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The Occurrence of CRISPR-encoding Genes in Extensively Drug-resistant *Escherichia Coli* Causing Urinary Tract Infection

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ABSTRACT

Background: The rapid proliferation of antimicrobial resistant superbugs, particularly multi-drug resistant (MDR) and extensively drug-resistant (XDR) *Escherichia* (*E. coli*), is a significant global public health issue.
Objectives: To investigate the prevalence and clinical relevance of CRISPR systems in MDR and XDR *E. coli* isolates from urinary tract infections (UTIs) and their association with antimicrobial resistance patterns.
Materials and methods: Eighty *E. coli* culture-positive clinical specimens from patients with UTIs were identified using the VITEK® 2 Compact System and submitted to antibacterial sensitivity surveillance using selected Gram-negative cards according to CLSI guidelines. *E. coli* ATCC 25922 was used as a quality control strain. CRISPR-2, -3, and -4 gene detection was performed using the polymerase chain reaction (PCR) method.
Results: Out of 120 clinical specimens, 80 yielded positive cultures, including 45 (56.25%) resistant *E. coli* isolates. Among these, 32 (71%) were MDR, 12 (26.7%) XDR, and one (2.2%) non-MDR. CRISPR-2, -3, and -4 were detected in 66.7%, 82.2%, and 22.2% of *E. coli* isolates, respectively. Co-occurrence of CRISPR-2 and -3 was found in 60%, CRISPR-3 and -4 in 22.2%, and CRISPR-2 and -4 in 15.6% of isolates. In MDR strains, CRISPR-2 and -3 were common, while CRISPR-4 was rare. In XDR isolates, the frequencies of CRISPR-2, -3, and -4 were 22.7%, 25%, and 20.5%, respectively.
Conclusion: CRISPR-2 and CRISPR-3 are strongly associated with MDR *E. coli*, while their reduced presence in XDR strains may indicate CRISPR loss or suppression during resistance evolution, suggesting a role in bacterial adaptation.
Keywords: CRISPR/Cas Systems; Multidrug-resistant *Escherichia coli*; Urinary tract infections; Antimicrobial resistance.

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INTRODUCTION

E. coli is the most recognized constituent of the human intestinal habitat. Bacterial infections caused by *E. coli* constitute a significant global issue in both clinical and community settings [1]. Among the more common uropathogens associated with the development of urinary tract infection (UTI) is uropathogenic *E. coli* (UPEC) [2]. When antibiotics are administered to eradicate of microbial infection, commensal *E. coli* resistance can develop after exposure [3]. This bacterium is a major reservoir for the transmission of antimicrobial resistance (AMR) to other pathogenic bacteria through plasmid exchange [4].

A worrying rise in antibiotic resistance among bacteria has been observed, potentially resulting in community-

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acquired or hospital-acquired illnesses. The multidrug resistance (MDR) pathogen *E. coli* is of particular importance [5]. The uropathogenic *E. coli* infection is categorized as MDR if it exhibits resistance to a minimum of three distinct classes of antibiotics, and as extensively drug resistance (XDR) if it is susceptible to only one class of antibiotics [6]. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) loci were first recognized over two decades ago and have since been discovered in the genomes of several bacteria [7]. The loci, together with their corresponding CAS proteins, constitute a system referred to as CRISPR/Cas, which is characterized as conferring adaptive immunity to bacteria by targeting harmful or detrimental invading deoxyribonucleic acid (DNA), including phages or plasmids [8]. CRISPR loci comprise short repeats of 21-47 bp intermingled by non-repeating sequences of comparable length known as spacers [9].

It is well recognized that repeat arrays are often, but not invariably, associated with CAS genes, which encode proteins crucial for the functionality of the CRISPR/Cas system [10]. This leads to an improved genome modification capability. Recent studies revealed a link between existence of CRISPR sequences and diminished antibiotic resistance, indicating that CRISPR presence inhibits the adaptability of the bacterium [11]. There is a huge controversy in the literature regarding the association of CRISPR/Cas system in problematic bacterial species causing medically important infection in addition for the presence or absence of this system in MDR and XDR bacterial species. Therefore, this study aims to identify the incidence of MDR and XDR *E. coli* in patients having UTIs, as well as to investigate the co-occurrence of CRISPR/Cas types in these resistant isolates.

