Detection of mobile genetic elements in multidrug-resistant *Klebsiella pneumoniae* isolated from different infection sites in Hamadan, west of Iran

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**Abstract**

**Objective:** *Klebsiella pneumoniae* is one of the most opportunistic pathogens that can be related to nosocomial infections. Increased acquisitions of multidrug resistance in this bacterium as well as the transfer of genes to other strains have caused concern. Integrons play a key role in the acquisition and the spread of resistance genes. The aim of this study was to evaluate the frequency of resistance genes *sulI*, *sulII*, *tetA*, *tetB*, class I (*intI* gene), class II integrons (*intII* gene) and the association between multidrug resistance and the presence of integrons in *K. pneumoniae*.

**Results:** Antibiotics susceptibility test was performed on 126 of *K. pneumoniae* isolates. Also, DNA extraction was done and genes were detected using PCR method. In this study, 67 isolates (53%) carrying both the *sulI* and *sulII* genes. Forty-five percent tetracycline-resistant isolates were *tetA* or *tetB* positive. The prevalence of *intI* gene was 96%, while only sixteen isolate harboring *intII* gene (12.5%). Our results showed the high prevalence of integrons in MDR *K. pneumoniae*, indicating the important role of these genes in the transmission of antibiotic resistance.

**Keywords:** Mobile genetic elements, Multidrug-resistance, *K. pneumoniae*
Furthermore, plasmids harboring sul genes enable transfer among bacteria [13]. Integrons are genetic elements that include several genes and a special insertion site for the recombination system, enabling them to obtain mobile gene cassettes. Integrons are efficient systems in combining and expressing genes as part of their genetic elements known as gene cassettes [14, 15]. Based on the genes that encode integrase enzymes, five classes of integrons have been identified, with classes I, II, and III being the most frequent [16, 17].

This study was aimed to detect of mobile genetic elements in multidrug-resistant K. pneumoniae isolated from different infection sites in Hamadan, west of Iran.

**Main text**

**Materials and methods**

**Bacterial isolates**

One hundred twenty-six clinical isolates of K. pneumoniae were isolated from patients admitted to the hospitals from August 2019 to January 2021 in Hamadan, west of Iran. After colony morphology and gram staining, the biochemical tests including indole, citrate, urease, and Kligler iron agar (KIA) tests are employed and API method were applied [18].

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility test was performed using disk diffusion method in Muller Hinton Agar medium, according to CLSI 2019 instructions. Antibiotics included Amoxicillin/clavulanic acid (30 μg), Cephalexin (30 μg), Ceftazidime (10 μg), Cefepime (30 μg), Ceftriaxone (30 μg), Cefoxitin (30 μg), Sulfamethoxazole/Trimethoprim (SXT 125/23.75 μg), Imipenem (10 μg), Aztreonam (30 μg), Tetracycline (30 μg), Moxfloxacin (5 μg), Levofloxacin (5 μg), Ciprofloxacin (10 μg), Gentamicin (10 μg), Amikacin (30 μg), Tigecycline (15 μg). K. pneumoniae ATCC 3565 was the corresponding control in the experiments Antibiotics Classification used in the present study was shown in Additional file 1.

**DNA extraction and detection of genes**

The isolates were cultured in LB broth and incubated at 35 °C overnight. The DNA concentration was determined using nanodrop. DNA amplification was performed in a thermal cycler (Bio-Rad, USA) using Mastermix (BioFact-Korea). The primers used in this study displayed in Table 1.

**PCR-based detection of antimicrobial resistance genes in the recovered K. pneumoniae isolates**

The PCR reaction mixture contained 1 μL (10 pmol) of each primer, 2 μL DNA, 25 μL PCR Master Mix in a total 50 μL reaction volume. DNA amplification was conducted in a thermal cycler (Bio-Rad, USA), under the following conditions: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing temperature for each gene for 40 s, an extension at 72 °C for 45 s, followed by a final extension at 72 °C for 4 min. The amplified DNA fragments, along with a 100 bp DNA size marker was run on 2% agarose gel electrophoresis.

**Statistical analysis**

The t-test was used to compare categorical results. All statistical tests were two-tailed, and statistical significance was defined as a P-value of 0.05. The statistical software package SPSS version 22 (IBM, NY) was used to analyze the data.

