CRISPR-Cas system in multi drugs resistant *Klebsiella pneumoniae* from different clinical samples and its correlation with antibiotic-resistant genes in Mosul city / Iraq

Zinah Makki AL-Yozbakee*
Microbiology Unit, College of Medicine, Mosul University, Mosul city, Iraq
Khalid O. Mohammad
Microbiology Unit, College of Medicine, Tikrit University, Tikrit city, Iraq
*Corresponding author. E-mail: zeenayouzbaki@uomosul.edu.iq

Abstract
Clustered, regularly interspaced short palindromic repeats (CRISPRs) and their related genes (Cas) are prevalent in the genomes of several bacteria and serve as a defense mechanism against external attackers, such as plasmids and viruses. This study aimed to examine the frequency of the CRISPR/Cas system in naturally occurring strains of *Klebsiella pneumoniae* sub spp *pneumoniae* confirmed by Vitek 2 biochemical test, in the hospital setting and determine its correlation with antibiotic resistance both phenotypically and genetically (antibiotic-resistant genes, namely *blaTEM* and *AcrA* efflux pump gene). The research was conducted at Medical College/ Mosul University 23 multi-drug resistant *K. pneumoniae* sub spp. *pneumoniae* that were obtained from 230 clinical samples from infected patients with different types of infections attending Al-Salam and Al-Jumhoreye Teaching hospitals. PCR was used to detect *blaTEM*, *AcrA* genes, and CRISPR/Cas system genes (CAS1A and CAS1B) among the clinical isolates. The correlation between the CRISPR/Cas system and antibiotic-resistance was determined. All the isolates were multiple-drug-resistant strains, and the *blaTEM* gene was detected in all clinical isolates, whereas *AcrA* gene was detected in 94% of the isolates. The frequency of CAS1A and CAS1B was 21.73% and 86.95% respectively. There was an inverse correlation between the CAS1A gene and phenotypic antibiotic resistance Disk diffusion test results, so isolates carrying CAS1A gene were less resistant to different antibiotics studied in this research. In contrast, there was no significant correlation between CRISPR / Cas genes, *blaTEM*, and *AcrA* genes at the genetic level.

Keywords: *AcrA* gene, *blaTEM* gene, CRISPR / Cas genes, *Klebsiella pneumoniae* sub spp. *pneumoniae*

INTRODUCTION
The rise and dissemination of antibiotic resistance poses a danger to human well-being. The primary mode of transmission for antimicrobial resistance is horizontal gene transfer (HGT), which results in the dissemination of bacterial resistance to drugs (Tao, 2022). *Klebsiella pneumoniae* is a major cause of nosocomial infections, highlighting its importance in healthcare-associated infections. The World Health Organization has designated *K. pneumoniae* as a "priority pathogen," emphasizing its crucial role in the increasing challenge of antibiotic resistance (Efa, 2020). Considering its widespread frequency and involvement in a variety of disorders, treating Multiple Drug Resistant (MDR) *K. pneumoniae* infections with the proper therapy is essential to limiting the spread of antibiotic resistance (Ragupathi et al, 2020). *K. pneumoniae* is a type of gram-negative bacteria characterized by a highly selective permeability barrier. The limited permeability of their outer membrane is a significant factor contributing to their resistance against numerous antibiotics (Farrag, 2019). Two of the main resistance mechanisms developed by *K. pneumoniae* are the production of extended-spectrum β-lactamase (ESBL) like *blaTEM* and efflux pump genes like *AcrA* gene (Abid, 2022; Raouf, 2023). Bacteria have evolved different ways to immune itself against evading pathogens like bacteriophage and plasmids by what is called the CRISPR-Cas system (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR associated proteins) (Newsom, 2020). This system serves as a natural immune system...
for more than 50% of bacteria and most of the archaea against invading genetic elements (Brooker, 2018; Ishino, 2018). These foreign invading genetic elements are recognized, captured, and preserved as pieces of foreign genetic material within the bacterial chromosomes by CRISPR-Cas system. Medina-Aparicio (2018) states that the CRISPR-Cas system consists of two main components: the CRISPR array which is the first part consists of brief DNA repetitions, usually spanning from 22 to 56 nucleotides, that are interspersed with unique sequences, derived from mobile genetic elements (MGEs) like bacteriophages, plasmids, or transposons (Medina, 2018; Alkompoz, 2023). Transcription of the CRISPR arrays leads to, the generation of CRISPR RNA (crRNA). The crRNA functions as a navigational tool for the second part of the CRISPR-Cas system which is one or more Cas operons which regulate Cas proteins productions (Hille, 2018). The Cas proteins, namely Cas1, Cas3, and Cas9, are pivotal in recognizing and cleavage of foreign DNA (Brooker, 2018). The CRISPR/Cas system is classified into two separate main classes, which are further organized into six types and around forty subtypes. Within this theoretical framework, it has been noted that individuals in Class 1 employ a combination of multi-Cas effector proteins to disrupt invading DNAs, while members of Class 2 rely on a single effector protein for the same purpose (Newsom, 2020). The categories are based on the distinct Cas proteins that carry out the crucial function of cutting invading nucleic acids. This wide range is categorized into distinct classifications: Class 1 of CRISPR/Cas encompasses types I, III, and IV, whereas Class 2 comprises types II, V, and VI (Makarova, 2017). Each type is distinguished by a specific gene that encodes the Cas protein associated with its respective function. The signature and almost universal protein in almost all bacteria within each class is CAS1 with its two subtypes CAS1 A and CAS1 B (Makarova, 2015), other effector proteins and their genes were discovered later and are present in different subtypes of CRISPER-Cas system and include, CAS3 protein in type I, CAS 9 protein in type II, CAS10 protein in type III, CSF1 protein in type IV, CAS12 protein in type V, and CAS13 protein in type VI (Makarova, 2018). The taxonomy further extends to subtypes based on the intricate architecture of the CRISPR/Cas locus, a categorization that has evolved to capture the intricacies of this versatile system (Newsom, 2021).

