# THE PREVALENCE OF INTEGRON CLASS I AND II AMONG MULTI-DRUG RESISTANCE PRODUCING *KLEBSIELLA* pneumonia

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**ABSTRACT**

**This study aimed to determine antibiotic resistance profiles and the distribution of Integron I, Integron II, and genes among uropathogenic *Klebsiella pneumonia*e isolated from patients suffering from urinary tract infection. The results revealed that one hundred and thirty-seven (91%) out of 150 clinical specimens were *Klebsiella pneumonia*e. The isolates (137 isolates) were given a higher percentage of resistance against Piperacillin (94.89%)and Rifampicin (93.43%) and the lowest resistance was with Meropenem (40.87%). According to PCR amplification for 15 isolates showed the proportion of class I (*intl1*) and class II (*intl2*) integrons were investigated. What are 100% (n=15) and 45% (n=7) of isolates had *intl1* and *intl2* genes, respectively. PCR products for (Five)isolates were subjected to direct sequencing, and both strands of PCR products were sequenced with an automatic sequencer. These sequences were analyzed for the presence of variance of these genes and the detection of the differences in the nucleotides.**

**Keywords: Bacteria, Antibiotics, Molecular**
**Part of the M.Sc. thesis of the 1st author**



# انتشار الصنف الأول والثاني من Integron بين عزلات *klebsiella pneumonia*e المنتجة مقاومة الادوية المتعددة

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**المستخلص**

**هدفت الدراسة إلى تحديد انماط مقاومة المضادات الحيوية وتوزيع Integron I, Integron II والجينات بين *Kelbsiella pneumoniae* المسببة للأمراض البولية المعزولة من المرضى الذين يعانون من التهاب المسالك البولية. أظهرت النتائج أن مائة وسبعة وثلاثين (91٪) من 150 عينة سريرية *كانت Kelbsiella pneumoniae*. أعطت العزلات (137 عزلة) أعلى نسبة مقاومة ضد بايبرسايكلين (94.89 %) , ريفامبيسين) (93.43% ) وأقل مقاومة مع ميروبينيم (40.87٪) حسب تضخيم تفاعل البلمرة المتسلسل لـ 15 عزلة أظهرت نسبة الصنف I (*intl1*) و II (*intl2*) تم فحصها. وهي 100٪ (ن = 15) و 45٪ (ن = 7) من العزلات تحتوي على جينات *intl1* و*intl2* ، على التوالي. خضعت نواتج تفاعل البلمرة المتسلسل لـ (خمسة) عزلات لتسلسل مباشر، وتم اجراء تسلسل كل من خيوط منتجات تفاعل البلمرة المتسلسل باستخدام جهاز تسلسل آلي. تم تحليل هذه التسلسلات لوجود متغيرات هذه الجينات وكشف الاختلافات في النيوكليوتيدات.**

**كلمات مفتاحية:بكتريا,مضادات حيوية,جزيئي**
**جزء من رسالة الماجستير للباحث الاول**

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## INTRODUCTION

Urinary tract infections are the most common bacterial infection in primary care, impacting 150 million people each year throughout the world(12). It is an inflammatory reaction caused by the invasion of pathogenic microorganisms in the urinary tract(3), which is a common infection among patients in recent years, and it is caused by the inappropriate use and abuse of broad-spectrum antibiotics for decolonization not only results in a decrease in the effectiveness of standard treatments but also leads to the emergence of multidrug resistance (14). Due to the high mortality rate in healthcare-associated nosocomial infections, the rapid appearance and effective dissemination of carbapenem-resistant *K. pneumoniae* (CR-KPN) have become a major public health concern around the world (31,19). Escherichia coli is responsible for 60 to 80 percent of UTIs, while *K. pneumoniae* is responsible for 3 to 10%(27). *K. pneumoniae* is a major pathogen that causes a variety of illnesses, including pneumonia and bloodstream infections, most commonly in newborns and patients in intensive care units(29). Carbapenems are the last line of defense against *K. pneumoniae* that produces an extended spectrum -of lactamase (ESBL), and their widespread use has resulted in the emergence and dissemination of CR-KPN, a clinically important carbapenem-resistant Enterobacteriaceae (CRE) (12). The discovery of a New Delhi Metallo-lactamase-1 (NDM-1) has generated worldwide interest. After being dubbed "superbugs" with a reputation for being tough to treat, these NDM-1-producing isolates became well-known in the media(31). In recent years, rising antibiotic resistance among urinary opportunistic bacteria like *K. pneumoniae* has produced a concerning situation in the treatment of urinary tract infections (UTIs). The rising occurrence of antibiotic resistance among UTI-causing organisms makes empiric treatment of these infections extremely difficult(19). The development of Multidrug Resistance (MDR) in the Enterobacteriaceae family has been aided by increased antibiotic usage and horizontal transfer of antibiotic resistance genes located on various types of mobile DNA elements such as plasmids, transposons, and gene cassettes in integrons(17). In recent years, rising antibiotic resistance among urinary opportunistic bacteria like *K. pneumoniae* has produced a concerning situation in the treatment of (UTIs). Integrons have a key role in the spread of antibiotic resistance genes (15), so researchers looked at class 1 and 3 integrons, as well as the resistance gene cassettes they correspond to, in urinary *K. pneumoniae* isolates. Based on the amino acid sequence homology of the intI gene, integrons are categorized into many classes, with classes 1, 2, and 3 being the most commonly recovered from clinical isolates(8). Different investigations have identified class 1 integron as the most common class found in clinical isolates (8). Class 1 integrons have variable gene cassette portions that are sometimes missing from the integron structure, flanked by two highly conserved sequences: 5′-conserved segment (5′-CS) and 3′-conserved segment (3′-CS) (9). Gene cassettes are promoterless variable sections of integrons positioned between the attC and attI regions that encode antibiotic resistance traits. Their expression is controlled by the integron promoter (Pc), which in the case of class1 integron relies on the 5′-CS. Various cassette arrays have been reported, with the majority having two or three gene cassettes (23). The study's goals are to isolate and identify K. pneumoniae from patients with urinary tract infections, as well as to look into antibiotic resistance in these isolates and to look for Integron genes Class I and II in *K. pneumoniae*.

