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GENOMIC ANALYSIS OF CRISPR-CAS3 (SUBTYPE I-F/YPEST) IN ACINETOBACTER BAUMANNII CLINICAL ISOLATES FROM PAKISTAN: INSIGHTS INTO PHAGE DEFENSE AND ANTIMICROBIAL RESISTANCE

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ABSTRACT

Underlining a major danger to healthcare systems all over, Acinetobacter baumannii is an opportunistic pathogen linked to multidrugresistant infections. CRISPRCas systems especially the Cas3 nuclease/helicase Subtype IF/YPEST significantly contribute to bacterial adaptive immunity against phages and influence horizontal gene transfer, therefore influencing antimicrobial resistance (AMR) profiles. CRISPRCas3 in clinical isolates of A. baumannii from Pakistan was examined to understand its function in phage defense and antibiotic resistance (AMR). Total 50 non duplicate clinical isolates yielded through stratified random sampling. CRISPRCas loci were found using whole-genome sequencing and bioinformatics methods. Phylogenetic research and correlation between CRISPR presence and resistance patterns were done using chi-square and logistic regression analysis. Results showed that 36% of isolates carried CRISPRCas3 Subtype IF/YPEST, with preserved spacers pointing to historical phage exposure. Isolate with CRISPRCas3 showed much less lateral acquisition. These results highlight the double importance of CRISPRCas3 in phage of plasmid-mediated resistance genes ($p < 0.05$), therefore implying a possible protective role against gene transfer. immunity and modulation of AMR offer insights for phage therapy methods and other ways to fight multidrugresistant A. baumannii in clinical environments.

INTRODUCTION:

A Gramnegative, opportunistic bacterium increasingly associated with hospital-acquired infections including bloodstream infections, ventilator-related pneumonia, and wound infections (Peleg et al., 2008). The emergence of multidrugresistant (MDR) strains has complicated treatment options and poses a significant worldwide public health concern (MunozPrice et al., 2013). Ahmed et al., 2020 show a considerable increase in resistance against carbapenems and other last-line antibiotics, hence Pakistan's MDR A. baumannii frequency has been slowly rising.

CRISPRCas systems, which provide protection against foreign genetic material including bacteriophages and plasmids (Barrangou et al., 2007), are among the adaptive immune systems used by bacteria and archaea. Among various kinds, the CRISPRCas3 mechanism (Subtype IF/YPEST) functions as a nuclease/helicase, so targeted degradation of intrusive DNA and eventually limiting lateral gene transfer (Makarova et al., 2015). Lower acquisition of antimicrobial resistance genes in A. baumannii has been linked to CRISPRCas3, therefore suggesting a potential contribution in controlling. the development of MDR strains from Touchon et al., 2012.

Clarification of mechanisms of phage immunity, lateral gene transfer, and antimicrobial resistance requires a knowledge of the genomic characteristics of CRISPRCas3 in clinical isolates. Genomic investigations (Shah et al., 2013) provide insights into spacer sequences, phylogenetic relationships, and the potential CRISPR-mediated protection against phage. This information is especially relevant in medical settings where MDR A. baumannii strains jeopardize patient safety and limit treatment options.

Although many studies have investigated CRISPR-Cas systems in A. baumannii and highlighted their ubiquity and functional relevance (Wright et al., 2016; Hasan et al., 2019), there Knowing regional variation in CRISPRCas3 occurrence, spacer diversity, and link with antimicrobial resistance profiles is limited as comprehensive genomic data from Pakistani clinical isolates is still absent.

Significance of Study:

While CRISPR-Cas systems are often known for their part in phage defense, their impact on A. baumannii antibiotic resistance is not much studied. Knowing how CRISPRCas3 influences gene transfer and resistance mechanisms is essential to creating strategies against MDR strains. The presence and functional effects of CRISPRCas3 (Subtype IF/YPEST) in clinical isolates of A. baumannii from Pakistan a nation with scant information on this characteristic are intended to be clarified by this study.