MATERIALS AND METHODS

The study design was cross-sectional. It was performed on 120 clinical specimens obtained from patients suffering from UTIs admitted to Ramadi Teaching Hospital and Ramadi Teaching Hospital for Maternity and Children, Ramadi City, Iraq. The study was conducted during the period from January to December, 2024. The study protocol received approval from the Ethical Approval Committee of the University of Anbar with reference number 205. Informed consent was obtained from all patients participating in the study.

According to Krejcie and Morgan's equation and standard table, the required sample size was calculated to be 92. Out of 120 collected specimens, only 80 culture-positive cases were obtained during the study period, reflecting the high and deliberate selectivity of the required samples.

In study patients, a UTI diagnosis generally relies on a combination of clinical symptoms, such as renal or lower abdominal pain, dysuria, fever, and an urgent need to urinate, as well as urinalysis findings. An expert urologist makes the diagnosis. In the presence of leukocytes in urine in more than 5/HPF (high power field) of the microscope, urine culture was performed for a definitive diagnosis of significant bacteruria.

The inclusion criteria of the study encompassed all study patients suspected of UTIs attending the assigned hospitals. Urine cultures were performed at the hospital laboratories. The exclusion criteria were as follows: anaerobic bacterial infections, fungal infections, polymicrobial growth, p and the presence of non-pathogenic microbes in addition to patients who had taken antibiotics within the last 72 hours. The urine clinical specimens have been obtained and collected using a sterile urine container and cultured immediately under aseptic conditions.

Bacteriological recognition of *E. coli* isolates

The microbiological laboratories of the selected hospitals managed the receiving and culturing of the specimens, in addition to performing all bacteriological and chemical investigations for further confirmation. MacConkey agar, Eosin methylene blue agar and Nutrient agar (Dams, Germany) have been used to culture all specimens, incubated for 24 to 48 hours at 37°C. Standard microbiological and biochemical techniques for *E. coli*, including the catalase test and methyl red test [12], were applied. *E. coli* isolates were unequivocally identified utilizing VITEK®2 GN ID cards in addition to VITEK®2 Compact B System (BioMérieux, France) [13].

Antimicrobial susceptibility testing

According to the manufacturer's instructions, the isolates underwent antimicrobial susceptibility testing (AST) utilizing the VITEK®-2 Compact B System with AST-GN cards. Antimicrobial susceptibility testing was conducted for the following antibiotics: Ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, imipenem, meropenem, aztreonam, gentamicin, tobramycin, amikacin, ciprofloxacin, pefloxacin, minocycline, rifampicin, trimethoprim/sulfamethoxazole, and colistin. Results were presented as minimal inhibitory concentrations (MICs) utilizing the VITEK® 2 Compact B System (BioMérieux, France), and the Clinical and Laboratory Standards Institute's (CLSI) recommendations were employed to determine bacterial susceptibility, intermediate susceptibility, or resistance [14]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has published guidelines and criteria for the interpretation of tigecycline susceptibility [15]. *E. coli* American Type Culture Collection (ATCC 25922) has been used as an international quality control to guarantee the integrity of the antibiotic susceptibility tests.

The isolates in the study were classified as MDR, XDR, and PDR according to the criteria mentioned above. Moreover, the unique AST card provided the processes contributing to resistance against the selected antimicrobial drugs [16].

Molecular part of the study

Extraction of DNA

SaMag-12TM, a system manufactured by SaMag, a Cepheid company, Italy, was used for automated genomic DNA extraction. The purity and concentration of genomic DNA were assessed using a QuantusTM Fluorometer (Promega, USA) [17].

CRISPR/Cas encoding genes amplification by polymerase chain reaction (PCR)

The PCR approach has been employed to identify the CRISPR/Cas encoding genes utilizing suitable primers (Table 1). The initial denaturation for Cas2 happened at 95°C for 2 minutes, followed by denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 1 minute, ending with a final extension at 72°C for 5 minutes, and a hold at 4°C for 5 minutes during the PCR cycle. A total of 35 cycles comprising denaturation, annealing, and extension were performed. The PCR consisted of initial denaturation for Cas3 and Cas4, which occurred at 95°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. Furthermore, it was finalized by final

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Table 1. The sequences of the primers used in this study.

