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MOLECULAR AND IN-SILICO CHARACTERIZATION OF BLANDM IN CARBAPENEM-RESISTANT KLEBSIELLA PNEUMONIAE ISOLATED FROM IMMUNOCOMPROMISED CANCER PATIENTS

Shahnaz Erfan Omar[1*],Amin Aziz Bakir[2], Souzan Hussain Eeassa[3]

[1,3.] Department of Preclinical Sciences, School of Medicine, UKH
[2], Department of Basic Sciences, College of Medicine, Hawler Medical University
* Corresponding Authors: Shahnaz.erfan@ukh.edu.krd

ABSTRACT
The emergence of New Delhi metallo-β-lactamase (blaNDM) variants makes carbapenem-resistant klebsiella pneumonaie (CRKP) a critical threat to immunocompromised cancer patients, using in-silico 3D modeling and integrated molecular diagnostics, this study showed the frequency of structural changes and genetic mutations of the blaNDM gene in clinical CRKP strains isolated from cancer patients. The obtained and identified carbapenem resistance from the screened clinical K. pneumoniae isolates was amplified by PCR to detect the blaNDM gene, then examined by Sanger sequencing, and simulated using AlphaFold 3 and PyMOL to assess localized structural modifications. In 75.00% of sequenced blaNDM isolates, genetic variation was detected, along with carrying multiple substitutions with 66.33%. In 100.00% of the mutated isolates, the non-synonymous alterations was seen A>C (Met154Leu), whereas the alteration of the G>T (Val88Leu) was found in 83.33% of mutated strains, showing a transition toward the highly effective NDM-5 phenotype. The overall globular fold remained unchanged despite the localized loop rearrangement happened next to positions 88 and 154 according to in-silico 3D modeling. The given study shows the high regional frequency of the optimized NDM-5 phenotype, the affinity of the zinc is known to be enhanced by the observed mutations. In the host-induced zinc starvation, this modification in the structure of carbapenemase maintains its function and preserve it, and this highlights the critical need for quick molecular diagnosis in oncology surveillance and monitoring.

KEYWORDS/ Carbapenem-resistance, Klebsiella pneumoniae, blaNDM gene, sequence variation

INTRODUCTION

Carbapenem-resistant Klebsiella pneumoniae (CRKP) is among the most problematic bacterial pathogens encountered in modern healthcare and represents a particular concern in immunocompromised patient populations, including individuals receiving chemotherapy or hematopoietic stem cell transplantation [1]. The World Health Organization (WHO) has clarified carbapenem-resistant Enterobacteriaceae as ”Priority 1:Critical group” because of the limited availability of efficient treatment options and continuing need for antimicrobial agents.

Although K. pneumoniae was once recognized primarily as a cause of community-acquired pneumonia, it has become one of the most prominent opportunistic pathogens responsible for hospital-acquired infections. These include bloodstream infections, ventilator-associated pneumonia, and urinary tract infections. In oncology and critical care units, where patients are commonly infected by multidrug-resistant (MDR) Gram-negative bacilli, carbapenem often remain the few remaining therapeutic option. However, the intensive clinical use of these agents has contributed to increasing carbapenem-resistance, particularly among members of Enterobacteriaceae family (CRE) [2].

The resulting situation constitutes a major public health alert. These organisms can now tolerate carbapenem concentrations once considered lethal. Contributing to mortality rates that commonly exceed 40% percent in vulnerable populations [3]. The global rise of the New Delhi metallo-β-lactamase gene (blaNDM) has pushed the antimicrobial resistance to new level, testing the limits of current infectious disease management. Encoding a class B metallo-β-lactamase (MBL), blaNDM relies on a zinc-dependent mechanism that enables efficient hydrolysis of virtually all β-lactam antibiotics, encompassing carbapenems, cephalosporins and penicillin. Unlike serine carbapenamases, NDM enzymes remain unaffected by available β-lactamase inhibitors such as avibactam, which considerably adds to the clinical danger [4].