## MATERIALS AND METHODS

**Urine collection:** Each patient's midstream urine was collected using a sterile (20 mL) calibrated screw-capped universal container that was given to them at the outset(16). The specimen was properly labeled, transferred to the laboratory as soon as possible, and analyzed within two hours after collection(29). A total of 150 clinical urine specimens were collected From November 2021 to the end of February 2022, from patients suffering from UTIs from four hospitals in Baghdad as follows, Al-Yarmuk Teaching Hospital, Al-Karama teaching Hospital, Al-Mahmoodia Hospital, and Al - Kadhimiya General Hospital.

**Culture media:** Blood agar, MacConkey agar, Eosin Methylene blue Agar (EMB Agar), and CHROMagar Orientation(10).

**Biochemical tests:** The following biochemical tests were used to identify bacterial isolates, IMVIC, urease, oxidase, and catalase test, in addition, to using the VITEV2 system.

**Susceptibility testing for antibiotics (ATS):** Kirby-Bauer method was followed to carry out the antibiotic's susceptibility test for 19 different antibiotics including Levofloxacin 30µg/disk, Aztreonam 30µg/disk, Cefepime 5µg/disk, Cefotaxime 5µg/disk, Ceftazidime 30µg/disk, Ceftriaxone 30µg/disk, Ciprofloxacin 10µg/disk, Gentamicin 10µg/disk, Imipenem 10µg/disk, Meropenem 10µg/disk, Piperacillin 100µg/disk, Ampicillin/sulbactam 30µg/disk, Tobramycin 30µg/disk, Tigecycline 5µg/disk, Trimethoprim/sulfamethoxazole 1.25/23.75µg/disk, Amikacin 30µg/disk, Colistin 10µg/disk, Rifampicin 5µg/disk, Tetracycline 10µg/disk.

**Primers sequence:** Primers utilized in this study are listed in Table (1).

**Table 1. Primers utilized in this Study**

| PRIMER NAME | | SEQUENCE (5´-3´) | PRODUCTION SIZE | ANNEALING TEMP |
|---|---|---|---|---|
| | F | GCAAGTCGAGCGGTAGCACAG | | |
| *16S RNA* | R | CAGTGTGGCTGGTCATCCTCTC | 260 BP | 58 |
| INTI1 | F | CAGTGGACATAAGCCTGTTC | 160 BP | 54 |
| | R | CCCGAGGCATAGACTGTA | | |
| INTI2 | F | CACGGATATGCGACAAAAAGGT | VARIABLE | 60 |
| | R | GTAGCAAACGAGTGTGACGAAATG | SIZE | |

**DNA extraction:** Bacterial DNA extraction is performed according to the Chang and Jiany method (2006). The bacterial culture was grown in five ml LB broth and, then one ml of the culture was transferred to Eppendorf tubes. These tubes were centrifuged for 3 min at 8000 rpm and the supernatant was discarded without touching the pellet. Genomic DNA extracted according to ABC DNA Isolation Kit. The amount and integrity of DNA were determined using the spectrophotometer (Nanodrop).

**Quantitation of DNA:** To detect the quality of samples for downstream applications, a Quantus Fluorometer was utilized to detect the concentration of extracted DNA. 1µL liter of DNA was combined with 199µL liters of diluted Quantifluor Dye. DNA concentration readings were determined after a 5-minute incubation period at room temperature.

**PCR reaction mix preparation and PCR thermocycling conditions:** Aseptically prepared the PCR reaction mix by using Taq Ready master mix Kit according to the manufacturer's instructions (Promega, USA). Polymerase chain reaction assays were performed at a reaction volume of 50 µl (table 2), and according to the PCR program (table 3).

