



Research Article

The relationship between class I and II integrons and antibiotic resistance in *Klebsiella pneumoniae* clinical strains isolated from patients in Isfahan hospitals

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ABSTRACT

Integrons are recognized for their capacity to transfer antibiotic resistance genes between pathogenic bacteria including *K. pneumoniae*. The objective of this study was to assess the correlation between class I and II integrons and the antimicrobial resistance among clinical strains of *K. pneumoniae*, in patients in Isfahan. A total of 96 strains of *K. pneumoniae* were procured from 200 clinical specimens. The identification of the isolates was carried out through biochemical analyses and tracing the 16S-23S ITS sequence. The antibiotic sensitivity pattern was assessed by disk diffusion. Detection of the genes encoding class I and II integrons in resistant clinical isolates was done by PCR. From 96 *K. pneumoniae* isolates, 81 isolates (84.4%), exhibited resistance to multiple antibiotics. The isolates exhibited the maximum level of resistance towards meropenem, which was evident in 73 of the isolates, followed closely by amikacin in 67 isolates. Conversely, gentamicin displayed the least level of resistance (in 12 isolates). Men from the emergency and intensive care units exhibited a higher frequency of isolates and associated drug-resistant strains. Elderly individuals exhibited a notably elevated frequency of the isolates and resistant variants. A significant proportion of resistant strains were found to possess both class I and II integrons (82.72% and 86.42%, respectively). Moreover, a relatively high percentage of resistant strains (70.37%) were found to harbor both classes of integrons. The results indicated a notable incidence of integron-dependent resistance, thereby emphasizing the need for an informed approach towards diagnosis and management of such infections

1. Introduction

K. pneumoniae strains are displaying an increasingly high level of resistance to a variety of antibiotics, resulting in septicemia and elevated mortality rates in multiple hospital departments. Consequently, available treatment options for infections caused by this bacterium are limited (Effah et al., 2020; Bassetti et al., 2018). *K. pneumoniae* exhibits various pathogenic mechanisms, such as the presence of

a capsule, fimbriae, lipopolysaccharide (LPS), outer membrane proteins (OMPs) and siderophores, as outlined in the research of Gan et al. (2022). Over the past twenty years, a diverse range of *K. pneumoniae* strains with resistance to multiple drugs have arisen, exhibiting a heightened capacity to prompt outbreaks on multiple continents and disseminate globally (Navon-Venezia et al., 2017; Rahimzadeh Torabi et al., 2021). The

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findings of the study indicate that cefotaxime exhibited the highest rate of resistance, at 79.2%. Subsequently, eztronam and cefepime were identified as having resistance rates of 73.3% and 72.6%, respectively. According to research conducted by Effah et al. (2020), a minimal resistance level of 2.9% has been observed amongst bacteria in response to colistin. The pathogen exhibits a vast resistance spectrum that encompasses both chromosomal and plasmid-encoded antibiotic resistance genes (ARGs). *K. pneumoniae* demonstrates a sustained propensity to accumulate ARGs in response to the selective pressure of antibiotics. This phenomenon effectively results in the emergence of highly resistant strains, characterized by novel mutations and acquisitions of transferable genetic elements and plasmids (Navon-Venezia et al., 2017).

Integrations are regarded as mobile genetic elements that facilitate the acquisition and subsequent dissemination of resistance genes. These genetic structures are capable of harboring gene cassettes within a diverse range of genomic contexts, such as plasmids, chromosomes, and transposons. The horizontal transfer of integrations is widely regarded as the foremost effective method of disseminating resistance genes and fostering the emergence of multi-drug resistant (MDR) species. The imperative nature of identifying resistance genes, situated on integrations, and their capability to be disseminated across distinct bacterial strains is of utmost significance. Integrations are considered crucial mobile genetic elements that facilitate the acquisition and propagation of antibiotic resistance genes. Integrations represent a class of genetic elements that possess a distinct recombination system capable of facilitating the acquisition and expression of diverse gene cassettes. As a consequence of this characteristic, integrations are widely recognized as a prominent reservoir of genetic material. The horizontal transmission of integrations is widely identified as a highly efficacious means of disseminating genes of resistance and fostering the emergence of microbial strains that exhibit multidrug resistance (Zeighami et al., 2014). Integrations are genetic elements that possess safeguarded DNA sequences, denoted as *int*, which carry the integrase gene. This gene is responsible for facilitating the incorporation and transfer of gene cassettes through recombination

mechanisms that occur at a unique site. Numerous studies have elucidated that, predicated upon variances in the sequence of the preserved DNA region within integrase, four primary integron groups have been identified in both gram-positive and gram-negative bacterial populations. The degrees of association between these classes extend from 15% to 41%. Class IV integrons have been exclusively detected solely within the bacterial species *Vibrio cholerae*. The drug resistance properties of Classes I, II, and III of integrons have been the subject of investigation, as reported by Sabbagh et al. (2021). Despite the clinical significance of *K. pneumoniae*, the current understanding of its antibiotic resistance and resistance transfer mechanisms remains limited (Effah et al., 2020). The current study sought to ascertain the antibiotic resistance status of isolated *K. pneumoniae* strains through an examination of antibiotic sensitivity patterns and identification of strains displaying multiple drug resistance patterns among clinical samples. In light of the significant contribution of integrations, specifically types I and II, towards the development of drug resistance in multidrug-resistant strains, an investigation was conducted into the association between the aforementioned integrations and clinical strains of *K. pneumoniae* extracted from patients in Isfahan hospitals.