The blaNDM gene has continued to evolve, producing several variants, among them, NDM-1 and NDM-5 have become dominant in clinical isolates [5]. Recent surveillance studies have continued to report NDM-5 among clinically important

high risk-lineages including ST167A sequence type, frequently linked to carbapenem resistance, international dissemination and successful clonal expansion [6].
Although the international spread of blaNDM-producing K. pneumoniae is well documented, relatively little detailed work has been done on isolates from oncology patients. Most studies in this population have concentrated on antimicrobial susceptibility testing and basic detection of resistance genes [7]. Few have explored sequence variations or structural characterization of NDM enzymes in any depth. This gap restricts our understanding of how these resistance determinants involve spread and function within this particularly vulnerable patient group.

MATERIAL & METHOD

Clinical Klebsiella pneumoniae isolates were collected from Nanakaly Hospital for Hematology & Oncology, Erbil, Iraq, from November 2025 until March 2026 from cancer patients. A total of 60 isolates from various Cancer cases were collected including urine from urinary tract infection and blood samples. Then, isolates were cultured on MacConkey agar and confirmed as K. pneumoniae through their morphological characteristics (large mucoid colonies with pink coloration), Klebsiella pneumoniae isolates were subsequently confirmed using the VITEK 2 Compact system. Confirmed isolates were then subjected to antimicrobial susceptibility testing using the VITEK-2 to confirm the availability of Carbapenem resistance. The DNA was extracted and purified from sixty samples for each isolate, following the protocol of (Thermo Fisher PureLink™ Genomic DNA Mini kit, Invitrogen™, USA) commercial kit. Polymerase chain reaction (PCR) was performed to detect the presence of the blaNDM gene in the extracted genomic DNA of carbapenem-resistant Klebsiella pneumoniae isolates. Gene-specific primers targeting blaNDM were used for amplification.
Following the PCR and confirming exact bands of expected PCR products by using gel electrophoresis, PCR products were prepared for sequencing with using forward primer (5-GGTTTGGCGATCTGGTTTTC-3) for blaNDM-1 gene from the positive Carbapenem-Resistant Klebsiella pneumoniae isolated from immunocompromised cancer patients and subsequently forwarded to Macrogen Labs, located in Korea, for detailed analysis. At Macrogen Labs, the samples underwent analysis through capillary electrophoresis. Then, mutation Surveyor Software version 5.1 (SoftGenetics, LLC) was used to evaluate the resultant sequences and align them with the reference sequence downloaded from NCBI. Finally, the amino acid sequences were aligned using Clustal Omega (multiple sequence alignment) to identify sequence variations and assess sequence conservation among the isolates. The predicted amino acid sequence was then analyzed using AlphaFold Powered by AlphaFold 3 to generate a three-dimensional (3D) structural model of the protein and evaluate the potential structural impact of the identified mutations.

RESULTS

PCR and Sanger sequencing identified genetic variation in blaNDM-1 in 75% of isolates, and 66.33% of variant-positive samples carried more than one mutation after alignment with reference sequence. Also, a total of 4 variation substitutions were identified, including A>C, G>T, G>GT and T>TG. The mutation profile showed that all detected sequence changes belonged to the substitution category. Among these different substitutions, the A>C substitution was observed in 100.00% of Carbapenem-Resistant Klebsiella pneumoniae isolated from 2mmune-compromised cancer patients, making it the most frequent variant identified. The G>T substitution was also highly prevalent, occurring in 83.33% of patients, whereas T>TG was 50.00%. In contrast, the G>GT variation was the least frequent, detected in only 16.66% of Carbapenem-Resistant Klebsiella pneumoniae isolated from immunocompromised cancer patients, as shown in **table 1**, four blaNDM-1 variants were detected.

Table.1. presents the frequency and codon-level effects of the detected variants

Variant	Frequency	Codon change	Amino acid change
A>C	100.00%	ATG to CTG	Met to Leu
G>T	83.33%	GTG to TTG	Val to Leu
G>GT	16.66%	GCG to GCT	Ala to Ala
T>TG	50.00%	GGT to GGG	Gly to Gly

Furthermore, the detected sequence variations in the blaNDM-1 gene were distributed across several positions within the gene and its corresponding amino acid sequence. Among the identified variants, the A>C substitution was detected at blaNDM-1 gene position 460 and corresponded to amino acid position 154 of the protein. The G>T substitution was observed at nucleotide position 262, affecting amino acid position 88. The G>GT variant was identified at position 414 and mapped to amino acid position 138, while the T>TG variant occurred at nucleotide position 231 and corresponded to amino acid position 77. The genomic and amino acid positions of the substitutions are shown in **Table 2.**

Table. 2. Positional characterization of substitutions in the blaNDM-1 gene.