**Table 2. Ready master mix Kit according to the manufacturer's instructions**

| MASTER MIX COMPONENTS | STOCK | UNIT | FINAL | UNIT | VOLUME 1 SAMPLE |
|---|---|---|---|---|---|
| MASTER MIX | 2 | X | 1 | X | 12.5 |
| FORWARD PRIMER | 10 | MM | 2.5 | MM | 1 |
| REVERSE PRIMER | 10 | MM | 2.5 | MM | 1 |
| NUCLEASE FREE WATER | | | | | 8.5 |
| DNA | 10 | NG/ML | 10 | NG/ML | 3 |
| TOTAL VOLUME | | | | | 25 |
| ALIQUOT PER SINGLE RXN | 22 ML OF MASTER MIX PER TUBE WAS ADDED | | | | |

**Table 3. PCR Program**

| STEPS | °C | M: S | CYCLE |
|---|---|---|---|
| INITIAL DENATURATION | 95 | 05.00 | 1 |
| DENATURATION | 95 | 00.30 | |
| ANNEALING | 54,58,60 | 00.30 | 30 |
| EXTENSION | 72 | 00.30 | |
| FINAL EXTENSION | 72 | 07:00 | |
| HOLD | 10 | 10:00 | 1 |

**Gel electrophoresis and sample preparation:** DNA and PCR products were detected using gel electrophoresis, which was observed using ethidium bromide and a UV transilluminator documentation system. Before electrophoresis, 7 µL of DNA extract sample were mixed with 3 µL of loading dye solution and loaded into the wells. whereas for PCR, each well was loaded with 10µL of PCR products on the gels. To detect PCR product size, DNA ladders (100bp) were always performed concurrently with each electrophoretic run. UV Transilluminator documentation system was used to visualize DNA bands (25).

**Standard sequencing:** PCR products were sent for Sanger sequencing using ABI3730XL, an automated DNA sequence, by Macrogen Corporation – Korea. The results were received by email and then analyzed using genius software.

**Statistical analysis:** The Statistical Analysis System- SAS program was used to test the effect of different factors on study parameters. Chi-square test was used to detect significant comparison between percentage and in this study

**RESULTS AND DISCUSSION**

Identification of the isolates was performed using growth-based conventional methods following their morphology in Gram's staining, Cultural characteristics, and biochemical properties. A total of 150 clinical specimens of urine were cultured on selective medium CHROMagar Orientation, Blood agar, EMB, and also on MacConkey agar plates. 137 isolates were obtained from these media and were identified according to the following characters which were observed. The isolates appeared as metallic blue or greenish colonies at 37°C for 24 hours of incubation as shown in (Figure 1), this result agreed with(26).

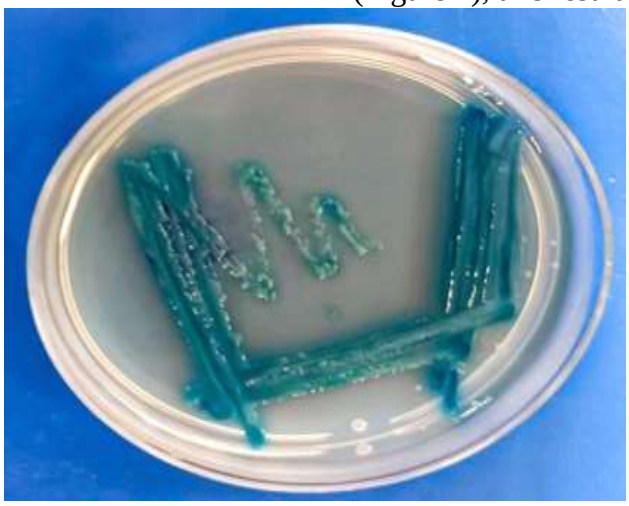

**Figure 1. *Klebsiella pneumoniae* on CHROMagar medium**

CHROMagar (CHR) is a highly selective growth medium for Klebsiella spp. isolation and is widely used in diagnostic and clinical laboratories. CHROMagar is a chromogenic, differential, and highly selective media for detecting Klebsiella pneumoniae in strikingly green colonies. The colony colors were the same as the CHROMagar colony colors. Only E. coli was easily distinguished by color check, whereas the other strains such as the KESC group (*Klebsiella*, *Enterobacter*, *Serratia*, and *Citrobacter*), required additional testing to distinguish them. On blood agar medium, the *Klebsiella pneumoniae* colonies are non-hemolytic (γ-hemolysis), and this is consistent with the results. The growth on MacConkey agar were bright pink colonies with a mucoid structure due to the lactose fermentation which is a characteristic feature of these bacteria and this agrees. On EMB the isolated bacteria produced large, mucoid, pink to purple colonies with no metallic green sheen. Biochemical tests clarified that all 137 *Klebsiella pneumoniae* isolates from the 150 samples were positive for Urease, Simmon's Citrate Utilization, and Catalase. Likewise, they were negative for the Indole test, Methyl red, Oxidase and Voges-Proskauer tests. The ability of a microorganism to ferment carbohydrates and produce hydrogen sulfide yielded negative results for all isolates under consideration. Yellow slant, yellow but, gas positive, no $H_2S$ generated by *K. pneumoniae*. All the 150 *K. pneumoniae* isolates were