Primer Name	Encoding genes	Primer sequence	Molecular size (base pair)	Annealing temperature
CRISPR2	ygcFygcE	F-GTCGATGCAAACACATAAATA R-AAATCGTATGAAGTGATGCAT	–	50
CRISPR3	clpA	F-GCCCACCATTCACCTGT R-GCGCTGGATAAAGAGAAAAAT	776	50
CRISPR4	infA cys4	F-GTACGACCTGAGCAAAG R-CTGAACAGCGGACTGATTTA	944	53

Table 2. The pattern of antimicrobial susceptibility for resistant clinical study isolates of *E. coli* using antimicrobial susceptibility test cards from the Vitek system.

Antimicrobial agent	Sensitive Number(%)	Intermediate resistant Number(%)	Resistant Number(%)
Ticarcillin	1 (2.2)	0.0	44 (97.8)
Ticarcillin/clavulanic Acid	27 (60.0)	1 (2.2)	17 (37.7)
Piperacillin	9 (20.0)	0.0	36 (80.0)
Piperacillin/tazobactam	34 (75.6)	1 (2.2)	10(22.2)
Ceftazidime	16 (35.6)	1 (2.2)	28 (62.2)
Cefepime	24 (53.3)	1 (2.2)	20 (44.4)
Aztreonam	14 (31.1)	0.0	31 (68.9)
Imipenem	37 (82.2)	3(6.7)	5 (11.1)
Meropenem	35 (77.8)	3 (6.7)	7 (15.5)
Amikacin	36 (80.0)	9 (20.0)	0.0
Gentamicin	24 (53.3)	0.0	21 (46.7)
Tobramycin	26 (57.8)	0.0	19 (42.2)
Ciprofloxacin	11 (24.4)	1 (2.2)	33 (73.3)
Pefloxacin	33 (73.3)	0.0	12 (26.7)
Minocyclin	27 (60.0)	8 (17.8)	10 (22.2)
Colistin	30 (66.7)	15 (33.3)	0.0
Rifampicin	33 (73.3)	0.0	12 (26.7)
Trimethoprim/sulfamethoxazole	10 (22.2)	0.0	35 (77.8)

extension at 72°C for 5 minutes and a hold at 4°C for 5 minutes. Subsequently, agarose gel electrophoresis using a 1.5% agarose gel involving 10% ethidium bromide was used. The established protocol involved performing electrophoresis in a 1X Tris/Borate/EDTA (TBE) buffer at 50 V for 5 minutes, followed by an increase to 100 V for 1 hour. The anticipated dimensions of the PCR amplicon band can be verified using a DNA ladder with 100 base pairs. An ultraviolet transilluminator (Vilber Lourmat, Marne-la-Vallée Cedex 3, France) was employed for the comparative analysis of the PCR product bands. The primers used in the PCR for identifying the frequency of CRISPR/Cas system components are specified in Table 1.

Statistical analysis

Statistical analysis was conducted using version 22 of the Statistical Package for the Social sciences (SPSS) software (IBM Corp., Armonk, NY, USA). An independent t-test was used to compare the means between study groups, while the chi-square test was used for analyzing categorical variables (e.g., resistance type comparisons). A P-value of < 0.05 was considered indicative of a statistically significant difference. Effect size estimation was performed using Cohen's d, which is calculated as:

$$\mathbf{d = M1–M2/SD\ pooled}$$

Where M1 and M2 are the means of the two groups, and SD pooled is the pooled standard deviation. The interpretation of Cohen's d was as follows: Small effect (d = 0.2), medium effect (d = 0.5), and significant effect (d $\geq$ 0.8). Additional tools, such as R or Python, were used for data visualization, and GraphPad Prism was utilised for supplementary analyses and figure generation.

RESULTS

The study patients' age ranged from 15 to 45 years and were divided into 42 (35%) males and 78 (65%) females, with a male-to-female ratio of 1:1.8.