2. Materials and Methods

2.1. Phenotypic identification of clinical isolates

This study was carried out as a master's thesis with the code 1722910905149721400162421927 in Falavarjan Branch, Islamic Azad University, Isfahan, Iran under the ethics code of IR.IAU.FALA.REC.1402.030. In this study, a total of 96 isolates of *K. pneumoniae* originating were obtained from various clinical samples across different departments in Alzahra hospital in Isfahan, in 6 months. The identification and verification of *K. pneumoniae* isolates were achieved through a series of standard biochemical tests. The specimens were maintained at a temperature of -70 °C in a freezer in preparation for subsequent investigations (Levinson and Jawetz, 1996).

To ascertain the definitive identification of species, molecular verification of isolates was carried out through the utilization of a pair of specific primers designed for the amplification

of 16S-23S internal transcribed spacer (ITS) fragment in *K. pneumoniae* genome, as presented in Table 1. The Polymerase Chain Reaction (PCR) test was employed for this purpose. The thermal regime employed during this stage comprises a sequence of 94°C for 300 seconds as an initial cycle, followed by 35 repetitive cycles of 95°C for 30 seconds, 58°C for 90 seconds, and 72°C for 90 seconds each. Lastly, a terminal cycle of 72°C was conducted for a duration of 600 seconds. The resultant PCR product was subjected to electrophoresis via 1% agarose gel as stipulated by Tavakol et al. in 2017.

The Kirby-Bauer standard method was utilized to ascertain the pattern of antibiotic sensitivity, in accordance with the Clinical and Laboratory Standard Institute (CLSI 2021) guidelines. The employed discs comprised of various antimicrobial agents, specifically amikacin at a concentration of 30 µg, piperacillin/tazobactam (tazocin) at a dosage of 5 µg, ceftazidime and cefepime at 5 µg and 10 µg respectively, gentamicin at 10 µg, cotrimaxazole at 15 µg, ciprofloxacin at 5 µg, meropenem at 10 µg, and ampicillin/sulbactam also at a concentration of 10 µg. The experimental protocols were conducted utilizing a standard strain of *K. pneumoniae* ATCC700603 as a quality control. Based on the antibiotic resistance pattern, isolates demonstrating resistance to at least an antibiotic in two or more classes of antibiotics were characterized as isolates possessing multiple drug resistance (CLSI 2021). The present investigation entailed the identification and tracking of integron class genes within resistant microbial strains.

The Table 1 catalogues the primers belonging to the IntI and IntII genes. The PCR mixture contained PCR buffer (1X), mgCl₂ (1.5 mM), dNTPs (0.2 mM), each primer (0.5 µM), and Taq DNA polymerase (1 unit) in a total volume of 50 µl. The protocol employed for this stage was characterized by a thermal program consisting of a series of steps. Specifically, the program comprised an initial cycle of 94°C for 5 minutes, followed by 35 repeated cycles consisting of 94°C for 45 seconds, 54°C for 45 seconds, and 72°C for 1 minute. Lastly, the program included a final cycle of 72°C for a total duration of 7 minutes. The amplified DNA fragment was subjected to electrophoresis on an agarose gel with a concentration of 1%. The bacterial strain ATCC 700603 was employed as a positive control in the experimental protocol.

2.1. Statistical analyses

The methodology employed in this study involved the replication of all experiments a minimum of two times, followed by the statistical analysis of the resultant data utilizing the SPSS software version 20 (Chicago SPSS, USA) and Excel 2010 (Microsoft Corporation, USA). The present study employed parametric independent T-test and one-way analysis of variance (ANOVA) statistical methodologies to calculate the mean difference between groups, aimed at assessing the relationship and significance of the study data. Within the confines of this investigative inquiry, a statistical significance of a p-value less than 0.05 was deemed pertinent.

Table 1. Features of the primers utilized in the present investigation for the molecular verification of *K. pneumoniae* and the primers used for the amplification of genes that encode integrons.

target gene	primer sequence	Reference
16S-23S ITS-F	3' ATTTGAAGAGGTTGCAAACGAT5'	Tavakol et al, 2017
16S-23S ITS-R	3' CCCGAGGCATAGACTGTA5'	
Int1-F	3' CAGTGGACATAAGCCTGTTC5'	Khosravi et al, 2017
Int1-R	3' CCCGAGGCATAGACTGTA5'	
Int2-F	3' ACGGATATGCGGACAAAAGGT5'	Khosravi et al, 2017
Int2-R	3' GTAGCAAACGAGTGACGAAATG5'	

3. Results

In this investigation, 96 *K. pneumoniae* isolates obtained from various clinical specimens collected from Isfahan teaching hospitals were analyzed. Upon conducting biochemical tests and amplification of the 16S-

23S ITS sequence, the isolates were conclusively identified based on their phenotypic characteristics. All the specimens were found to be positive, displaying a band consisting of 130 base pairs. The findings are depicted in Fig. 1.

