitalized patients and 37% isolates were from outpatients. Among the 100 isolates, 81%, 14% and 5% were isolated from urine, sputum and blood respectively.

Antimicrobial susceptibility test

Results of antimicrobial susceptibility determined the percentage of antibiotic resistance as follow (Table 2): imipenem (10%), ceftazidime (40%), cefotaxim (41%), cefepim (28%), amoxiclav (30%), sulfamethoxazole/ trimethoprim (49%), tetracycline (28%), gentamycin (29%), amikacin (21%), tobramycin (28%). Thirty (30%) of strains were susceptible to all tested antibiotics. All (100%) of the isolates were susceptible to colistin. According to the antibiogram test, 25 (25%) out of *Klebsiella* isolates were multidrug resistance (MDR). Of 25% MDR isolates, %23 were isolated from hospitalize patients and 2% from outpatient. Three isolates was found to be extensive drug resistance (XDR). Also, MDR isolates were mainly isolated from urine samples.

Table 2. Antimicrobial resistance pattern of *Klebsiella pneumoniae* isolates.



Detection of class I and II integron genes

Evaluation of two class of integrons by PCR method showed that 46 (46%) and 40 (40%) isolates carried class I and II integrons, respectively (Fig. 1).

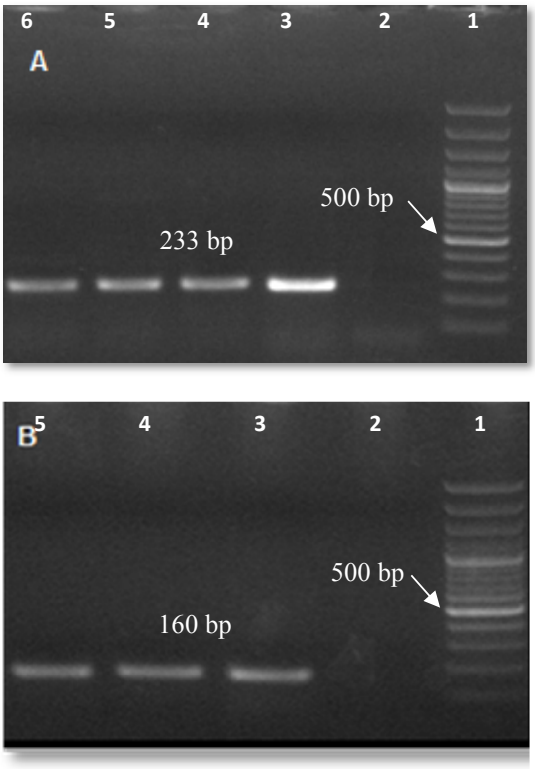


Figure 1. A) Analysis of PCR products of *intI* gene on 1% agarose gel. Lane 1: DNA marker 100 bp; Lane 2: Control negative; Lane 3: Positive control; Lane 4-6: Positive samples with a 233 bp product. B) Analysis of PCR products of *intII* gene on 1% agarose gel. Lane 1: DNA marker 100 bp; Lane 2: Control negative; Lane 3: Positive control; Lane 3-5: Positive samples with a 160 bp product.

Forty isolates (40%) were simultaneously carried class I and II integrons. More than 90% of isolates that carried class I integron were isolated from urine samples of hospitalized women patients. There was significant association between resistance to tetracycline, gentamycin, amikacin, imipenem, tobramycin, amoxiclav, cefepime, cefotaxim and ceftazidime antibiotics and presence of class I integron (p value = 0.001).

Discussion

Increasing antibiotic resistance of bacteria such as *Klebsiella pneumoniae* has led to many problems in the treatment of infectious diseases [11-13]. Antibiotic resistance genes can be transmitted and the integron plays an important role in this transmission [14, 15]. Integrons, genetic elements carrying resistance genes to different antibiotics, have been described initially by Hall and Stokes (16). According to the reports, most of clinical isolates have class I integron genes and are multidrug-resistance. In the present study the frequency of Class I integron gene in *Klebsiella pneumoniae* isolates was 46% that was consistent with the studies of Karimi *et al*., and Molana *et al*., with 48% and 36.6% frequency, respectively [17, 18]. In another study reported by Rezaee *et al*., the frequency of Class I integron gene was 78.5% that is different from our findings. This difference likely was resulted from various sample source, number of samples and region of study (19). In contrast of previous studies that have a more limited distribution of class II this report showed a high rate (40%) of distribution (20). The results of this study showed that all strains of *Klebsiella pneumoniae* with multidrug-resistance are positive for class I integron which similar with the results of Firoozeh *et al*., (21). Also in other studies, the association between the presence of the integron genes and antibiotic resistance, especially in *Klebsiella pneumoniae* isolated from clinical specimens, has been reported (22-24). The results of this study indicated a high prevalence of Integron in *Klebsiella* especially among hospitalized patients, and also there was a significant correlation between the presence of integron genes and increased resistance to many antibiotic groups. The role of integrons has been identified as moving genetic elements in the horizontal transmission of antibiotic resistance.

Conclusion

According to the findings, there is a risk of rapid spread of resistance genes, especially in clinical specimens. Therefore, the implementation of infection control programs and the prevention of the spread of resistant strains are of great importance.

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