cultured on a CHROMagar medium. The prevalence of CRE colonization was 137 isolates. Among 137 the occurrence of *K. pneumoniae* isolates to gender was found to be statistically significant, with 91 isolates (66.42%) obtained from female patients and 46 (33.58%) recovered from male patients as shown in Table (4). The study by Tran *et al*., (26) showed the prevalence of CRE colonization was 46.1%. Male and 40.0% in eight patients were CRE-positive. Although, *K. pneumoniae* isolates accounted for 16.2 percent of all UTIs. Males (23.4%) had substantially higher *K. pneumoniae* levels than females (15.4%) (P=0.03) (22). Despite this, 19 (7.6%) *K. pneumoniae* isolates were found in 150 clinical specimens, including 5 (5.61%) and 14 (8.69%) males and females, respectively. The isolates were shown to have high antibiotic resistance, particularly to penicillins and cephalosporins. *K. pneumoniae*, on the other hand, demonstrated very little resistance to imipenem and amikacin (1).

**Table 4. Statics results of *K. pneumoniae* isolate according to the gender**

| Male No. (%) | Female No(%) | Total No. (%) |
|---|---|---|
| 46(33.57) | 91(66.43) | 137(100) |

Among 137 *K. pneumoniae isolates*, 91(66.43%) were obtained from female patients and 46(33.57%) recovered from male patients. The highest infection of *K. pneumoniae in* the females' age group (20-29) years was 30(32.96%) followed by the age group (50-59) years was the infection ratio 19(20.87%), while the highest male infection in age group (30-39) years was 15(32.60%) followed by age group (20-29) years was 12(26.08%) (Table 5).

**Table 5. Results of *K. pneumoniae* isolates according to the age**

| AGE (YEAR) | MALE NO. (%) | FEMALE NO. (%) | TOTAL |
|---|---|---|---|
| 10-19 | 6 (13.04) | 6 (6.59) | 12 |
| 20-29 | 12 (26.08) | 30 (32.96) | 42 |
| 30-39 | 15 (32.60) | 18 (19.78) | 33 |
| 40-49 | 6 (13.04) | 15 (16.48) | 21 |
| 50-59 | 3 (6.52) | 19 (20.87) | 22 |
| 60-69 | 2 (4.34) | 3 (3.29) | 5 |
| 70-79 | 1 (2.17) | 0 (0) | 1 |
| 80-89 | 1 (2.17) | 0 (0) | 1 |
| TOTAL (%) | 46 (100) | 91 (100) | 137 |

In this study, an antibiotics susceptibility test of *Klebsiella pneumoniae* was conducted on nineteen antibiotics. Among 137 isolates were shown that these bacteria were resistant to Piperacillin, Rifampicin and Ceftazidime (94.89%), (93.43%) and (91.97%) respectively, while Ceftriaxone, Cefotaxime, Aztreonam and Tetracycline (89.78%),(82.48%), (82.48%) and (78.10 %) respectively,although Ampicillin/ Sulbactam followed by, Cefepime, Tigecycline, Amikacin and Trimethoprim (77.37%), (76.64%) , (75.18%), (72.26%) and (71.53%) respectively, whereas, Tobramycin, Levofloxacin, Ciprofloxacin, and Gentamicin (68.61 %), (68.61%), (65.69%), and (61.31 %) respectively, also, Imipenem (57.66%) and Colistin, Meropenem (45.25%), (40.87%) respectively. Those percentages showing the higher resistance were with Piperacillin (94.89%) and the lowest resistance was with Meropenem (40.87%). Statistically, there was a highly significant difference (Table 6). These results were agreed with Wang, *et al*.(28) who recorded that *Klebsiella pneumoniae* resists a wide range of antibiotics such as Cefotaxime (97.6%), Ceftazidime (91.8%), and Levofloxacin (79.3%), which is similar to our

study result. According to experts, ceftazidime may not be effective against organisms with a certain combination of beta-lactamases and alterations in outer-membrane porins. The increase of *K. pneumoniae* resistance to Ceftazidime has led to concerns that the drug might not be effective against this strain of bacteria (5, 13). Meropenem and Imipenem were shown to be effective antibiotics in this investigation, and the results showed that the majority of isolates were susceptible to them, which is consistent with the findings of many prior studies. These studies demonstrate that all isolates showed high resistance rates against ceftazidime (96.72%) and amikacin (80.87%). Showed their results that *K. pneumoniae* had high resistance against Cefotaxime 94.2%, Ceftazidime 94.2%, Aztreonam 79.7%, trimethoprim 76.8%, and Amikacin 62.3%, while Nimnoi *et al.*, (22), found that enterobacterial strains presented high resistant against Ampicillin about (76.2%) which close to this result while the resistance to the other result not related to this study.