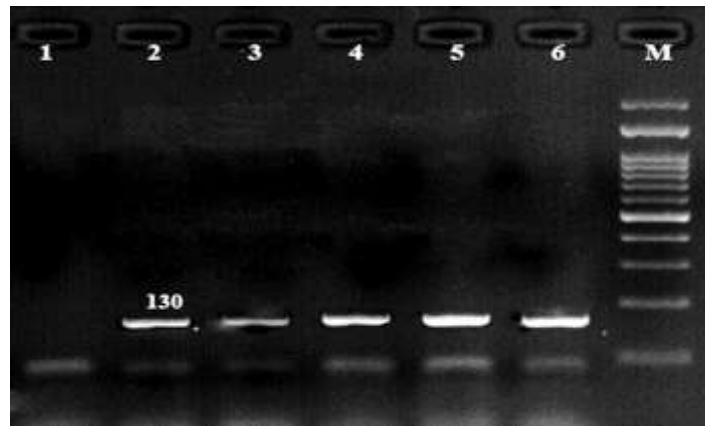


Figure 1. The PCR products of amplification targeting the 16S-23S ITS sequence was subjected to examination via electrophoresis on a 1% agarose gel, thereby producing a fragment of 130 base pairs (lanes 2-6 which belong to 5 isolates among the 96 isolate). NC: negative control (lane 1), M: indicates a 100 bp DNA size marker.

The distributions of isolates in different clinical samples and among females and males are presented in table 3. According to these findings, the frequency of strains in men was 16.66 times compared to female with a significant difference ($P = 0.01$) The results from detection of the frequency distribution of *K. pneumoniae* strains across various sample types showed that the isolates had a higher frequency in urine (37.5%) and respiratory trachea (28.12%) when compared to sputum, joint fluid, abscess, and feces. The statistical analysis underscores a substantial difference ($P = 0.005$) in the frequency distribution of the pathogenic strain across the stated specimen types. The isolation of strains has yielded a very low percentage of only 1.04%. The present study found that, among the 96 strains of *K. pneumoniae* examined, a significant number were observed to be prevalent in individuals between the ages of 61 and 80 years. Conversely, the minority of such strains were detected among individuals between the ages of 1 to 10 and 11 to 20 years. There was a lack of statistically significant difference noted in this instance ($P = 0.86$) The findings of the study pertaining to the allocation of *K. pneumoniae*

strains among various units of the hospital revealed that among the 96 strains identified, the highest proportions were found in the Intensive Care Unit departments (39.58%) ($P = 0.011$) and the Emergency department (23.96%) ($P = 0.013$), while the lowest proportions were detected in the CCU, accounting for 0% of the total strains noted. The present study reveals that the departments of surgery, selected, heart and glands and skin, neurology and hematology, and gastroenterology, nephrology, infectious, lung and pediatrics displayed moderate frequencies of occurrence, with percentages of 10.42%, 5.21%, 4.17%, 3.13%, and 2.08%, respectively.

2.1. Antibiotic sensitivity of strains

The findings of this study revealed that the frequency distribution of strains indicated a notable resistance to the antibiotic meropenem, with 73 isolates showing resistance, followed by amikacin, with 67 resistant isolates. The lowest resistance was observed towards gentamicin, with only 12 isolates showing resistance. Moreover, the findings of frequency distribution analysis indicated that 81 out of the isolated strains categorized as MDR demonstrated multi-

antibiotic resistance, accounting for 84.4% of the total strains. Conversely, 15 strains (25.6%) were found to be susceptible to antibiotics. Based on the results of the study, it was found that strains exhibiting multi-antibiotic resistance were 3.31 times more prevalent compared to strains that were sensitive to antibiotics.

2.2. Distribution of isolated MDR strains

The findings of the present study indicated that gender is a discriminating factor in the prevalence of drug-resistant *K. pneumoniae* infections, whereby male individuals exhibited a greater susceptibility to multi-drug resistant strains compared to females. However, the observed difference in gender distribution did not reach statistical significance at the predetermined level of significance ($P = 0.12$) as shown in Fig. 2.

The frequency distribution of resistant strains was analyzed between different departments of the hospital, various types of clinical samples and age. Fig. 3 illustrates the observed results of this investigation. The present study identified a distribution pattern in which the highest percentage of isolated strains was associated

with the intensive care unit (ICU) and the lowest frequency was belonged to the critical care unit (CCU) which had no isolated strains. The outcomes of the chi-square test revealed a noteworthy variation in the allocation of strains among diverse hospital departments ($P = 0.01$).