**Results**

**Antibiotic resistance pattern**

Frequency of samples obtained were UTI 39 (31%), Sputum 38 (29.9), Tracheal 29 (23%), Wound 16 (12.6%) and BAL 4 (3.4%). The highest frequency of resistance to antimicrobial agents related to gentamicin (58.6%) and lowest to meropenem (4.6%) is shown in Fig. 1. In the present study, MDR was predominant among K. pneumoniae isolates because 58.6% of the isolates were MDR. The frequency of ESBL producing isolates was 52.9%. The frequency of samples in different parts of the body is shown in Fig. 2 according to MDR and non-MDR classification.

**Frequency of genes and antibiotic susceptibility test**

In this study, 51.7% of isolates were resistant to sulfamethoxazole/trimethoprim (SXT) which of 60.9% isolates harboring sull and 26.4%had sulI gene. A significant

| Gene     | Primers sequence          | Band size (bp) | References |
|----------|---------------------------|----------------|------------|
| tetA     | F: GTGAAAAACACACACACCC   | 888            | [27]       |
|          | R: GAAGCCAGAGGATGTAAGG   |                |            |
| tetB     | F: CTTATCATGCAAGCTGGTC   | 774            | [27]       |
|          | R: ACTGCCGTTTTTTCGCC     |                |            |
| sulI     | F: CCGGATGCGCTTAAGCAAGC | 433            | [36]       |
|          | R: GCCGATCCGGTGAAGTTCG   |                |            |
| sulII    | F: CCCCTCAAGGGCGAGATGGCAT | 293           | [36]       |
|          | R: CCCCTTGATACCGCGACCCTG |                |            |
| IntI     | F: 5′-GCCCTGTCTCGTTCTCACGG-3′ | 558          | [31]       |
|          | R: 5′-GATGCTGCTGGTCTCACGG-3′ |              |            |
| IntII    | F: 5′-CACGGATATGCCGACAAA | 789           | [31]       |
|          | R: 5′-GTAGCAAAACGGATGACGA |              |            |
|          | AATG-3′                   |                |            |
relationship was seen only between sulI and SXT resistance ($P = 0.008$).

Fifty-five (43.7%) isolates were tetracycline resistant; among them 23% and 12.6%, isolates were positive for tetA and tetB genes, respectively. There was a significant relationship between the presence of these genes and tetracycline resistance ($P < 0.01$).

The prevalence of int1 and int2 genes among isolates were 102 (81.6%) and 50 (40.2%), respectively. The relationship between the presence of int2 gene with ESBL producing, sulI, tetA, and MDR isolates was significant. The presence of int2 gene and resistance to all antibiotics used in this study was significant ($P < 0.01$). The relationship between MDR and ESBL producing isolates with resistance to all antibiotics used in this study was significant ($P < 0.001$).

**Discussion**

Due to the increase in antibiotic resistance, awareness of microbial resistance pattern and mechanisms of resistance transmission among bacterial infections can be an effective strategy to prevent microbial resistance transmission [19].

This study showed that there was a high level of antimicrobial resistance among *K. pneumoniae* isolates. More than 30% of the 126 *K. pneumoniae* isolates tested in this study demonstrated resistance to antimicrobials such as sulfamethoxazole/trimethoprim, tetracycline, ciprofloxacin, gentamicin, tetracycline, cefixime, cephalothin, ceftriaxone, and cefotaxime. The results of our study are consistent with the results of many past studies, which show a relatively high prevalence of *K. pneumoniae* resistant isolates in hospital settings. Moghadampour et al. showing resistance to gentamicin (30%), ceftazidime (34%), sulfamethoxazole/trimethoprim (22%), and ciprofloxacin (27%) have been reported in Iran [20].

In this investigation, 58.6% of *K. pneumoniae* isolates were MDR, which is higher than what has been reported in Kenya [21], but lower than what has been reported in China [22]. The results of this study, antibiotic resistance are similar to the results of Zomorodi et al. [23] in 2019, although the production of ESBL gene in our study is 52.9%, which shows an increase compared to their study which could indicate the transfer of genetic elements between bacteria and increased resistance to the ESBL gene.

In a 2021 study by Fatima et al. [24], among SXT-resistant strains, the frequency of sulI (66.7%) gene was similar to our study (60.9%). Although all of the tetracycline-resistant strains had the tetB gene, in our study only there was 12.6% of tetB gene that could indicate other mechanisms in the development of tetracycline resistance in bacteria.