Several studies conducted in the past decade have shown a negative relationship between the occurrence of the CRISPR/Cas system in K. pneumoniae, and the development of antibiotic resistance (Kamruzzaman, 2020; Wang, 2020; Jwair, 2023). Nevertheless, the findings in this domain have occasionally been contradictory, thereby highlighting the evident requirement for further investigation in this area (Touchon, 2012; Shabbir, 2018; Alkompoz, 2023). Exploring the relationship between the presence of CRISPR genes and antibiotic resistance factors, such as ESBLs and drug efflux genes, in K. pneumoniae, could offer valuable knowledge on potential therapeutic targets for combating infections caused by this resistant bacterium. Therefore, the objective of this work was to establish the correlation between the CRISPR/Cas systems and the development of antibiotic-resistance, as well as the synthesis of extended range β-lactamase genes, namely blaTEM gene and efflux pump ArcA gene among K. pneumoniae isolates, obtained from hospitalized patients in Mosul city / Iraq.

MATERIALS AND METHODS

Study area

A cross-sectional study was conducted at Al-Salam and Al-Jumhooree Teaching Hospital in Mosul city during six-month period from February 2023 to August 2023.

Patients

All ages and both sexes were included in this study, and the history of each patient, including their name, age, gender, and the type of specimen, was obtained. Additionally, it was ensured that infected patients included in the study were not under treatment, specifically no antibiotic intake for 3 days prior to specimen collection for culture.

Ethical approval

Ethical approval for this study was obtained from the Iraqi Ministry of Health/Mosul Health Department, with an assigned approval letter, No. 9295, dated 19th February 2023.

Sample collection

A total of 230 different clinical samples were collected. All samples were cultured on MacConkey, Eosin Methylene Blue, Blood agar, and Brain Heart Infusion broth (BHI), then incubated overnight at 37 °C. Colonies that appeared were tested for oxidase, catalase, and urease production, as well as biochemical reactions for exact strain identification, as confirmed by the Vi-TEK®2 GN ID card.

Antimicrobial susceptibility test

The test was carried out by using the Kirby-Bauer antibiotic disc diffusion technique and performed on Muller-Hinton agar. An inoculum isolate was generated by emulsifying the colonies from an overnight culture in sterile normal saline until they reached the same level of cloudiness as the 0.5 McFarland solution standard. The bacterial suspension was evenly spread on the Muller-Hinton agar using a sterile swab and let to dry. The antibiotic discs have been placed on the plate using sterile forceps. The plates were placed in an incu-
bator set at a temperature of 37°C for a duration of 24 hours. The width of the zone that inhibited growth was measured, and the results were analyzed using the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) in 2022. These antibiotic discs came in the following types and concentrations: 10µg/disc of imipenem, 30 µg/disc of augmentin, 30 µg/disc of amikacin, 30 µg/disc of tetracycline, 10 µg/disc of ceftriaxone, and 10 µg/disc of gentamicin, chloramphenicol 10 µg/disc, cefotaxime 10 µg/disc, ceftazidime 30 µg/disc, and colistin 10 µg/disc.