The frequency distribution outcomes of resistant strains as categorized by the sample type may be observed in Fig. 4. The findings of the study indicate that the highest prevalence of antibiotic resistance was observed in cases of urinary tract infections (UTI). Fig. 5 presented the frequency distribution of resistant strains according to the age of the patients. The present study conducted an analysis of 81 strains of *K. pneumoniae* that exhibited resistance to multiple drugs. The results revealed a higher prevalence of *K. pneumoniae* strains among individuals belonging to the ages of 71-80 and 81-90 years than those aged 11-20 and 1-10 years. Based on the outcomes of the chi-square test, a notable dissimilarity exists in the strain allocation among the distinct age cohorts of individuals.

Table 2. Types of samples from patients with *K. pneumoniae* infection

Sampling location	Gender		Total
	Female (%)	Male (%)	
Throat	1 (1.04)	4 (4.16)	5 (5.20)
Respiratory Trachea	8 (8.30)	19 (19.76)	27 (28.12)
CSF	3 (3.12)	2 (2.08)	5 (5.20)
Urine	13 (13.52)	23 (23.92)	36 (37.50)
Blood	6 (6.25)	3 (3.12)	9 (9.37)
Pleural Fluid	0 (0.00)	1 (1.04)	1 (1.04)
Wound	1 (1.04)	4 (5.20)	6 (6.24)
Pus	1 (1.04)	2 (2.08)	3 (3.12)
Sputum	0 (0.00)	1 (1.04)	1 (1.04)
Synovial Fluid	1 (1.04)	0 (0.00)	1 (1.04)
Abscess	1 (1.04)	0 (0.00)	1 (1.04)
Feces	1 (1.04)	0 (0.00)	1 (1.04)
Total	36 (37.5)	60 (62.40)	96 (100.00)

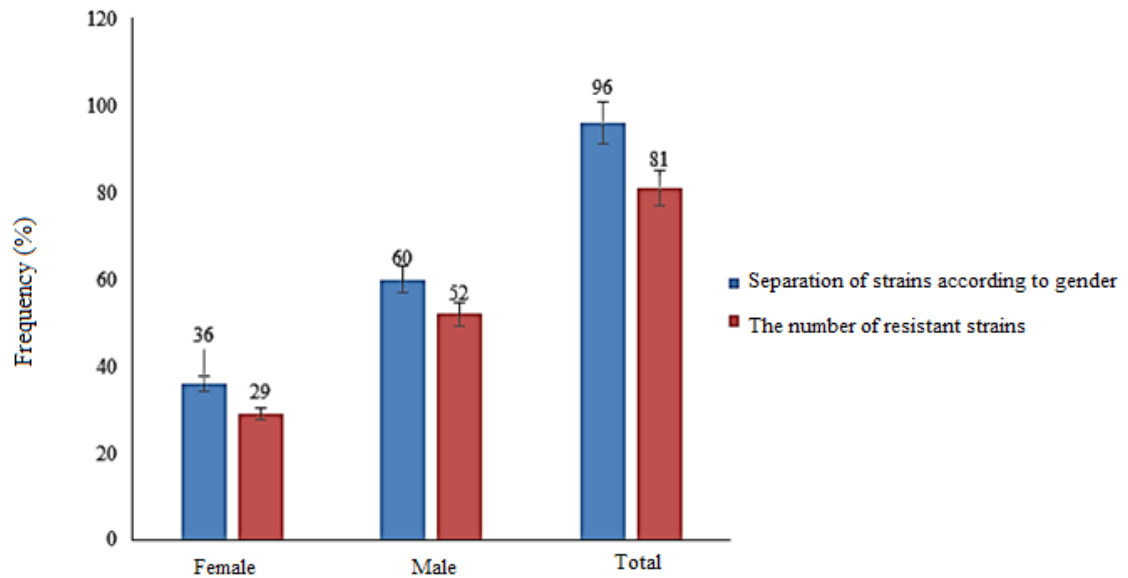


Figure 2. Frequency distribution of strains isolated in the present study based on gender and drug resistance