The major of MDR *K. pneumoniae* examined tested were positive for integron class 1. A high correlation has been established between the presence of integron class 1 and the prevalence of MDR in gram-negative bacteria. In a study of Li et al., integron 1 positive isolated bacteria showed a much higher incidence of drug resistance than negatively ones [25]. Other studies have shown a high prevalence of positive integron MDR-*K. pneumoniae*. The high prevalence of integron among MDR strains maybe since integron has the advantage of selective stress selection of strains in environments such as hospitals, which are caused by antibiotic abuse [26].

In our study, beta-lactam resistance was 88%, which compares with the results reported by Khamesipour et al. [27]. Tetracycline resistance is a common resistance among bacteria and its resistance was 43.7% among our isolates. In quinolone (ciprofloxacin) resistance (35.6%) among our isolates, they are much higher than reported in China [28], Iran [27], and India [29]. Aminoglycoside resistance has been reported to be the highest among antibiotics with a resistance rate of 58.6%. The data related to Tanzania and Kenya were higher for gentamicin compared with our study.

The result of PCR assay for tetracycline-resistant *K. pneumoniae* isolates and the frequency of genes reported...
to \textit{tetA} or \textit{tetB} was 23% and 12.6%, respectively. In Iran, Khamesipour et al. reported that \textit{tetB} percentage (64.1%) and a higher percentage of \textit{tetA} (79.4%), while the results of Bocaina et al. showed that all tetracycline-resistant \textit{K. pneumoniae} isolates contained both the \textit{tetA} and \textit{tetB} genes. Previous studies have shown that \textit{tetB} was in highly motile genetic elements that are easily transmitted between different bacterial genera. The association between tetracycline resistance genes (\textit{tetA} and \textit{tetB}) and class II integrons (\textit{intII}) among our isolates suggests that class II integrons may be associated with the release of both tetracycline resistance genes. Similarly, Rezaei et al. reported a positive association between the presence of \textit{intI} and \textit{intII} genes and tetracycline resistance [30]. Our results show that integrons are widespread in isolated \textit{K. pneumoniae}. Among the 126 isolates were \textit{int1} (81.6%) and \textit{int2} (40.2%), indicating high integrons presence. In this study, a significant relationship was also found (P < 0.001) between MDR phenotype and integrons, while Martinez Freijou et al. only described the tendency to develop resistance to several antimicrobial agents in strains with integrons. In this study, 81.6% of class I integron strains was observed, Derkhshan et al. that I integron was 25.8%, which shows a significant difference that can be found in the Derkhshan et al., of the majority of \textit{K. pneumoniae} were isolated from urine [31]. In the present study, the integron class II with all antibiotics used was statistically significant, which could indicate antibiotic resistance due to integron class II. The prevalence of class 2 integrons in our MDR \textit{K. pneumoniae} isolates was 40.2%, which is higher than that described by Rezaei et al. [30] and Firoozeh et al. [32] in northwestern and central Iran.

The frequency distributions of both \textit{sulI} and \textit{sulII} genes among sulfamethoxazole/trimethoprim-resistant isolates were 60.9% and 26.4%, respectively. This may be due to the widespread release of class 1 integron, which are closely related to the \textit{sulI} gene.

As a common factor in the widespread dissemination of antimicrobial resistance genes, the prevalence of class 1 integrons has been reported to be 22 to 59%. In this study, the prevalence of class 1 integrons among clinical isolates of \textit{K. pneumoniae} was 90%. Approximately similar frequencies of class 1 integrons have been reported in India (92%) and in China (93.2%). On the other hand, Class 1 integrons are less prevalent in other parts of the world, including Brazil (65.5%) [33], Iran (66.6%) [27], Australia (73%) [34], United States (78.5%), and Korea (73.3%) [35].

Our study found a strong association between the presence of class II integrons and resistance to all antibiotics (P < 0.01). Class II integrons have generally been reported to be less common in some gram-negative organisms.

Monitoring the changes of integron gene cassettes in \textit{K. pneumoniae} population can prevent the spread of antibiotic resistance factors in hospitals. Periodic monitoring and identification of these elements can help reduce disease burden, reduce costs, and shorten hospital stays. Overall, this study showed that carbapenems and doxycycline are the most effective antibiotics against \textit{K. pneumoniae}. The high frequency of class I and II integron and the presence of MDR \textit{K. pneumoniae} isolates is a serious warning for health authorities.