Susceptibility to antimicrobial agents

Of the 120 clinical specimens, 80 positive culture specimens yielded. Of these, 45 (56.25%) were resistant *E. coli*, 10 (12.5%) represented sensitive isolates of *E. coli* to all study antimicrobial agents, while 12 (15%), eight (10.0%), and five (6.25%) were *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. The remaining specimens showed no growth of pathogenic aerobic microorganisms or were sterile. According to CLSI criteria, the study clinical isolates were classified according to their responses to antimicrobial agents as follows: 32 (71%) were MDR and 12 (26.7%) were XDR. Only one isolate (2.2%) was non-multidrug resistant (non-MDR).

According to the CLSI, the pattern of antimicrobial susceptibility tests for the selected antimicrobial agents among the 44 resistant clinical study isolates of *E. coli* using the AST cards in the Vitek system is represented in Table 2. It is important to remember that an international quality control isolate of *E. coli* ATCC 25922 was sensitive to all antimicrobial agents used in this study.

In the molecular part of our study, it was found that after amplification of the ygcFygcE gene, which encodes for CRISPR-2, 28 (63.6%) were found to be positive for the presence of this gene, while the remaining 16 (36.4%) were negative. A range of bands representative of the CRISPR-2 encoding gene were observed, including at 650 bp, 944 bp, 1706 bp, 1902 bp, 1906 bp, 2023 bp, and 3681 bp, as reflected in Figure 1.

It was further found that after amplification of the *CAS* 1 *clpA* gene, 37 (84.1%) of the samples were positive for the presence of this gene, while seven (15.9%) were negative for this CRISPR-3 encoding gene with a base pair at 776 bp (Figure 2).

In CRISPR-4 encoding gene with base pair at 944 bp, it was found that after amplification of the *infA cys*4 gene, 10 (22.7%) of samples were positive for the presence of this gene, while 34 (77.3%) were negative, as reflected in Figure 3.

The study results revealed that CRISPR-2, CRISPR-3, and CRISPR-4 were detected in 30 (66.7%), 37 (82.2%), and 10 (22.2%) samples of *E. coli*, respectively. The co-existence of both CRISPR-2 and CRISPR-3 was dominant in MDR (64.3% of MDR isolates). CRISPR-3 and CRISPR-4 combinations were more frequent in XDR (58.3% of XDR isolates). Furthermore, CRISPR3 was most prevalent in both MDR (81.2%) and XDR (91.7%). CRISPR4 prevalence was significantly higher in XDR (75%) compared to MDR (21.9%) with a P-value of 0.002. It is interesting to report that CRISPR-2 and CRISPR-3 showed strong positive associations with MDR (P-value < 0.05), while CRISPR-4 correlated with XDR (P-value $= 0.002$). Effect sizes indicate moderate to considerable clinical significance (Figure 4).

It is essential to take note of Figure 5, in which the study distributed the CRISPR-positive and negative cases among resistant *E. coli* isolates for the 12 selected antimicrobial agents (as previously described). It is essential to note that the P-value = 0.00026 refers to the presence of a highly significant difference between the existence and absence of CRISPR-2 in resistant study isolates of *E. coli*.

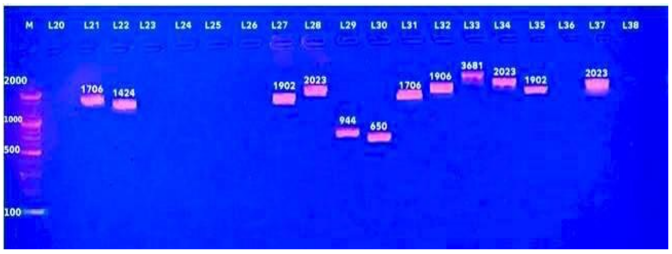

Figure 1. Polymerase chain reaction result of CRISPR-2 gene sequences (Variable base pairs). M denotes a deoxyribonucleic acid marker comprising 100 bases. Various *E. coli* isolates, each possessing distinct CRISPR systems, are designated by numerical characters. The electrophoresis was performed on 1.5% agarose at a field gradient of 5 V/cm.