**Table 6. Results of Antibiotic resistance of *Klebsiella pneumoniae* isolates**

| ANTIBIOTICS | SENSITIVE NO.(%) | INTERMEDIATE NO.(%) | RESISTANT NO.(%) | TOTAL NO.(%) |
|---|---|---|---|---|
| MEM | 57(41.60) | 24(17.51) | 56(40.87) | 137 (100) |
| CL | 35(25.54) | 40(29.19) | 62(45.25) | 137(100) |
| TGC | 12(8.75) | 22(16.05) | 103(75.18) | 137(100) |
| AM | 15(10.94) | 16(11.67) | 106(77.37) | 137(100) |
| TE | 22(16.05) | 8(5.83) | 107(78.10) | 137(100) |
| SXT | 21(15.32) | 18(13.13) | 98(71.53) | 137(100) |
| AK | 19(13.86) | 19(13.86) | 99(72.26) | 137(100) |
| ATM | 13(9.48) | 11(8.02) | 113(82.48) | 137(100) |
| FEP | 17(12.40) | 15(10.94) | 105(76.64) | 137(100) |
| IPM | 39(28.46) | 19(13.86) | 79(57.66) | 137(100) |
| CIP | 24(17.51) | 23(16.78) | 90(65.69) | 137(100) |
| LEV | 22(16.05) | 21(15.32) | 94(68.61) | 137(100) |
| CTX | 20(14.59) | 4(2.91) | 113(82.48) | 137(100) |
| CN | 49(35.76) | 4(2.91) | 84(61.31) | 137(100) |
| TOB | 37(27.00) | 6(4.37) | 94(68.61) | 137(100) |
| CRO | 10(7.29) | 4(2.91) | 123(89.78) | 137 (100) |
| CAZ | 6(4.37) | 5(3.64) | 126(91.97) | 137 (100) |
| RA | 4(2.91) | 5(3.64) | 128(93.43) | 137 (100) |
| PRI | 2(1.45) | 5(3.64) | 130(94.89) | 137 (100) |

**Genomic DNA extraction:** Quantus Fluorometer technique and PCR analysis proved to be beneficial in the measurement of both quantity and purity of DNA in the samples. Using a genomic DNA purification kit, genomic DNA was extracted from *Klebsiella pneumoniae* isolates. Extraction of genomic DNA from 137 isolates was confirmed as bands by gel electrophoresis. Similar to the results which purified and quantified the samples using the Quantus Fluorometer technique and PCR analysis, the adequate yielded satisfactory purity values (1.89 for A260/A230 and 1.65 for A260/A280), as well as concentration ratios (476.78 ng/µl) with low variability. Pre-lysis, post-extraction purification, and preparation of nucleic acids are suggested for use in potential molecular applications. The concentration and purity of DNA from urine samples are greatly improved when using a commercial kit to prepare samples for analysis.

**Identification of *K. pneumoniae* by conventional PCR technique:** For identification of all 137 isolates of *Klebsiella pneumonia* was done depending on the use of specific primers for 16sRNA which confirm the results of the biochemical test and VITEK 2 system. The product of conventional PCR for 15 isolates was detected detect by using gel electrophoresis as shown in fig. (2). Because it is more potent, reproducible, and exact than results acquired using traditional approaches, the 16S rRNA gene is utilized as a marker for identifying distinct bacteria at the genus level. Bacterial taxonomy and phylogeny can be studied using 16S rRNA sequencing. For a variety of reasons, it is currently the most widely utilized genetic marker. It is found in practically all bacteria, frequently in operons or multigene families, and has a consensus sequence with the function of the 16S rRNA region that has remained unchanged across time. Out of 50 specimens collected from each

organ of 50 camels, isolates were identified as *Klebsiella pneumoniae* using traditional methods (microscopic and culturing examinations, as well as biochemical tests) and molecular techniques employing the 16SrRNA gene. The results presented here coincided with the results recorded by Budiarso, *et al*. (4) who collected 120 samples of street foods and drinks sold at schools and several public places. According to traditional methods and molecular techniques using the 16SrRNA gene, only 11 out of 120 bacterial isolates belonged to *K. pneumoniae.*

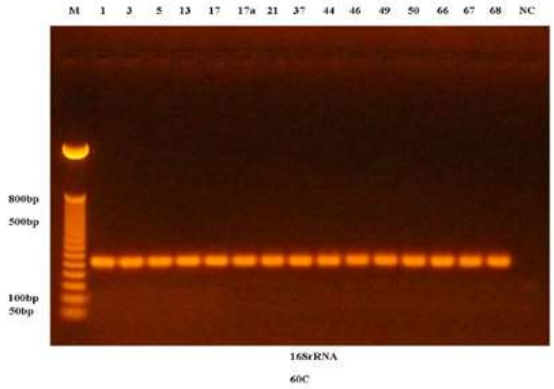


**Figure 2. The amplification results of the 16srRNA genes of Klebsiella pneumoniae samples fractionated on 1.5% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker. NC: Negative control 100v /mAmp**