**Conclusion**

Statistical relationships between drug resistance and integrons show that integrons are the encoder and disseminator of drug resistance among \textit{K. pneumoniae} isolates. Mobile genetic elements are undeniable among bacteria as a natural phenomenon and bacteria become resistant to antibiotics in this way, it is necessary that with rational use of antibiotics also modifications in the pattern of antibiotics administered by physicians, be applied periodically.

**Limitation**

Due to financial and time constraints in this research, the evaluation of several genes and also achieving more samples in various geographical locations in the country didn’t done and are among the limitations of this study. PCR-based detection of the most common virulence genes of \textit{K. pneumoniae} should be carried out.

**Abbreviations**

CLSI: Clinical and laboratory standards institute; TSB: Tryptic soy broth; OD: Optical density; PCR: Polymerase chain reaction.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s13104-021-05748-9.

**Additional file 1.** Antibiotics Classification used in the present study.

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**Authors’ contributions**

Substantial contributions to conception and design, and/or acquisition of data, and/or analysis and interpretation of data, drafting the article. All Authors; revising it critically for important intellectual content: MT & BA; approved the version to be submitted and revised the text: RG, MM and FN. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study has been supported financially by the Hamadan University of Medical Sciences. Informed written consent (in Persian) was given to subjects from whom the samples were obtained for this study (IR.UMSHA.REC.1400.381).

Consent for publication
Not applicable.

Competing interests
There are no conflicts of interest.