Objectives:

1. Prevalence Assessment: Determine the prevalence of CRISPR-Cas3 (Subtype I-F/YPEST) in A. baumannii clinical isolates from Pakistan.
2. Genomic Characterization: Analyze the genomic features of CRISPR-Cas3 loci, including spacer sequences and associated genes.
3. Functional Implications: Investigate the role of CRISPR-Cas3 in horizontal gene transfer and its correlation with antimicrobial resistance profiles.
4. Comparative Analysis: Compare the findings with global data to assess regional variations and potential implications for treatment strategies.

Research Questions:

What is the prevalence of CRISPR-Cas3 (Subtype I-F/YPEST) in A. baumannii clinical isolates from Pakistan?

How do the genomic features of CRISPR-Cas3 loci correlate with antimicrobial resistance profiles in these isolates?

Does the presence of CRISPR-Cas3 influence the horizontal gene transfer mechanisms in A. baumannii?

Rationale of Study:

This research closes the knowledge gap by conducting genomic analysis of CRISPRCas3 (Subtype IF/YPEST) in Pakistan's clinical A. baumannii isolates. The goal is to clarify the function of the system in phage defense, horizontal gene transfer, and modulation of antimicrobial resistance, thereby providing insights that could guide infection control and phage treatment techniques.

LITERATURE REVIEW:

CRISPRCas Methods in Bacteria

Bacteria and archaea have adaptive immune systems called clustered regularly interspersed short palindromic repeats (CRISPR) and their associated Cas proteins that offer resistance against bacteriophages. Plasmids as well (Makarova et al., 2015; Barrangou et al., 2007). Based on their structure and functional components, CRISPRCas systems are categorized into two groups, six forms, and many subtypes (Koonin et al., 2017). Among these, Class 1 Type I systems, especially Subtype IF/YPEST, use the Cas3 nucleasehelicase, which causes targeted DNA degradation and constrains horizontal gene transfer (Sinkunas et al., 2011).

CRISPRCas in the Acinetobacter baumannii

Many studies have demonstrated the widespread use and functional importance of CRISPR-Cas systems in A. baumannii clinical isolates (Touchon et al., 2012; Wright et al., 2016). Reduced intake of plasmidborne antibiotic resistance genes related to CRISPRCas3 in A. baumannii points therefore to a protective role against horizontal gene transfer. Hasan Additionally, spacer analysis shows past exposure to bacteriophages, so pointing to adaptive immunity and therefore potential phage resistance (Shah et al., 2013).

Horizontal gene transfer and phage immunity

By including short sequences from infiltrating phages into CRISPR arrays, which direct Cas nucleases to identify and degrade matching, CRISPRCas systems help to build phage immunity. Barrangou et al., 2007; Brouns et al., 2008; phage DNA. Research have shown that isolates with active CRISPRCas loci have lower rates of horizontal gene transfer, therefore limiting the spread of antibacterial resistance (Makarova et al., 2015; Touchon et al., 2012).

Antibiotic resistance in A. baumannii

Often recorded (Peleg et al., 2008; MunozPrice &), MDR A. baumannii is a major worry in medical environments because of resistance to carbapenems, aminoglycosides, and colistin. The interplay between CRISPR-Cas systems and antimicrobial resistance gene acquisition has been seen; CRISPR-positive strains typically show fewer plasmid-mediated resistance genes (Hasan, 2013). 2019; Wright et al., 2016). This connection emphasizes the CRISPR-mediated mechanisms' potential as a natural barrier to spread of resistance.

Gaps in Current Investigations

Limited information exists on the genomic profile of CRISPRCas3 in Pakistani A. baumannii isolates despite worldwide research on CRISPRCas systems. Few studies have examined functional consequences for phage immunity and horizontal gene transfer in clinical situations (Ahmed et al., 2020), most of them have concentrated on prevalence and spacer diversity. Understanding the role of CRISPRCas3 in regional strains calls for thorough genomic investigations combining phylogenetic profiling, spacer characterization, and association with antimicrobial resistance.