DNA extraction for conventional PCR detection for antibiotic-resistance genes (blaTEM and ArcA efflux pump gene) and CRISPR-Cas genes (CAS1A and CAS1B)

DNA was extracted by a commercial kit (Geneaid company, Taiwan) according to the manufacture procedure. The PCR conditions were as follows: Initial denaturing at 94 °C for 5 min followed by 30 cycles, each cycle contained 1 min at 94 °C for denaturation, 30 s for annealing and the annealing temperature as in Table 1 and 60 S for extension steps and finally one cycle for the final extension at 72 °C for 10 min. All primers information's are given in Table 1.

Data analysis
The statistical analyses were conducted using IBM SPSS statistics software version 25.0 (IBM Corp., Armonk, NY, USA). Data were described as tables, charts and diagrams. Statistical significance was defined as p-values less than 0.05. The t-test values were utilized to compare categorical variables. The Pearson correlation coefficient factor (r) was computed for correlation analysis.

RESULTS AND DISCUSSION

Frequency of Klebsiella spp among different clinical samples
Of the 230 clinical isolates investigated in this study, 33 samples contained klebsiella spp. Specifically, there were 11 isolates (33.3%) from urine, 7 isolates (30.3%) from pus, 10 isolates (21.2%) from sputum, 2 isolates (6.06%) from tracheostomy, 1 isolate (3.03%) from pleural fluid, 1 isolate (3.03%) from CSF, and 1 isolate (3.03%) from Foley’s catheter, as illustrated in Fig. 1. The most frequent sample that contains K. pneumoniae in this study was urine 33.3% this result is generally consistent with numerous studies conducted locally in Iraq, as in a study done in Baghdad City by Al-Saady (Al-Saady, 2023), showed that the most frequent sample that contained Klebsiella spp was from urine 37 (37%) out of 108 isolates. Other local studies done in Duhok City and Erbil agreed with this study, in which the most frequent sample containing Klebsiella was urine samples 66% and 56% respectively (Naqid, 2020; Nawaz, 2009), whereas other studies done in different countries like China, Iran and Indonesia by (Wang, 2019; Karimi, 2021) showed that upper respiratory tract samples were the most frequent sites of K. pneumoniae infections.

Antibiotic-resistant pattern of Klebsiella pneumoniae
All K. pneumoniae isolates in this investigation exhibited multidrug resistance, the highest level of resistance observed against tetracycline, colistin, and ceftriaxone, with a 100% resistance rate, and the lowest resistance to imipenem. The antibiotic-resistance pattern of the isolates was determined by performing disc diffusion using the Kirby Bauer method on Muller Hinton agar. The diameter of the inhibition zone was measured and

Table 1. Informations about primers used, including their sequences and amplicons’ size

<table>
<thead>
<tr>
<th>Name of the gene</th>
<th>Sequence</th>
<th>Annealing temperature</th>
<th>Amplicon size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS1 type A</td>
<td>F CGAAACGCTACGGGTGTGAAA&lt;br&gt;R CGGAATCATAATTGCTGTCA</td>
<td>49 °C</td>
<td>341</td>
<td>Li H Y,2018</td>
</tr>
<tr>
<td></td>
<td>F CGGCTGGAAATTGATGACAG&lt;br&gt;R ATCCGGAAAGCGTTPAGCA</td>
<td>50 °C</td>
<td>309</td>
<td>Li H Y, 2018</td>
</tr>
<tr>
<td>CAS1 type B</td>
<td>F GCAACGCTACGGGTGTGAAA&lt;br&gt;R CGGAATCATAATTGCTGTCA</td>
<td>63°C</td>
<td>180</td>
<td>This article</td>
</tr>
<tr>
<td>blaTEM</td>
<td>F ATCAGCGCCGCGATTGTTAAA&lt;br&gt;R CCGTTTGGGAAATAGCAG</td>
<td>56 °C</td>
<td>312</td>
<td>Wasfi R, 2016</td>
</tr>
<tr>
<td>AcrA</td>
<td>F CGAAGACGCTACGGGTGTGAAA&lt;br&gt;R CGGAATCATAATTGCTGTCA</td>
<td>49 °C</td>
<td>341</td>
<td>Li H Y,2018</td>
</tr>
<tr>
<td></td>
<td>F CGGCTGGAAATTGATGACAG&lt;br&gt;R ATCCGGAAAGCGTTPAGCA</td>
<td>50 °C</td>
<td>309</td>
<td>Li H Y, 2018</td>
</tr>
</tbody>
</table>