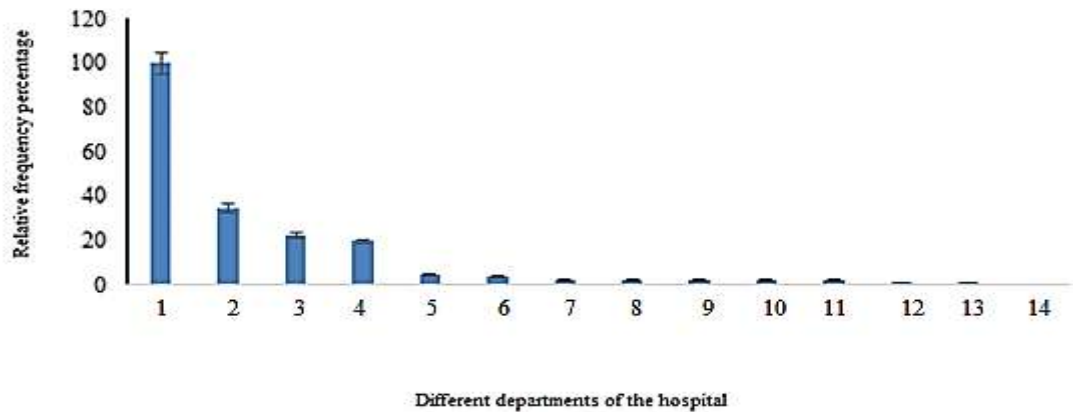


Figure 3. Distribution of total MDR strains of *K. pneumoniae* isolated among different hospital departments (1. Total, 2. ICU, 3. Emergency, 4. Surgery, 5. Haemodialysis, 6. Neurology, 7. Cardiology- Endocrinology- Dermatology, 8. Nephrology, 9. Hematology, 10. Infectious - Pulmonary, 11. Pediatrics, 12. Gastroenterology, 13. Operating room, 14. CCU)

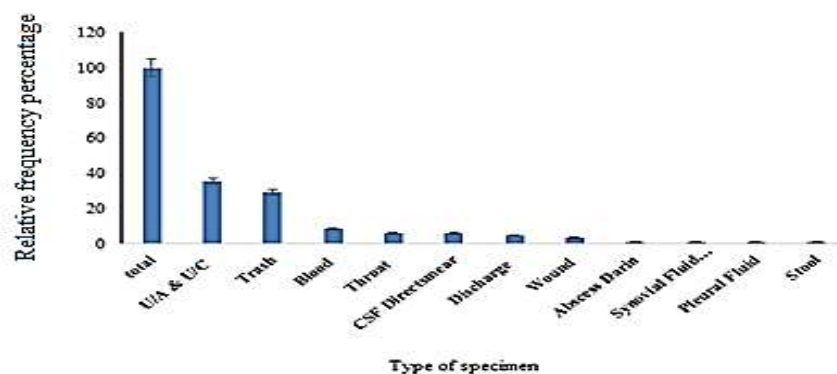


Figure 4. Distribution of total MDR strains of *K. pneumoniae* isolated by sample type

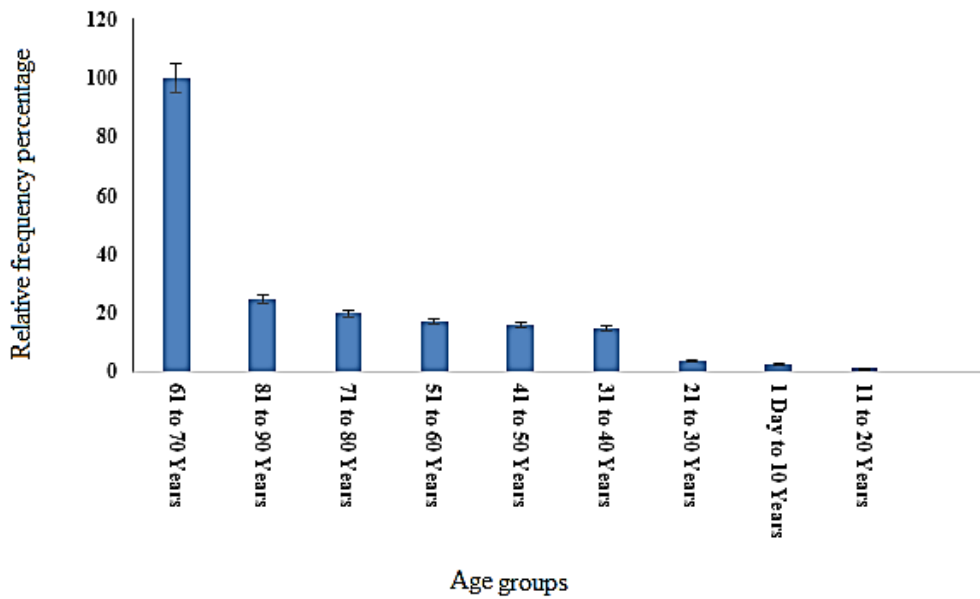


Figure 5. Distribution of total MDR strains of *K. pneumoniae* isolated by age groups

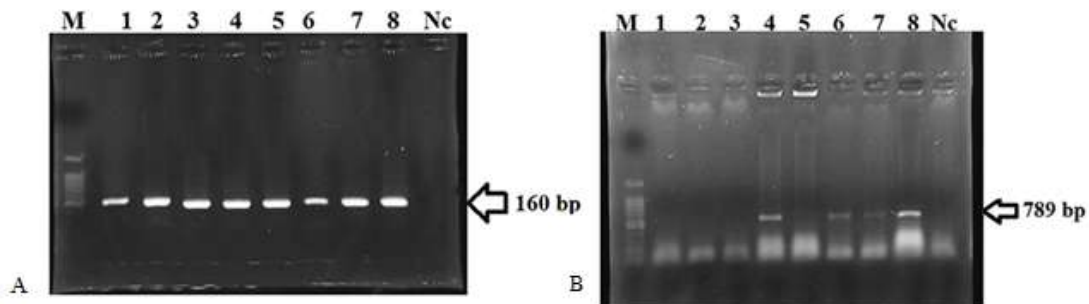


Figure 6. The examination of the PCR products resulting from the amplification of a segment of the integron I gene, which yielded a fragment of 160 bp (A), and a segment of the integron II gene, which produced a fragment of 789 bp (B) in 1% agarose gel (160 bp fragment). NC: negative control, M: 100 bp DNA ladder.