METHODOLOGY:

The research was carried out in diagnostic microbiology laboratories to examine the prevalence and genomic features of CRISPRCas3 (Subtype IF/YPEST) in clinical isolates of Acinetobacter baumannii from With clinical isolates gathered from patients with proven infections. Over the course of the study, 50 clinical isolates chosen via stratified random sampling to guarantee representation from several hospital departments comprised the total. Only isolates verified as A. baumannii from blood, urine, wound swabs, and respiratory samples; from ICU, surgical, and medical wards; from different specimen types. While contaminated or nonviable isolates and samples without full clinical data were excluded, MALDITOF mass spectrometry and biochemical testing were included.

Bacterial identification and isolation were performed using standard microbiological techniques, and DNA was extracted from confirmed isolates for whole-genome sequencing to detect CRISPR-Cas3 systems and associated spacer sequences. Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disk diffusion method according to CLSI 2023 guidelines. Bioinformatics tools, including CRISPRCasFinder and CRISPRDetect, were used to identify CRISPR arrays and Cas3 genes, and phylogenetic analyses were conducted using MEGA-X software.

Data were analyzed with SPSS version 26. Descriptive statistics, including frequencies, percentages, means, and standard deviations, were used for demographic and clinical variables, while chi-square tests assessed associations between CRISPR presence and antimicrobial resistance genes. Independent t-tests and ANOVA were applied to compare CRISPR-positive and CRISPR-negative isolates, with significance set at $p < 0.05$. Ethical approval was obtained from the Institutional Review Board of the participating hospitals, and informed consent was secured for the use of clinical samples, ensuring patient confidentiality throughout the study.

Results: Genomic Analysis of CRISPR-Cas3 in Acinetobacter baumannii

Table 1: Demographic and Clinical Characteristics of Patients (n = 50)

Characteristic	Frequency (n)	Percentage (%)
Gender – Male	28	56
Gender – Female	22	44
Age Group (18–30)	8	16
Age Group (31–50)	22	44
Age Group (51–70)	20	40
Source of Isolate – Blood	15	30
Source of Isolate – Urine	12	24
Source of Isolate – Respiratory Secretions	18	36
Source of Isolate – Wound Swab	5	10

The study included 50 clinical isolates of A. baumannii, with a slightly higher prevalence in males. The majority of isolates were from patients aged 31–50 years, and respiratory secretions were the most common source of isolation

Table 2: Prevalence of CRISPR-Cas3 (Subtype I-F/YPEST) in Clinical Isolates

CRISPR-Cas3 Status	Frequency (n)	Percentage (%)
Present	18	36
Absent	32	64

CRISPR-Cas3 Subtype I-F/YPEST was detected in 36% of clinical isolates, indicating moderate prevalence among A. baumannii strains in Pakistan.

Table 3: Distribution of Antibiotic Resistance Genes in Isolates

Resistance Gene	CRISPR-Cas3 Present (n=18)	CRISPR-Cas3 Absent (n=32)	Total (n=50)
bla_OXA-23	4	15	19
bla_NDM-1	2	10	12
bla_TEM	3	8	11
bla_SHV	1	5	6
bla_VIM	0	4	4

Isolates harboring CRISPR-Cas3 showed lower frequency of plasmid-mediated antibiotic resistance genes compared to CRISPR-negative isolates, supporting a potential role of CRISPR-Cas3 in limiting horizontal gene transfer.

Table 4: Phylogenetic Analysis of CRISPR-Cas3 Positive Isolates

Isolate ID	Closest Reference Strain	Sequence Similarity (%)	Spacer Count	Notable Phage Targets
AB01	ATCC 17978	98	5	Phage φAB1, φAB2
AB07	AB5075	97	6	Phage φAB3
AB12	ACICU	95	4	Phage φAB1, φAB4
AB18	ATCC 19606	96	5	Phage φAB2, φAB5
AB25	AB0057	97	7	Phage φAB3, φAB6

Phylogenetic analysis revealed high sequence similarity with reference A. baumannii strains. Spacer sequences suggested historical phage exposure and potential immunity against multiple phages.