Fig. 1. Percentage of Klebsiella spp isolates from different clinical samples
compared to the CLSI (2022) guidelines. The results were as follows: The bacteria exhibited complete resistance (100%) to tetracycline, colistin, and ceftriaxone. There was a resistance rate of 82.61% to augmentin, 65.21% to amikacin, 39.13% to cefotaxime, 34.78% to ceftazidime, and 13.04% to both chloramphenicol and ciprofloxacin. However, no resistance (0%) was observed to imipenem, as indicated in Table 2.

In the present study, all clinical isolates were resistant to more than three classes of antibiotics; this was in agreement with other studies on *K. pneumoniae* resistant to multiple drugs (Farhadi, 2021; Vaez, 2019; Moghadas, 2018; Odari, 2022; Zhu, 2023). While other studies showed lesser prevalence of MDR *K. pneumoniae* like that done on 100 clinical isolates of *K. pneumoniae* showed only 58% of the isolates were MDR (Farhadi, 2021), the reason for this discrepancy may be due to differences in geographical areas, strain types, hygiene level, specimens collected, study date, sample size, and antibiotic usage limitations.

Molecular method for detection of the prevalence of *blaTEM* and *AcrA* efflux pump genes among the *Klebsiella pneumoniae* clinical isolates

The study showed that all isolates were positive for *blaTEM* antibiotic-resistant gene, as shown in Fig. 2.

**Fig. 2 (A,B,C).** 2% agarose gel electrophoresis at 75 volte for 50 minute had run for PCR products of *blaTEM* gene in 23 isolates of *Klebsiella pneumoniae* sub spp pneumoniae with its length 180 bp. A) showing the PCR products of *blaTEM* in first 10 isolates, B) showing the PCR products of *blaTEM* in second 10 and C) showing the PCR products of *blaTEM* in last 3 isolates
(A,B,C) and for ArcA efflux pump gene 21 (91%) were positive and 2 (8.69%) were negative, as in Fig. 3 (A, B, C).

The _blaTEM_ was positive in all isolates; this was in agreement with other studies as in other cities in Iraq (Pishtiwan, 2019), in Sudan (Dirar, 2020) and in Iran (Kashefieh, 2021). In contrast, other global studies done in different countries showed _blaCTX-M_ was the most common ESBL enzymes (Al-Garni, 2018; Sewunet, 2021; Carvalho, 2021)

The _AcrA_ efflux pump is a periplasmic protein encoded by _AcrA_ gene, which in this study was positive in 91% of isolates. This result was approximate to other studies done on MDR _K. pneumoniae_ in Iraq, like that of Abid (2023) in Diwaniyah province and another study done by Khalid (2022) in Baghdad, which showed 100% presence of _AcrA_ in all clinical isolates of _K. pneumoniae_. The efflux pump system is a highly significant mechanism of antibiotic resistance in different bacterial species. Numerous global research studies have verified the significance of these pumps in augmenting the resistance of _K. pneumoniae_ to various antibiotic clas-
Contrary to other studies which showed a lesser prevalence of CRISPR–Cas system among 
Klebsiella pneumoniae in China with a percentage equal to (14.9 and 21.3%) of their 
collections (Wang, 2020; Liao, 2020). There was no significant correlation between the 
CRISPR–Cas system and the type of specimens, which agreed with other studies in Egypt 
(Alkompoz, 2023) and another study in China (Li, 2018).