**Detection of the *intl-1* and *intl-2* genes by PCR:** The fraction of class I (*intl1*) and class II (*intl2*) integrons were studied using PCR findings. Figure (3) and [Table 7] show that 100 percent (n=15) and 45 percent (n=7) of the isolates investigated possessed intl1 and intl2 genes, respectively. The intl1 integron is the most prevalent among *K. pneumoniae* strains. according to the findings of many investigations. In two separate investigations conducted in Iran, the fraction of intl1 was reported to be 91 percent and 100 percent, respectively, which is the closest to the gene frequency in our study (21, 11). Delarampour *et al*. (7) found Class I and II integrons in 65 *K. pneumoniae* isolates (45.8%) and 1 (0.7%) isolate, respectively. It is probably that the spread of integron-positive isolates will lead to the spread of multi-drug resistant isolates in hospitals. According to the World Health Organization (WHO), these mobile genetic components usually contain a large number of drug resistance genes that can be transmitted horizontally between bacteria(30). The *int1* and *int2* genes were detected in 96 percent of isolates, but the intII gene was found in just sixteen isolates, according to Asghari *et al*., (2). (12.5 percent). However, according to Maina *et al*., (18), carriage of class one integrons intI1 (23 percent) was more common than intl2 (3 percent) in non-fastidious Gram-negative bacteria collected in Nairobi, Kenya. Our findings revealed a significant incidence of integrons in MDR *K. pneumoniae*, implying that these genes play an essential role in the transmission of antibiotic resistance. Asghari and colleagues (2).

**Table 7. Frequency of genes among 15 isolates**

| No. | *Intl1* | *Intl2* |
|---|---|---|
| **1** | **Positive** | **Negative** |
| **2** | **Positive** | **Negative** |
| **3** | **Positive** | **Negative** |
| **4** | **Positive** | **Negative** |
| **5** | **Positive** | **Negative** |
| **6** | **Positive** | **Negative** |
| **7** | **Positive** | **Negative** |
| **8** | **Positive** | **Positive** |
| **9** | **Positive** | **Negative** |
| **10** | **Positive** | **Positive** |
| **11** | **Positive** | **Positive** |
| **12** | **Positive** | **Positive** |
| **13** | **Positive** | **Positive** |
| **14** | **Positive** | **Positive** |
| **15** | **Positive** | **Positive** |
| | **Percentage of genes** | |
| | ***Intl 1*** | ***Intl 2*** |
| **Positive** | **100%** | **47%** |
| **Negative** | **0%** | **53%** |

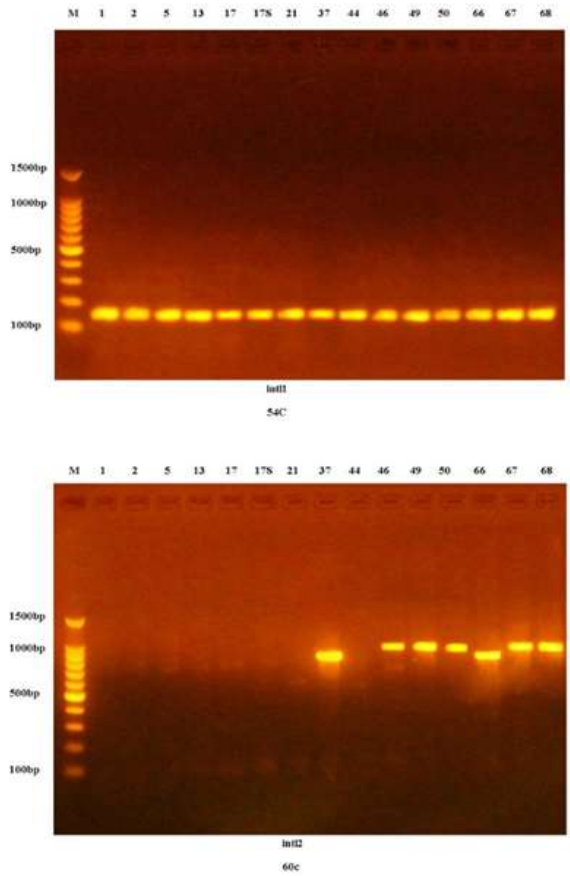


**Figure 3. The amplification results of the *Klebsiella pneumoniae* genes fractionated on 1.5% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker 100v /mAmp**