Variation	Variants	blaNDM-1 gene position	Amino acid position
Substitution	A>C	460	154
Substitution	G>T	262	88
Substitution	G>GT	414	138
Substitution	T>TG	231	77

Moreover, alignment of the deduced amino acid sequences from of Carbapenem-Resistant Klebsiella pneumoniae isolated from immunocompromised cancer patients with the reference NDM-1 amino acid sequence demonstrated a similarity as well as observing 2 changes in amino acids. The conservation of amino acid residues was reflected by the continuous asterisk marks in the alignment output **(figure 1).**

Figure 1. Alignment of the NDM-1 amino acid sequence from of Carbapenem-Resistant Klebsiella pneumoniae isolated from immunocompromised cancer patients with NCBI Reference Sequence.

Regarding 3D structures, the predicted 3D structures of the reference and mutant NDM-1 proteins are shown in **figure 2.** The mutant protein retained an overall folding pattern that was largely comparable to the reference structure, and both displayed the typical globular conformation characteristic of NDM metallo-β-lactamase.

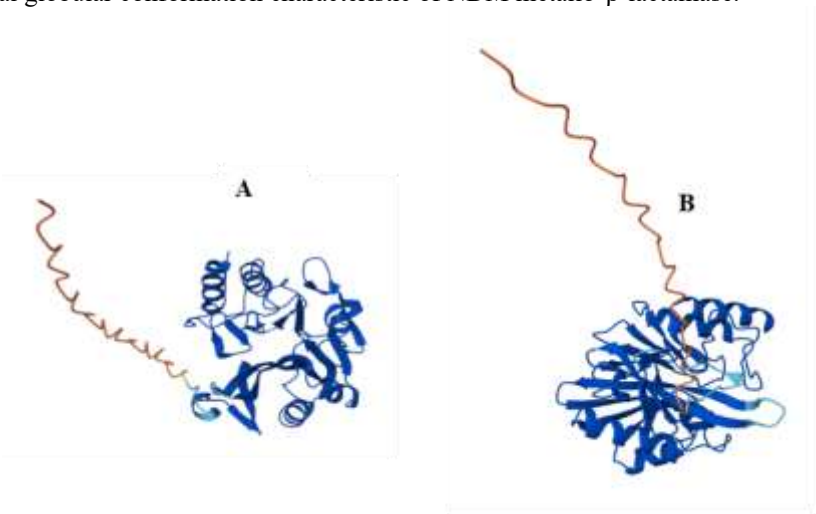

Figure 2. Predicted 3D structures of the NDM-1 protein. (A) Reference NDM-1 protein predicted from the reference blaNDM-1 nucleotide sequence. (B) Predicted structure of the NDM-1 protein translated from the blaNDM-1 sequence of carbapenem-resistant Klebsiella pneumoniae isolated from immunocompromised cancer patients, containing two amino acid substitutions.

DISCUSSION

Carbapenem-resistant Klebsiella pneumoniae (CRPK) remains a major problem in hospital practice, with a particular relevance to oncology units where many patients are immunocompromised because of chemotherapy, neutropenia, prolonged admission, or invasive procedures. In these settings, carbapenems are often used as a key agent for empirical or targeted treatment of severe Gram-negative Bacilli infections. The emergence of CRKP therefore has direct clinical consequences as it reduces the reliability of one of the few remaining therapeutic options for high-risk patients. In the present study, 29 out of 60 clinical K. pneumoniae isolates obtained from immunocompromised patients showed resistance to carbapenem. This finding supports the concern that sustained antimicrobial exposure in specialized healthcare environments, including cancer hospitals, may contribute to the selection and persistence of multidrug-resistant K. pneumoniae lineages. Sequence analysis of blaNDM gene identified substitutions of particular importance. The A>C substitution and nucleotide position 460 was detected in all variant isolates and resulted in methionine to leucine change at a minor position 154 (M154L). A second substitution, G>T at the nucleotide position 262 was present in 83.33% of the mutated isolates and produced valine to leucine change at position 88 (V88L). Together, these amino acid replacements are consistent with the transition from NDM-1 background towards the NDM-5 variant. The high frequency of these substitutions present collection suggests that NDM-5 associated alleles may be circulating among CRKP isolates recovered from this oncology system.