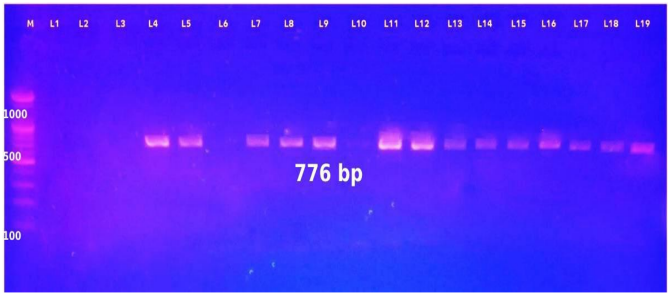

Figure 2. Polymerase chain reaction result of CRISPR-3 gene sequences (776 bp). M indicates a deoxyribonucleic acid marker having 100 bases. Various *E. coli* isolates have unique CRISPR, which are represented by the assigned numbers. The electrophoresis was performed on 1.5% agarose at 5 V/cm.

Figure 3. Polymerase chain reaction product of CRISPR-4 encoding genes (944 bp). M denotes a deoxyribonucleic acid marker comprising 100 base pairs. Many *E. coli* isolates possess distinct CRISPR reflected by numerical characters. The electrophoresis was performed on agarose (1.5%) at a field gradient of 5 V/cm.

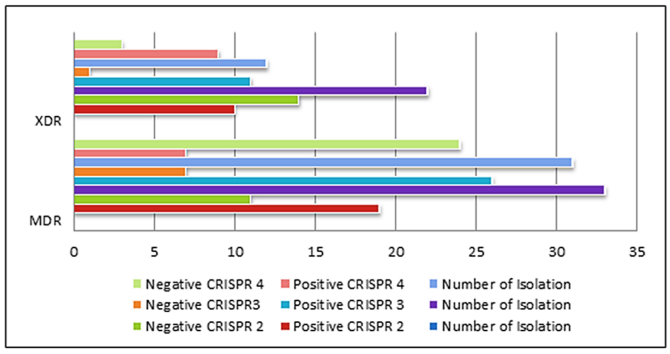

Figure 4. Distribution of positive and negative CRISPR-2, 3, and 4 in two forms: alone and as co-existence form among multidrug-resistant and extensively drug-resistant study isolates of *E. coli*. XDR: Extensively drug-resistant, MDR: Multidrug-resistant.

The most common combination in MDR isolates was CRISPR-2+CRISPR-3 (40.63%), while in XDR isolates, it was higher at 66.67%. Isolates with all three CRISPR genes (CRISPR-2+CRISPR-3+CRISPR-4) were less frequent but exhibited moderate resistance levels (18.2%). Isolates with no CRISPR genes had the lowest average resistance (14.1%). The combination of CRISPR-2 and CRISPR-3 ap-

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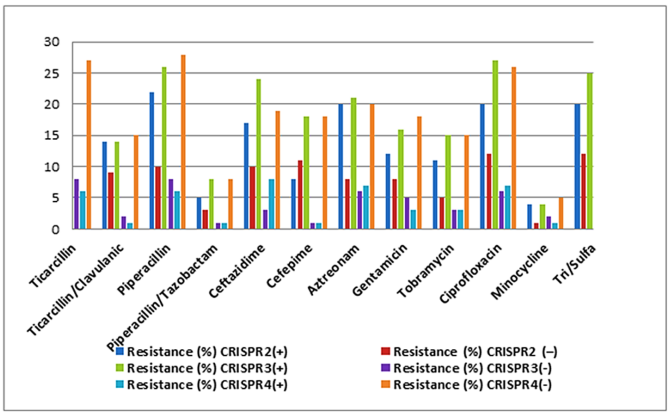

Figure 5. The presence or absence of CRISPR-2 and CRISPR-3 systems among resistant *E. coli* isolates for the selected antimicrobial agents.

pears strongly linked to higher resistance, particularly in XDR isolates, suggesting a synergistic role in resistance mechanisms. However, the presence of all three genes doesn't markedly increase resistance, hinting that CRISPR-4 might not amplify resistance as much as CRISPR-2 or CRISPR-3 (Table 3).