**Analysis of sequencing of PCR products (*intl-1* and *intl-2* genes):** Products of PCR were sequenced and named (OMAH22) *intl*-1 gene for class 1 integron integrase, (OFK1) *intl*-1 gene for class 1 integron integrase and (OFK1 DNA) similar to class 2 integron integrase deposited in NCBI with accession numbers (LC705340, LC705341, and LC705343) respectively. PCR products for 5 selected *intl*-1 genes of the *K. pneumoniae* isolates and PCR products for 2 selected *intl*-2 genes of the *K. pneumoniae* isolates which had a high level of resistance to all clinically available aminoglycosides for systemic (Figure 4) and (Figure 5), were detected by agarose gel electrophoresis and the sequencing was carried out using the Applied (Seoul, Korea: Macrogen Research). Direct sequencing of PCR products was performed, with both strands of PCR products being sequenced with an automated sequencer. The Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov) was used to analyze DNA sequences and find similarities. Also, these sequences were analyzed for the presence of variants of these genes and detection of the differences in the nucleotides (mutations). In the results of the alignment of the *intl-1* sequences of the local isolates with the *intl*-1 gene, it was found that the most of sequences had high similarity (100 %) but at the same time contained new mutations at the positions not recorded in the previous studies in the sample NO. 44 with Query (767864) (strain Danio rerio) revealed the presence of 1 substitution of the pyrimidine nucleotides (C to T) in different positions of the sequence (Figure 6, 7) these variations led to changes in the several amino acids of the produced protein. the result is similar to the Cronan *et al*., (6) the alignment of *itln-1* or called *intl-1* gene from the local isolate RK4 with the reference 1(bases 1 to 1256) (strain Danio rerio) revealed the presence of 3 substitutions of the pyrimidine nucleotides (C to A, G, T) in different positions of the sequence. BLASTn searches for these genes in the relevant genome assembly graphs revealed that intI1 were coharbored on a single replicon, which formed a closed circularised path through the graph and was later shown to carry an IncN plasmid replicon marker, implying that this structure represented an antimicrobial resistance plasmid, and a BLASTn search revealed that the IntI1 integron sequence was an identical match to >100 *K. pneumoniae*(20).

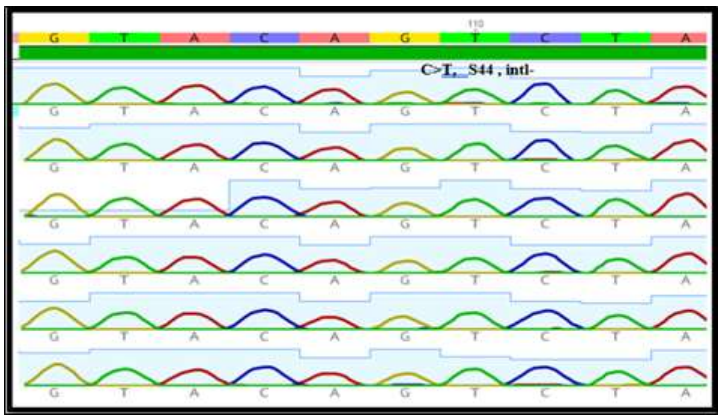


**Figure 4. The chromatogram profile of the intl-1 locus genetic variations found in K. pneunmniae bacterial isolates. The positions of the detected substitution mutations in the PCR amplicons are marked. ">" stands for "substitution" mutation, while "S" stands for sample code**

intl-1 primer

| Description | Max Score | Total Score | Query Cover | E value | Per. Ident | Accession |
|---|---|---|---|---|---|---|
| Klebsiella pneumoniae strain NordR_R85 plasmid pR85_1, complete sequence | 219 | 438 | 100% | 8e-55 | 100.00% | CP091583.1 |
| Klebsiella pneumoniae strain Nord5-1_R48 plasmid pR48_2, complete sequence | 219 | 657 | 100% | 8e-55 | 100.00% | CP091591.1 |
| Klebsiella pneumoniae strain Nord77_R886 plasmid pR886_1 | 219 | 438 | 100% | 8e-55 | 100.00% | CP091602.1 |
| Klebsiella pneumoniae strain Nord77_R886 plasmid pR886_3 | 219 | 219 | 100% | 8e-55 | 100.00% | CP091600.1 |
| Klebsiella pneumoniae strain Kp11-81 plasmid p220F, complete sequence | 219 | 219 | 100% | 8e-55 | 100.00% | CP082155.1 |
| Klebsiella pneumoniae strain M169-3 plasmid pM169-3.2, complete sequence | 219 | 438 | 100% | 8e-55 | 100.00% | CP063880.1 |
| Klebsiella pneumoniae strain S165-1 plasmid pS165-1.1, complete sequence | 219 | 219 | 100% | 8e-55 | 100.00% | CP058649.1 |
| Klebsiella pneumoniae strain S161-2 plasmid pS161-2.3, complete sequence | 219 | 219 | 100% | 8e-55 | 100.00% | CP058647.1 |
| Klebsiella pneumoniae strain S161-2 plasmid pS161-2.2, complete sequence | 219 | 219 | 100% | 8e-55 | 100.00% | CP058646.1 |
| Klebsiella pneumoniae strain JINKPN30 plasmid pJNKPN30_NDM, complete sequence | 219 | 219 | 100% | 8e-55 | 100.00% | CL389795.1 |
| Klebsiella pneumoniae strain WSCRKP plasmid pWSCRKP-3, complete sequence | 219 | 219 | 100% | 8e-55 | 100.00% | CP051071.1 |
| Klebsiella pneumoniae strain BA29665 plasmid pIncHI1B_FIB, complete sequence | 219 | 438 | 100% | 8e-55 | 100.00% | CP038469.1 |
| Klebsiella pneumoniae strain KPN142 plasmid pn142_1, complete sequence | 219 | 219 | 100% | 8e-55 | 100.00% | CP053876.1 |
| Klebsiella pneumoniae strain KP697 plasmid unnamed3, complete sequence | 219 | 219 | 100% | 8e-55 | 100.00% | CP066154.1 |
| Klebsiella pneumoniae strain KP697 chromosome, complete genome | 219 | 219 | 100% | 8e-55 | 100.00% | CP066151.1 |
| Klebsiella pneumoniae strain 200 plasmid pKp2002, complete sequence | 219 | 219 | 100% | 8e-55 | 100.00% | CP063211.1 |
| Klebsiella pneumoniae strain ONE259 plasmid p1194_11_5, complete sequence | 219 | 219 | 100% | 8e-55 | 100.00% | CP090936.1 |
| Klebsiella pneumoniae strain ONE259 plasmid p1194_11_1, complete sequence | 219 | 219 | 100% | 8e-55 | 100.00% | CP090932.1 |