Additional nucleotide changes were also observed at lower frequencies. These include G>GT and T>TG changes, detected in 16.66% and 50.00% of the relevant isolates, respectively. Although these substitutions altered codon composition, including changes corresponding to Ala138 and Gly77, they did not modify the predicted amino acid sequence. Their biological effect is therefore likely to be limited at the protein-structure level. However, these silent changes may still reflect local genetic drift, codon usage variation or the accumulation of lineage specific mutations within circulating strains.

Structural modeling using AlphaFold 3 followed by visualization in PyMOL indicated that the predicted metallo-β-lactamase structure retained its overall globular fold despite the presence of the V88L and M154L substitutions. The overall tertiary structure was retained, which is consistent with the position of these institutions and their limited effect on the core fold of the enzyme. Small local changes were noted around the substituted residues, particularly in regions that may influence loop positioning, residue packing, or the flexibility of nearby secondary-structure elements. These alterations may help explain the resistance phenotype observed in antimicrobial sensitivity testing. Although modeling alone cannot establish a direct functional effect, confirmation would require enzyme-kinetic analysis using purified protein.

The combined presence of V88L and M154L is widely recognized as defining characteristics of blaNDM-5 allele when compared with blaNDM-1. Since its initial report in the United Kingdom in 2011, blaNDM-5 has been detected in multiple regions, including Asia, Middle East, and Europe [8]. The predominance of NDM-5 associated substitutions in the present isolates is therefore consistent with the broader international expansion of this allele and suggest possible establishment of NDM-5 producing CRKP in high-risk clinical environments. This is particularly concerning in oncology units, where antimicrobial exposure, immune suppression, and prolonged hospitalization may create conditions that favor both acquisition and persistence of resistant strains.

Previous biochemical and structural work has shown that substitutions found in NDM variants can influence enzyme stability, zinc binding, and activity under zinc-limited conditions [9]. The M154L substitutions is of particular interest because previous work has linked to improve zinc binding and greater stability of NDM enzymes under zinc-limited conditions. This may be relevant during infection where host nutritional immunity can restrict availability of metal ions at the site of bacterial growth. NDM variants carrying M154L, either alone or with additional substitutions, have also been associated with changes β-lactam resistance, including resistance to carbapenems. In the present study, the detection of both V88L and M154L in carbapenem-resistant isolates provide a plausible molecular basis for a strong resistance profile observed among the sequenced CRKP isolates.

Taken together, the data suggests that carbapenem-resistant K. pneumoniae isolates from immunocompromised patients was associated with blaNDM variants carrying substitutions characteristic of NDM-5. The preservation of the predicted global enzyme structure, together with localized structure changes around V88L and M154L suggests that these variants retain structural framework required for β-lactam hydrolysis while potentially acquiring features that enhance stability or activity under clinically relevant conditions. These data add to the growing evidence of NDM-5 producing CRKP represents an important threat in vulnerable patient population and highlights the need for continuous molecular surveillance in oncology settings.

CONCLUSION

In conclusion, in clinical cancer wards this study offers an important structural and molecular characterization of carbapenem-resistant klebsiella pneumoniae carrying the modified blaNDM variants. The results shows that the significant majority 75.00% of the clinical blaNDM isolates have been moved away from the wild-type genotype, developing a highly conserved mutations (Met154L and Val88Leu). The overall globular proteins structure was maintained despite the production of the loop rearrangement next to catalytic pocket from the non-synonymous mutations from the observed

AlphaFold 3 and PyMOL structural stimulation. The affinity of the vital zinc cofactors can be increased from these localized changes from the enzyme architecture, and this shows a change in evolution mechanism, that allows the pathogen to bypass the surveillance of the host immunity that will do the zinc deprivation coming from the nutritional immunity and maintain the high resistance to carbapenem. The high prevalence of these changes in oncology settings is quite concerning, as it makes the therapeutic options infective. Evolving and changing from the slow, growing-dependent phenotype screening to more rapid and PCR-based genotypic diagnosis in healthcare systems along with developing new therapeutic combinations, is a must to preserve last-line clinical interventions.

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