DISCUSSION

Hospitals are now recognized as the primary settings of numerous MDR pathogenic bacteria, including *E. coli* [18]. The uncontrolled and unregulated use of antibiotics for treating infections significantly contributes to this phenomenon by imposing selective pressure on the invading microbe allowing to develop resistance. The spread of resistance encoding genes among bacteria in hospitals and healthcare facilities through various channels significantly enhances the transmission of resistance into the population [11]. To our knowledge, there has been limited research in Arab countries, and this is the first publication in Iraq addressing the CRISPR/Cas system and its correlation with MDR and XDR *E. coli*. The majority of third-generation cephalosporins are ineffective against extended spectrum β-lactamase (ESBL) producing bacteria. The most important bacterium responsible for the creation of ESBLs is *E. coli*. Patients with bacteremia caused by ESBL-producing Enterobacteriaceae isolates exhibited an approximately 57.0% higher risk of mortality compared to those infected with non-ESBL-producing isolates [19].

The research categorized resistant *E. coli* isolates into MDR and XDR, accounting for 71% and 26.7%, respectively. No isolates demonstrating pan-drug resistance have been detected. Recently, a growing number of resistances encoding genes have been identified in clinical isolates of *E. coli*. Multiple studies reveal that both commensal and pathogenic *E. coli* are prevalent in broiler hens, with the majority of isolates demonstrating resistance to ampicillin, tetracycline, ciprofloxacin, nalidixic acid, and sulfamethoxazole-trimethoprim [3].

Of our study isolates, 28 (62.2%) were resistant to ceftazidime, while 20 (44.4%) were resistant to cefepime. The resistance to the above cephalosporins allows for the possibility of ESBL-production. *E. coli* can produce ESBLs, which inactivate antimicrobial agents such as third-generation cephalosporins, which are commonly advised to treat systemic problematic microbial infections [20]. ESBL-producing bacteria may move between humans or from animals to humans via the food chain or direct contact [21]. Three closely linked clusters of broiler and human isolates were identified, indicating that poultry and slaughterhouse farms are major reservoirs of ESBL-producing bacteria. The consequences of *E. coli* infections caused by ESBL-producing isolates in animals remain ambiguous. To successfully manage this hazard, it is crucial to assess the animals' capacity as reservoirs for these pathogens from an approach known as One Health [22].

Recent documentation indicates that prokaryotes adopt adaptive immunity represented by the CRISPR-Cas system to protect bacterial cells from invading pathogens, such as phages and plasmids. Immunity is by seizing tiny DNA pieces, or spacers, from foreign nucleic acids (protospacers) and incorporating them into the host CRISPR locus [23]. In the molecular segment of our analysis, following amplification, the specifically chosen genes associated with CRISPR-2, CRISPR-3, and CRISPR-4 were confirmed as positive in 28 (62.2%), 37 (82.2%), and 10 (22.2%) isolates, respectively. Our study results consistently indicated that the prevalence of CRISPR4 in MDR study isolates was significantly lower than that of CRISPR-2 and CRISPR-3, which were substantially higher. Our findings also showed that the lack of the CRISPR-4 system was adversely correlated with MDR clinical isolates of *E. coli*, consistent with the work of Jawir and colleagues [11]. Tao and colleagues [24] determined that the lack of the CRISPR/Cas system was inversely related to the level of resistance in clinical isolates of *Enterococcus faecalis*, as well as in Enterococcus faecium, and was negatively correlated with the presence of antibiotic resistance genes. The presence of CRISPR-2 and CRISPR-3 may be favorably associated with MDR study isolates. The protospacer of the CRISPR-3 locus is well-documented to have evolved from genes linked to AMR. The results suggest that the CRISPR sequence type may improve antimicrobial resistance in bacteria and refine strategies for addressing the intricacies of antimicrobial resistance in bacterial pathogens [25]. Furthermore, it is proposed that CRISPR/Cas acts as an important constraint to horizontal gene transfer, and the evolutionary mechanisms that ensure its maintenance or drive its loss are key to the pathogen's ability. The evolutionary mechanisms that ensure the maintenance or drive the loss of the CRISPR/Cas system are key to the adaptability of this pathogen. It is proposed that CRISPR/Cas functions as a crucial barrier to horizontal gene transfer. The evolutionary mechanisms that ensure its maintenance or drive its loss are key to the pathogen's ability to adapt to new niches and stressors [26]. The most striking findings when examining antimicrobial resistance pathways in the assigned strains are that the absence of the CRISPR-Cas system and their encoding genes in these isolates was reflected by the existence of huge and significant numbers of resistant genes Furthermore, whole Genome Sequencing of antibiotic resistant *Pseudomonas aeruginosa* can lead to genomic approach fully understanding [27].