**Figure 5. Results of alignments of *Intl-1* gene of samples NO. 5,17,44,49,66 *K. pneunmniae* local isolates by BLASTn analysis of NCBI**

intl-2 primer

| Description | Max Score | Total Score | Query Cover | E value | Per. Ident | Accession |
|---|---|---|---|---|---|---|
| Klebsiella pneumoniae strain KP89 plasmid pKP89, complete sequence | 1290 | 1290 | 100% | 0.0 | 100.00% | MK284770.1 |
| Klebsiella pneumoniae strain KP_NORM_BLD_2014_104014 plasmid unnamed1, complete sequence | 1290 | 1290 | 100% | 0.0 | 100.00% | CP034046.1 |
| Klebsiella pneumoniae strain KP_NORM_BLD_2015_112126 plasmid unnamed1, complete sequence | 1290 | 1290 | 100% | 0.0 | 100.00% | CP034054.1 |
| Klebsiella pneumoniae strain KP30635 plasmid unnamed5, complete sequence | 1290 | 1290 | 100% | 0.0 | 100.00% | CP027700.1 |
| Klebsiella pneumoniae strain A64477 plasmid pKP64477d, complete sequence | 1290 | 1290 | 100% | 0.0 | 100.00% | MF150120.1 |
| Klebsiella pneumoniae subsp. pneumoniae strain 234-12 plasmid pKpn23412-362, complete sequence | 1290 | 1290 | 100% | 0.0 | 100.00% | CP021314.1 |
| Klebsiella pneumoniae strain ONE259 plasmid p1194_11_3, complete sequence | 1290 | 1290 | 100% | 0.0 | 100.00% | CP090934.1 |
| Klebsiella pneumoniae strain 1194 plasmid p1194_3, complete sequence | 1290 | 1290 | 100% | 0.0 | 100.00% | CP089430.1 |
| Klebsiella pneumoniae subsp. pneumoniae Kp13 plasmid pKP13d, complete sequence | 1290 | 1290 | 100% | 0.0 | 100.00% | CP003997.1 |
| Klebsiella pneumoniae strain B109 plasmid pKpnB109, complete sequence | 1290 | 1290 | 100% | 0.0 | 100.00% | MK582106.1 |
| Klebsiella pneumoniae strain D-03 plasmid IncN class 2 integron, partial sequence | 1290 | 1290 | 100% | 0.0 | 100.00% | HM043675.1 |
| Klebsiella pneumoniae strain A2293 plasmid pA2293-Cr2, complete sequence | 1273 | 1273 | 100% | 0.0 | 99.57% | MN310379.1 |
| Klebsiella pneumoniae strain KP1278 plasmid pIAC-KLUC, complete sequence | 1273 | 1273 | 100% | 0.0 | 99.57% | MH176940.1 |
| Klebsiella pneumoniae strain A1761 plasmid pA1761-Cr2, complete sequence | 1273 | 1273 | 100% | 0.0 | 99.57% | MG764652.1 |
| Klebsiella pneumoniae strain 3 class 2 integron, partial sequence | 1258 | 1258 | 97% | 0.0 | 100.00% | KF810144.1 |
| Klebsiella pneumoniae strain MRK9 chromosome | 1072 | 1072 | 83% | 0.0 | 100.00% | CP043859.1 |
| Klebsiella pneumoniae subsp. pneumoniae strain M5 chromosome, complete genome | 1072 | 1302 | 100% | 0.0 | 100.00% | CP031734.1 |
| Klebsiella pneumoniae strain NHG1 chromosome, complete genome | 1072 | 1200 | 100% | 0.0 | 100.00% | CP034408.1 |

**Figure 6. Results of alignments of *Intl-2* gene of samples NO. 49,66 *K.pneunmniae* local isolates by BLASTn analysis of NCBI**

| Gene ID | Symbol | Gene name | Gene type | Scientific name | Transcripts | | Query |
|---|---|---|---|---|---|---|---|
| 767684 | itln1 | intelectin 1 | protein-coding | Danio rerio | NM_001076622.2... | 2 | 767684 |

**Figure 7. Danio rerio intelectin 1 (*itln1*), reference 1 (bases 1 to 1256)**

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