3.3. The results of the existence of class I and II integrons in *K. pneumoniae* strains resistant to several drugs

The PCR amplification products for specific regions of integron I and II genes in the examined strains have been depicted in Fig. 6. Among the total of 81 strains exhibiting resistance genes, 82.72% (67 strains) possessed class I integrons, while 86.42% (70 strains) harbored class II integrons. Furthermore, 70.37% (57 strains) of the specimens under investigation manifested both classes of integrons. The outcomes of the chi-square test demonstrate that, within the context of this

investigation, the frequency proportion of Int II exceeded that of Int I, notwithstanding the absence of a statistically significant disparity.

The present study analyzed the frequency distribution of genes carrying integrons across a population of 96 isolates of *K. pneumoniae*. Results indicated that a vast majority of the strains, precisely 81 (84.4%), harbored multiple genes conferring antibiotic resistance. Conversely, 15 strains (25.6%) demonstrated sensitivity towards antibiotics (Fig. 7). The findings of the chi-square statistical analysis demonstrate a significant variation in the prevalence of integron-carrying genes amongst the isolated strains ($P = 0.008$).

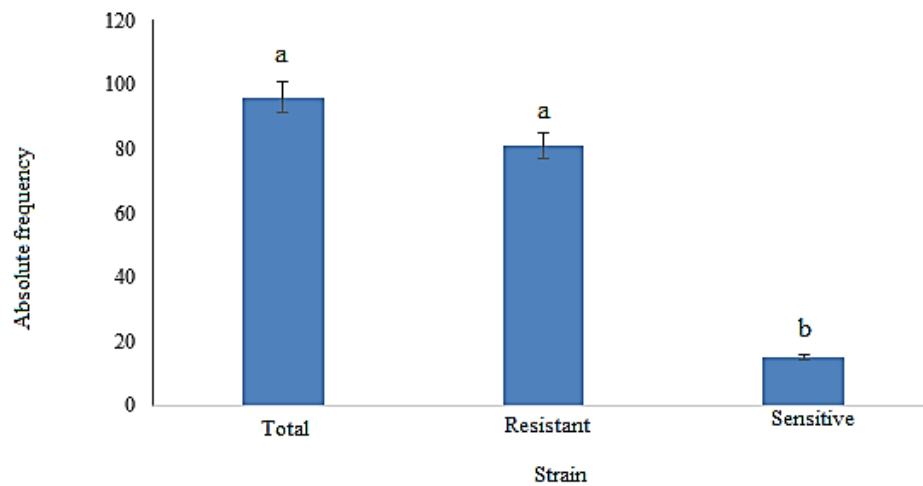


Figure 7. Frequency distribution of integron carrying genes in all resistant strain

Discussion

In the current study, a subset of *K. pneumoniae* strains displaying MDR were identified. Out of the total strains evaluated, 81 (84.4%) exhibited resistance to multiple antibiotics while 15 (25.6%) demonstrated sensitivity towards the antibiotics employed. Based on the findings, it was discovered that strains displaying multi-antibiotic resistance exhibited a frequency that was 3.31 times greater than that of the antibiotic-sensitive strains. The analysis of the frequency distribution of strains with regards to antibiotic resistance revealed that a notable proportion of the isolates displayed resistance towards meropenem (73 isolates) followed by amikacin (67 isolates). Conversely, a significantly smaller number of isolates displayed resistance towards the antibiotic gentamicin (12 isolates). In the research conducted by Liu et al., a total of 6493 Gram-negative strains were isolated from patients, among which *K. pneumoniae* constituted 1272 (19.59%) of the strains. The findings of the research indicated that *K. pneumoniae* exhibited a mounting trend of resistance towards piperacillin/tazobactam and ampicillin/sulbactam.

In the research conducted by Fatima et al. (2021), it was discovered that 21 out of a total of 107 isolates, representing 19.6%, were characterized as pathogenic *K. pneumoniae*. The results of the antibiotic susceptibility testing, which were conducted on a diverse panel of 17