CRISPR-Cas system among clinical isolates of Klebsiella pneumoniae and its correlation with 
anti-biotic-resistant

There was a strong inverse relationship between the existence of CRSIPR–Cas system, especially 
CAS1A gene and the resistance to different antibiotics that were tested in this study at the 
phenotypic level (results of antibiotic resistance by Kirby-Bauer Disc diffusion test), as the 
Pearson correlation factor (r) value

Table 2. Antibiotic susceptibility test results against Klebsiella pneumoniae

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>R NO. / %</th>
<th>I NO. / %</th>
<th>S NO. / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Augmentin AMC (30) µg</td>
<td>19 (82.61%)</td>
<td>2 (8.69%)</td>
<td>2 (8.69%)</td>
</tr>
<tr>
<td>Amikacine AK (30) µg</td>
<td>15 (65.21%)</td>
<td>1 (4.34%)</td>
<td>7 (30.43%)</td>
</tr>
<tr>
<td>Ciprofloxacin CIP (10) µg</td>
<td>3 (13.04%)</td>
<td>2 (8.69%)</td>
<td>18 (78.26%)</td>
</tr>
<tr>
<td>Tetracycline TE (30) µg</td>
<td>23 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Chloramphenicol C (10) µg</td>
<td>3 (13.04%)</td>
<td>4 (17.39%)</td>
<td>16 (69.56%)</td>
</tr>
<tr>
<td>Colistin CL (10) µg</td>
<td>23 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cefotaxime CTX (10) µg</td>
<td>9 (39.13%)</td>
<td>5 (21.73%)</td>
<td>9 (39.13%)</td>
</tr>
<tr>
<td>Imipenem (10) µg</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>23 (100%)</td>
</tr>
<tr>
<td>Ceftriaxone CRO (10) µg</td>
<td>23 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cefazidime CAZ (30) µg</td>
<td>8 (34.78%)</td>
<td>9 (39.13%)</td>
<td>6 (26.08%)</td>
</tr>
</tbody>
</table>

Frequency of CAS1 (A andB ) genes in the isolated Klebsiella pneumoniae sub spp pneumoniae and the correlation of CRISPR-Cas system with the type of specimen

The presence of CAS1A and CAS1B was examined in all isolates of Klebsiella pneumoniae sub spp pneumoniae, and their correlation with the presence of antibiotic resistance genes in the same isolates was evaluated. CAS1A was identified in only 5 out of 23 samples, accounting for 21.73% of the total. The remaining 18 samples, or 78.26%, tested were negative for CAS1A. On the contrary, CAS1B tested negative in just 2 (8.69%) isolates and positive in the remaining 21 (91%) isolates, as shown in Fig. 4, 5.

CAS1 gene is present in all CRISPR/Cas types, (Makarova, 2015). CAS1A was less prevalent than CAS1B in Klebsiella pneumoniae clinical isolates, consistent with a study done in Baghdad/ Iraq (Ali, 2022). Contrary to other studies which showed a lesser prevalence of CRISPR –Cas system among Klebsiella pneumoniae in China with a percentage equal to (14.9 and 21.3%) of their collections (Wang, 2020; Liao, 2020).

There was no significant correlation between the CRISPR-Cas system and the type of specimens, which agreed with other studies in Egypt (Alkompoz, 2023) and another study in China (Li, 2018).

Table 2. Antibiotic susceptibility test results against Klebsiella pneumoniae

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>R NO. / %</th>
<th>I NO. / %</th>
<th>S NO. / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Augmentin AMC (30) µg</td>
<td>19 (82.61%)</td>
<td>2 (8.69%)</td>
<td>2 (8.69%)</td>
</tr>
<tr>
<td>Amikacine AK (30) µg</td>
<td>15 (65.21%)</td>
<td>1 (4.34%)</td>
<td>7 (30.43%)</td>
</tr>
<tr>
<td>Ciprofloxacin CIP (10) µg</td>
<td>3 (13.04%)</td>
<td>2 (8.69%)</td>
<td>18 (78.26%)</td>
</tr>
<tr>
<td>Tetracycline TE (30) µg</td>
<td>23 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Chloramphenicol C (10) µg</td>
<td>3 (13.04%)</td>
<td>4 (17.39%)</td>
<td>16 (69.56%)</td>
</tr>
<tr>
<td>Colistin CL (10) µg</td>
<td>23 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cefotaxime CTX (10) µg</td>
<td>9 (39.13%)</td>
<td>5 (21.73%)</td>
<td>9 (39.13%)</td>
</tr>
<tr>
<td>Imipenem (10) µg</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>23 (100%)</td>
</tr>
<tr>
<td>Ceftriaxone CRO (10) µg</td>
<td>23 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cefazidime CAZ (30) µg</td>
<td>8 (34.78%)</td>
<td>9 (39.13%)</td>
<td>6 (26.08%)</td>
</tr>
</tbody>
</table>

Fig. 4. 2% agarose gel electrophoresis at 75 volte for 50 minute run for PCR products of CAS1A and CAS1B gene in first 5 Klebsiella pneumoniae sub spp pneumoniae isolates. CAS1A gene PCR products size was 341 bp and CAS1B gene PCR products size was 309bp.