To the best of my knowledge, this study represents the first report in Iraq which deals with the CRISPR system among uropathogenic *E coli*. A noteworthy aspect of our study was that the results confirmed earlier findings for CRISPR-2, CRISPR-3, and CRISPR-4, emphasizing their negative link with severe or XDR. *E. coli* study isolates demonstrate an advantageous relationship between CRISPR-2 and, to a lesser degree, CRISPR-3 and diminished MDR. Owaid and

Table 3. Combined CRISPR gene presence and resistance profile*.

CRISPR Combination	MDR Isolates (n=32)	XDR Isolates (n=12)	Average Resistance (%)
CRISPR-2+CRISPR-3+CRISPR-4	5 (15.63%)	2 (16.67%)	18.2
CRISPR-2+CRISPR-3	13 (40.63%)	8 (66.67%)	20.5
CRISPR-2 only	1 (3.13%)	0 (0%)	15.8
CRISPR-3 only	3 (9.38%)	1 (8.33%)	17.3
No CRISPR Genes	3 (9.38%)	1 (8.33%)	14.1

* Average resistance percentage is calculated across the 12 antimicrobial agents used, reflecting the mean proportion of resistance in isolates with each CRISPR combination.

Al-Ouqaili [28] Posited that the CRISPR/Cas system is rarely present in *K. pneumoniae* genomes, and a significant correlation exists between CRISPR/Cas and resistance to antimicrobial drugs. Comparable results were observed for the distribution of antibiotic resistance plasmids and related ARGs, which were also independent of CRISPR/Cas spacer quantities and do not undermine the CRISPR/Cas systems' capacity to inhibit plasmid acquisition. Faure and associates [29] In their study, the authors concluded that the substantially expanding range of CRISPR-Cas components carried by mobile genetic elements and documented that many prokaryotic viruses also carry CRISPR mini-arrays. Some of these are recognised by other viruses and are implicated in inter-virus conflicts, while solitary repeat units could inhibit host CRISPR-Cas systems.

On the other hand, there are two limitations to be considered in this study. First, the sample size was relatively small. This limitation will be addressed in future research, as a small sample size may impact the validity and generalizability of the findings. It may not be representative of the wider population, thereby limiting the ability to detect true associations, such as the presence and influence of CRISPR types in MDR and XDR isolates. Second, the antimicrobial susceptibility tests used in this study assess antimicrobial activity under laboratory conditions (*in vitro*), without for host-related factors. Therefore, the results may not accurately predict clinical outcomes, as an antimicrobial agent that inhibits bacterial growth *in vitro* may not necessarily be effective *in vivo*.

CONCLUSION

This study suggests a strong association between CRISPR-2 and CRISPR-3 loci and MDR *E. coli* strains, indicating that these CRISPR elements may play a role in the development or maintenance of antimicrobial resistance. Conversely, their reduced presence or negative association in XDR strains points to a potential loss or down regulation of these loci as bacteria acquire higher levels of resistance. This pattern may reflect an evolutionary trade-off, where the presence of specific CRISPR systems is diminished to favor the accumulation of resistance genes, possibly through increased horizontal gene transfer. These findings underscore the need for further research to elucidate the functional role of CRISPR elements in resistance evolution. Future studies should explore the regulatory mechanisms governing CRISPR activity in MDR and XDR bacteria, investigate the impact of CRISPR loss on genome plasticity, and assess the potential of targeting CRISPR systems as a novel approach to combat antimicrobial resistance.

ETHICAL DECLARATIONS

Acknowledgments

None.

Ethics Approval and Consent to Participate

The study was approved by the Ethical Approval Committee of the University of Anbar (Reference number 205). Informed consent was obtained from the participants.

Consent for Publication

Not applicable, as no individual data, images, or personal details are included in this manuscript.

Availability of Data and Material

Data generated during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

Funding

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Authors' Contributions

Al-Ouqaili MTS contributed to the conceptualization, supervision, writing, and formal analysis of the study. Jameel NQ was involved in the development of the methodology and performed the statistical analysis. Sabri MM contributed to the manuscript writing. All authors reviewed and approved the final version of the manuscript.

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