antibiotics indicated that a significant proportion of the isolates exhibited resistance to multiple antimicrobial agents. The results of their study indicated a complete resistance to amoxicillin, cefixime, amoxicillin-clavulanic acid, cefotaxime, and ceftriaxone in all the isolated strains. Additionally, tetracycline exhibited a high resistance rate of 95.2%, followed by ciprofloxacin and gentamicin with a resistance rate of 76.2%, and a lower resistance rate of 7% was observed to sulfamethoxazole. The study findings report that the isolates were found to be sensitive to amikacin, chloramphenicol, and imipenem, while nalidixic acid (61.9%), norfloxacin (42.9%), piperacillin-tazobactam (23.8%), cefoprazone-sulbactam (19%), and cefotaxime-clavulanic acid (33.3%). The persistence of genes that confer resistance to tetracycline, sulfamethoxazole and β -lactamase was reaffirmed through the utilization of distinct gene targets. In line with previous research findings, the current investigation reveals that resistance to beta-lactam antibiotics is at the forefront of *K. pneumoniae* resistance, whereby beta-lactamase production potentially serves a crucial function in the domain. Over the past few decades, the emergence of broad-spectrum β -lactamases within gram-negative bacilli has become a paramount concept with regards to antibiotic resistance. Carbapenems constitute the preferred class of antibiotics for addressing infections in patients affected by broad-spectrum β -lactamase-producing bacterial strains, notwithstanding the noticeable higher trend of

carbapenem resistance in bacteria as reported in Li et al. (2022) study. Therefore, more comprehensive studies are warranted with respect to exploring the underlying mechanisms of resistance in this bacterial genus. The research findings indicate that the strains isolated from urinary infections exhibited the highest levels of antibiotic resistance. This trend may be attributed to insufficient vigilance towards the disease symptoms during the initial stages, leading to delayed treatment and the administration of potent antibiotics during advanced stages of the infection. Urinary tract infection is a widespread infectious ailment that afflicts individuals across all age brackets. The absence of prompt diagnosis and management can result in critical complexities, including urinary tract abnormalities, hypertension, renal ailments, uremia, and untimely childbirth among expectant women (Langarizadeh et al., 2010; Henshke-Bar-Meir et al., 2006). In contrast to the community, the prevalence of *K. pneumoniae* carriage is significantly elevated among hospitalized patients (Jondle et al., 2018).

The current investigation revealed that a predominant gender composition of the patient population, specifically males, yielded the majority of the 96 *K. pneumoniae* isolates and MDR strains within the sample set. The outcome presented aligns with the findings of Osagie et al. (2017) on collected samples from five primary health care centers located in Nigeria, and subsequently reported that there existed a higher occurrence of *K. pneumoniae* infection among the male population when compared to their female counterparts. Furthermore, Akter et al. (2014), reported that the propensity for contracting *K. pneumoniae* infection is comparatively elevated in male patients when compared to their female counterparts. The correlation between gender and *K. pneumoniae* infection was found to be connected to unfavorable lifestyle decisions in the aspect of tobacco use and excessive alcohol intake. The previous investigations failed to reveal any statistically significant disparity between the genders, whereas the contemporary inquiry demonstrated marked dissimilarity. The observed contrast may be attributed to variations in the behaviors and customs of males within the diverse societies under examination.

In this investigation, 96 isolates of *K. pneumoniae*, including MDR strains, were examined. The data revealed that there was a significant difference between the incidence of cases associated with emergency departments and intensive care units (ICUs) compared to that observed with gastrointestinal departments, operating rooms, and cardiac care units (CCUs). This discrepancy may be attributed to the number of individuals that require the use of the operating room and the strict aseptic procedures enforced therein. The frequency in question is notably influenced by the existence of these settings, as outlined in the study conducted by Nirwati et al. (2019) According to Liu et al. (2022), the departments demonstrating the greatest levels of *K. pneumoniae* prevalence included respiratory, neurology, ICU, and cardiovascular departments. This finding is largely congruent with the current study. The majority of individuals in these specific departments exhibited indications of chronic illness, advanced age, hospitalized status, and diabetes, and underwent extended antimicrobial therapy along with recurrent instances of invasive procedures including tracheal intubation, catheterization, and mechanical ventilation. The present findings suggest that *K. pneumoniae*, functioning as an opportunistic pathogen, poses a significant threat to patients who exhibit compromised health states, such as diminished immune system efficacy, diabetes, or malignancy. Consequently, rigorous cautionary measures are imperative for the appropriate management and care of such individuals.

Among the 96 isolates of *K. pneumoniae*, the highest frequency of isolates was observed in the age cohort ranging from 61 to 70 years and from 71 to 80 years, whereas the lowest incidence of isolates was documented in the age brackets ranging from 1 to 10 years and 11 to 20 years. In the present study, it was observed that the age groups of 71 to 80 and 81 to 90 years exhibited the highest incidence of MDR strains. Conversely, the age cohorts of 11 to 20 and 1 to 10 years displayed the lowest occurrence of MDR strains. Although in the study of Nirwati et al. (2019) in Indonesia, most *K. pneumoniae* isolates were obtained from patients aged 18 to 65 years, in the study of Yang et al. (2008) in China, most *K. pneumoniae* isolates were obtained from patients over 70. Concurrently, a recent investigation has demonstrated that the

age range of 40 to 65 years was associated with the most substantial occurrence of *K. pneumoniae* isolates amongst patients (Zheng et al., 2018). The age distribution of patients may be attributed to a decline in the potency of the immune system that is anticipated to occur with advancing age. Individuals who are below 40 years of age exhibit a more robust immune response while advancing age is associated with a greater susceptibility to *K. pneumoniae* attributed to an augmented prevalence of comorbidities (Gunn et al., 2016).