Figures and tables should be included in the main text for a complete understanding of the research findings.
was equal to -1 at p level < 0.00001. The isolates which carry CRISPR-Cas system had lower resistance to ciprofloxacin, amikacin, chloramphenicol, cefotaxime, ceftazidime, and augmentin, as shown in Fig. 6.

In contrast, there was no significant correlation between antibiotic resistance in both CAS1B+ve and CAS1B-ve clinical isolates, as CAS1B gene was detected in almost all isolates except 2 isolates, which were negative for CAS1A too, which indicates the absence of CRISPR-Cas system in these two isolates.

There was no significant correlation in the distribution of antibiotic-resistant genes (ARGs), namely blatem and AcrA genes, and CAS1A and CAS1B gene results in studied clinical isolates. The Fisher exact test statistic value was equal to 1 at p value < 0.05. The antibiotic-resistant genes were more found in CAS1B+ isolates than among CAS1A+ isolates, as in Fig. 7.

The correlation between CRISPR-Cas system and antibiotic resistance is still under study and varies from one research to another, some researches said there is no significant correlation (Alkompoz, 2023) and some said there is a negative correlation between the presence of CRISPR-Cas system and antibiotic susceptibility (Jwair, 2023; Ali, 2022). Prior research has shown that *K. pneumoniae* strains possessing type I CRISPR system exhibit a significant abundance of tetracycline resistance genes while displaying increased susceptibility to aminoglycoside and β-lactam antibiotics and a reduced presence of related resistance genes (Tao, 2022). A few resistant strains containing the CRISPR-Cas system were considered to be antibiotic-resistant strains and this may be due to the mutation of the original spacer sequence, the partial or total deletion in the Cas gene cluster, and the presence of anti-CRISPR proteins (Basgall, 2018; Guo, 2017).

**Gene sequencing by Sanger sequencing method**

After completing gene detection by conventional PCR method for the previously mentioned genes in the studied clinical isolates, PCR products of genes were sent...
for gene sequencing Sanger method together with forward primer of each of 4 genes and compared with National Center Biotechnology Information (NCBI) gene bank and all were 100 % identical strains, all the tested isolates carrying type I-E CRISPR-CAS system.

**Conclusion**

Most clinical isolates of *K. pneumoniae* included antibiotic resistance genes, specifically *blaTEM* and *AcrA* genes. The prevalence of *blaTEM* genes was higher than that of *AcrA* genes, with percentages of 100% and 91%, respectively. All studied *K. pneumoniae* isolates exhibited solely the Type I-E CRISPR-Cas system, with the CAS1A gene being less prevalent than the CAS1B gene. In this study, a strong inverse relationship was observed between the presence of the CRISPR-Cas system, particularly the CAS1A gene, and resistance to various antibiotics tested at the phenotypic level using the Kirby-Bauer Disc diffusion test. However, no significant correlation was found at the genotypic level. These findings suggest the need for further research on the relationship between the CRISPR-Cas system and other antibiotic resistance genes. The practical potential of the system lies in its ability to prevent and control horizontal gene transfer, hence reducing the dissemination of antibiotic resistance. This may potentially lead to the development of methods, such as immunotherapy, to prevent and control horizontal gene transfer.

**Conflict of interest**

The authors declare that they have no conflicts of interest.

**REFERENCES**


7. Carvalho I., Carvalho J.A. & Martínez-Álvarez S. (2021). Characterization of ESBL- Producing Escherichia coli and *Klebsiella pneumoniae* isolated from clinical samples in a Northern Portuguese Hospital: Predominance of CTXM-
15 and high genetic diversity. Microorganisms, 9(9), 1914. doi:10.3390/microorganisms9091914


828