Table 5: Correlation Between CRISPR-Cas3 Presence and Horizontal Gene Transfer

Feature	CRISPR-Cas3 Present (n=18)	CRISPR-Cas3 Absent (n=32)	p-value (Chi-square)
Acquisition of Plasmid Genes	2	15	0.008*
Conjugation Frequency (events/10)	1.5 ± 0.5	3.2 ± 1.1	0.001*

CRISPR-Cas3 positive isolates showed significantly lower plasmid acquisition and reduced conjugation frequency compared to CRISPR-negative isolates, indicating a potential inhibitory effect on horizontal gene transfer.

DISCUSSION:

Focusing on phage immunity, horizontal gene transfer, and antimicrobial resistance, this study examined the genomic features of CRISPRCas3 (Subtype IF/YPEST) in clinical isolates of Acinetobacter baumannii from Pakistan. 36% of isolates contained the CRISPRCas3 system, therefore suggesting a low prevalence, consistent with earlier findings on erratic CRISPR distribution among A. baumannii strains (Wright). 2016; Hasan et al., 2019) This variation points to variances in adaptive immune systems among clinical isolates.

High sequence conservation and distinctive spacer sequences found through phylogenetic analysis point to past phage exposure. These conclusions support prior findings on spacer Diversity may indicate prior contacts with bacteriophages and adaptive immunity (Shah et al., 2013; Touchon et al., 2012). Consistent with the concept that CRISPR systems Compared to CRISPRnegative strains,

CRISPRCas3positive isolates showed less plasmid-mediated antibiotic resistance genes and slower conjugation rates. Hasan et al. (2019) and Makarova et al. (2015) wrote about horizontal gene transfer.

The Pakistani isolates show comparable CRISPR-mediated phage resistance and gene transfer limitation when comparing their results with worldwide data. In CRISPR-positive U.S. hospital isolates, Wright et al. (2016) found less plasmid acquisition and phage sensitivity, matching our results. Touchon et al. (2012) also observed an inverse link between CRISPR presence and horizontal gene transfer in E. coli, implying preserved functional roles across species.

These findings highlight how CRISPRCas3 serves both phage defense and horizontal gene transfer restriction, hence indirectly affecting antimicrobial resistance profiles. Developing phage therapy approaches and managing multidrug-resistant A. baumannii in healthcare situations depends on an awareness of these processes in regional isolates. CRISPRCas3 suggests that phage-based therapies might be used to target CRISPR-negative isolates.

A comparatively small sample size and single-region concentration limit the generalizability of future studies, thus altering it. Functional tests on larger, multicenter cohorts should thus be undertaken in following research. confirm CRISPRCas3 activity in gene transfer suppression and phage resistance

CONCLUSION:

A thorough genomic study of CRISPRCas3 (Subtype IF/YPEST) in clinical Acinetobacter baumannii isolates from Pakistan is presented here. CRISPRCas3's presence in 36% of isolates emphasizes its mild prevalence and possible part in mediating phage immunity and limiting horizontal gene transfer. Phylogenetic and spacer studies point toward historical contact with several bacteriophages, therefore mirroring adaptive immunological activity. Strikingly, CRISPRCas3positive isolates showed decreased acquisition of plasmid-mediated antimicrobial resistance genes, therefore implying that CRISPRCas systems may operate as natural obstacles to resistance gene distribution**.**

These findings have significant clinical implications. Understanding the functional role of CRISPR-Cas3 can inform phage therapy approaches by identifying susceptible CRISPR-negative isolates. Additionally, knowledge of CRISPR-mediated restriction of gene transfer can aid in the development of strategies to mitigate the spread of multidrug-resistant A. baumannii in hospital settings.

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