Given the pivotal role that integrons, particularly types I and II, play in MDR strains (Effah et al., 2020), the current study aimed to elucidate their association with clinical strains of *K. pneumoniae* isolated from patients in the hospitals of Isfahan. The current investigation revealed that a considerable portion of the strains exhibiting resistance genes, amounting to 82.72%, portrayed class I integrons, whereas 86.42% possessed class II integrons. Additionally, a significant percentage of 70.37% of the strains harbored both classifications of integrons. Within the framework of this investigation, the frequency ratio pertaining to Int II exhibited a marked proclivity towards elevation in comparison to Int I, albeit lacking statistical significance. Among the isolated bacterial strains, it was found that 84.4% displayed resistance to multiple antibiotics, while only 25.6% exhibited antibiotic sensitivity. The results indicate a statistically significant discrepancy in the prevalence of integron-containing genes across all analyzed strains. Fadare et al. (2023) found that among *K. pneumoniae* isolates which were obtained from wastewater, hospital, and animals; 98% of the isolates harbored an integrase gene; among them 80% detected as *intI1* and the remaining 20% harbored *intI1* and *intI2*. They did not detect *intI3* and other integrons in the isolates. These findings are coincident with the findings of the present study regarding to the integron class I prevalence. Xuan et al. (2017), conducted a study to examine the prevalence of integrons in clinical isolates of *K. pneumoniae* and elucidate the underlying molecular pathways of integron-mediated multidrug resistance in this microorganism. Class I and II integrons were detected in 60.1% and 1.7% of the isolates, respectively. However, the prevalence of these integrons in the present study was significantly

higher, particularly in regards to class II integrons. Firoozeh et al. (2019) evaluated the occurrence of class I, II, and III integrons in MDR *K. pneumoniae* isolates in Kashan, Iran. All of the MDR *K. pneumoniae* isolates analyzed were found to possess the *Int I* gene, whereas 36.7% of the isolates also carried the *Int II* gene. There was a lack of class III integrons among the MDR isolates of *K. pneumoniae*. The results of the aforementioned investigation, alongside those of the current research, demonstrate a notable incidence of class I and II integrons in multidrug-resistant *K. pneumoniae* isolates sourced from hospital-acquired infections. Furthermore, it appears that class III integrons have a noteworthy involvement in the absence of antibiotic resistance. Jahanbin et al. (2020), in a study which was conducted in Yasouj, a southwestern region of Iran, collected 196 cases of *K. pneumoniae* from patients diagnosed with urinary tract infections. Of these isolates, it was found that only 12.2% were positive for the presence of the *Int I* gene. Out of the total 28 bacteria identified as having multi-drug resistance, 60.7% (or 17 cases) were reported to possess the *Int I* gene, in stark contrast to the non-MDR isolates wherein only 2.4% of such isolates demonstrated the presence of the aforementioned gene. Shakib et al. (2020) determined the occurrence rate of class I integrons in isolates of *K. pneumoniae* from hospitals in Sanandaj, located in the Kurdistan province of Iran. Out of the 70 isolated *K. pneumoniae*, 13 cases (18.5%) were found to harbor the class I integron. Additionally, within a population of 28 drug-resistant isolates, it was observed that 11 of them exhibited positive indications of class I integron presence. The findings of their study indicated that the isolates possessing class I integrons exhibit significantly greater resistance to antibiotics relative to those lacking such integrons. In the present investigation, an examination of 96 strains of *K. pneumoniae* obtained from patients was conducted. The results indicated a correlation between multidrug resistance and the occurrence of class I and II integrons. Notably, 84.4% of the isolated strains exhibited resistance to multiple antibiotics, while 25.6% manifested antibiotic susceptibility. A significant difference was observed between these two categories of strains. Based on these research findings, the

consistent surveillance and characterization of integrons and correlated gene cassettes hold promise for managing the burgeoning growth of antibiotic resistance.

Conclusion

The findings of the current study indicated a notable prevalence of antibiotic resistance and the existence of MDR *K. pneumoniae* strains among clinical samples obtained from various sources within the geographical region of Isfahan city. The strains obtained from urinary infections, emergency departments and ICUs, exhibited the most extensive antibiotic resistance. The majority of the strains were obtained from individuals within the age range of 71 to 80 years. The present study reveals that the prevalence of class I and II integrons in MDR strains was 82.72% and 86.42%, respectively, while 70.37% of the strains carried both classes of integrons. This high prevalence of integrons in MDR strains indicates a global trend toward drug resistance among bacterial pathogens, as evidenced by previous studies. The present investigation reveals a remarkable prevalence of integron-mediated antimicrobial resistance in *K. pneumoniae* isolates from the urban locality of Isfahan, thereby emphasizing the significance of incorporating the identification and management of integron-dependent resistance mechanisms in the diagnostic and therapeutic approaches.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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