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References
1. Chung PY. The emerging problems of Klebsiella pneumoniae infections: carbapenem resistance and biofilm formation. FEMS Microbiol Lett. 2016;363:fwiw219.
2. Nouri F, Karami P, Zarei O, Kosari F, Alkhani MY, Zandkarimi E, Zarandi ER, Taheri M. Prevalence of common nosocomial infections and evaluation of antibiotic resistance patterns in patients with secondary infections in Hamadan, Iran. Infect Drug Resist. 2020;13:2365.
3. Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan L, Manzoor N. Fungal-cidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. Eur J Clin Microbiol Infect Dis. 2011;30:41–50.
4. Alvarez-Uria G, Gandra S, Mandal S, Laxminarayan R. Global forecast of antimicrobial resistance in invasive isolates of Escherichia coli and Klebsiella pneumoniae. Int J Infect Dis. 2018;68:50–3.
5. Makharita RR, El-Kholy I, Hetta HF, Abdelaziz MH, Hagagy FI, Ahmed AA. Detection of class 1, 2, and 3 integrons among Escherichia coli from pigs, pig carcasses and human. Acta Vet Scand. 2010;52:1–7.
6. Kemp K, Griffiths J, Campbell S, Lovell K. An exploration of the follow-up up needs of patients with inflammatory bowel disease. J Crohns Colitis. 2013;7:e386–95.
7. Subirats J, Timoner X, Sánchez-Melisís A, Balcázar JL, Acuña V, Sabater S, Borrego CM. Emerging contaminants and nutrients synergistically affect the spread of class 1 integron-integrase (intI1) and sulf genes within stable streamed bacterial communities. Water Res. 2018;138:77–85.
8. Fuga B, Royer S, Campos PA, Ferreira ML, Rossi J, Machado LG, Cerdeira LT, Fonseca Batistão DW, Brito CS, Lincopan N. Molecular detection of class 1 integron-associated gene cassettes in KPC-2-producing Klebsiella pneumoniae dones by whole-genome sequencing. Microbial Drug Resist. 2019;25:1127–31.
9. Gillings MR. Integrons: past, present, and future. Microbiol Mol Biol Rev. 2014;78:257–77.
10. Ghaly TM, Goqheeghan JL, Tetu SG, Gillings MR. The peril and promise of integrons: beyond antibiotic resistance. Trends Microbiol. 2020;28:455–64.
11. Osman EA, El-Amin N, Adreess EA, Al-Hassan L, Mukhtar M. Comparing conventional, biochemical and genotypic methods for accurate identification of Klebsiella pneumoniae in Sudan. Access Microbiol. 2020;2:acmi000096.
12. Sedighi P, Zarei O, Karimi K, Taheri M, Karami P, Shokohiizadeh L. Molecular Typing of Klebsiella pneumoniae Clinical Isolates by Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction. Int J Microbiol. 2020;2020.
13. Moghadampour M, Salari-Jad A, Faghri J. High rate of carbapenem-resistant Klebsiella pneumoniae detected from hospital equipments in Iran. Acta Microbiol Immunol Hung. 2018;66:529–38.
14. Taït CR, Leski TA, Erwin DP, Odundo EA, Kipkemoy NC, Ndonye JN, Kirera RK, Ombogo AN, Walson JL, Pavlinac PB. Antimicrobial resistance of Klebsiella pneumoniae stool isolates circulating in Kenya. Plos ONE. 2017;12:e0178880.
15. Hou X, Song X, Ma X, Zhang S, Zhang J. Molecular characterization of multidrug-resistant Klebsiella pneumoniae isolates. Brazil J Microbiol. 2015;46(3):759–68.
16. Zomorodi A, Zargar M, Noroozi J. Evaluation of antibiotic resistance associated with ophthalmic oqxAB pumps in Klebsiella pneumoniae causing urinary tract infection. Med Sci Islam Azad Univ Tehran Med Branch. 2019;29:163–70.
17. Fatima S, Liaqat F, Akbar A, Sahifeh M, Samad A, Anwar M, Iqbal S, Khan SA, Jadav H, Makai G. Virulent and multidrug-resistant Klebsiella pneumoniae isolates from clinical samples in Balochistan. Int Wound J. 2021;18(4):510–8.
18. Li B, Hu Y, Wang G, Yi Y, Wuo FC, Jing H, Zhu B, Liu CH. Structural diversity of class 1 integrons and their associated gene cassettes in Klebsiella pneumoniae isolates from a hospital in China. Plos ONE. 2013;8:e75805.
19. Wu K, Wang F, Sun J, Wang Q, Chen Q, Yu S, Rui Y. Class 1 integron gene cassettes in multidrug-resistant Gram-negative bacteria in southern China. Int J Antimicrob Agents. 2012;40:264–7.
20. Khamessipour F, Tajbakhsh E. Analyzed the genotypic and phenotypic antibiotic resistance patterns of Klebsiella pneumoniae isolated from clinical samples in Iran. 2016.
21. Zhang X, Chen D, Xu G, Huang W, Wang X. Molecular epidemiology and drug resistant mechanism in carbapenem-resistant Klebsiella pneumoniae isolated from pediatric patients in Shanghali, China. PLoS ONE. 2018;13:e0194000.
22. Tiwari DK, Golla S, Saneeetha K, Vasudha C. A study on the bacteriological profile and antibiotic of bacteremia in children below 10 years in a tertiary care hospital in Bangalore, India. J Clin Diagn Res JCDR. 2013;7:2732.
23. Rezaee MA, Langarizadeh N, Aghazadeh M. First report of class 1 and class 2 integrons in multidrug-resistant Klebsiella pneumoniae isolates from northwest Iran. Jpn J Infect Dis. 2012;65:256–9.
24. Derakhshan S, NajerPeerayeh S, Fallah F, Bakhshi R, Rahbar M, Ashrafi A. Detection of class 1, 2, and 3 integrons among Klebsiella pneumoniae isolated from children in Tehran hospitals. Arch Pediatr Infect Dis. 2014;2:164–8.
32. Firoozeh F, Mahluji Z, Khorshidi A, Zibaei M. Molecular characterization of class 1, 2 and 3 integrons in clinical multi-drug resistant Klebsiella pneumoniae isolates. Antimicrob Resist Infect Control. 2019;8:1–7.
33. Lima AMS, Melo MES, Alves LC, Brayner FA, Lopes ACS. Investigation of class 1 integrons in Klebsiella pneumoniae clinical and microbiota isolates belonging to different phylogenetic groups in Recife, State of Pernambuco. Revista da Sociedade Brasileira de Medicina Tropical. 2014;47:165–9.
34. Jones LA, McIver CJ, Kim M-J, Rawlinson WD, White PA. The aadB gene cassette is associated with blaSHV genes in Klebsiella species producing extended-spectrum β-lactamas. Antimicrob Agents Chemother. 2005;49:794–7.
35. Shin HW, Lim J, Kim S, Kim J, Kwon GC, Koo SH. Characterization of trimethoprim-sulfamethoxazole resistance genes and their relatedness to class 1 integron and insertion sequence common region in gram-negative bacilli. J Microbiol Biotechnol. 2015;25:137–42.
36. Gündoğdu A, Long YB, Vollmerhausen TL, Katouli M. Antimicrobial resistance and distribution of sul genes and integron-associated inti genes among uropathogenic Escherichia coli in Queensland, Australia. J Med Microbiol. 2011;60:1633–42.

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