Journal of Progressive Medical Sciences
ISSN: 3052-8518
J.P.M.S
DOI: https://doi.org/10.63939/640r0h03
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Vol. 2 , No. 7 , November 2026

RESEARCH Open Access

Molecular Detection of blaNDM-1 with Structure-Based Evaluation of Captopril and a Designed Thiosemicarbazone Derivative (PMIT) in Carbapenem-Resistant *Klebsiella pneumoniae*

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Abstract

Background: The emergence of Carbapenemase-producing *Klebsiella pneumoniae* (CPKP) is a major clinical problem because of the spread of Carbapenemase-encoding genes and scarce clinical treatments. This study investigated antimicrobial re sistance patterns and Carbapenemase genes in clinical *K. pneumoniae* isolates and evaluated the predicted molecular interactions of D-captopril and a designed compound, PMIT, with New Delhi Metallo-β-lactamase-1 (NDM-1). **Methods:** All 314 clinical specimens were analyzed and 54 were identified as *K. pneumoniae* isolates. Susceptibility was determined by disk diffusion, and 41 selected isolates underwent PCR screening for Carbapenemase-encoding genes. The crystal structure of NDM-1 was used for molecular docking of D-captopril and PMIT using CB-Dock3. **Results:** All 54 isolates exhibited resistance to imipenem, gentamicin, amikacin, and trimethoprim/sulfamethoxazole. Resistance to meropenem, chloramphenicol and ciprofloxacin was 90.7%, 77.8% and 70.4%, respectively. Among the 41 screened isolates, *blaIMP* was detected in 100%, followed by *blaNDM-1* (36.6%) and *blaOXA-48-like* (26.8%). Molecular docking identified highest-ranked Vina scores of -8.4 kcal/mol for D-captopril in cavity C5 and -8.5 kcal/mol for PMIT in cavity C3. The ligands displayed distinct predicted interaction profiles involving different crystallographic protein chains. **Conclusion:** The findings demonstrate the high-level antimicrobial resistance and the presence of more than one Carbapenemase

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determinants in the clinical isolates of *K. pneumoniae*. PMIT docking score was similar to D-captopril.

Keyword: NDM-1, *blaIMP, Klebsiella pneumoniae,* Carbapenem-resistance, molecular docking, D-captopril, PMIT.

1- Introduction

The emergence of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is significant public health issue and public health management to effective control of bacterial infections [1]. *K. pneumoniae* is an opportunistic pathogen that is a clinically significant cause of serious HAPs such as pneumonia, bloodstream infections, and urinary tract infections, especially in hospitalized patients [2]. The clinical relevance of CRKP is also highlighted by being included in the World Health Organization (WHO) 2024 Bacterial Priority Pathogens List, which classifies CR *Enterobacterales* as critical-priority pathogens requiring urgent research and therapeutic development [3]. While carbapenems are still valuable drugs to treat infections caused by multidrug-resistant Gram-negative bacteria, the increasing prevalence of carbapenem resistance has substantially restricted their clinical utility [4]. One of the mechanisms of this resistance is the production of carbapenem-hydrolyzing β-lactamases, which is a major therapeutic challenge, and novel approaches to the study resistance determinants in bacteria [5].

Among the Carbapenemase associated with antibiotic resistance, New Delhi Metallo-β-lactamase-1 (NDM-1) is a clinically significant class B1 Metallo-β-lactamase capable of hydrolyzing a broad spectrum of β-lactam antibiotics, including carbapenems [6]. Unlike serine-dependent β-lactamases, NDM-1 relies on a binuclear zinc center within its active site, where zinc ions facilitate the activation of a water-derived hydroxide nucleophile responsible for β-lactam ring hydrolysis [6]. Structurally, NDM-1 possesses a relatively open catalytic pocket surrounded by flexible loops that contribute to substrate recognition and accommodate chemically diverse β-lactam compounds [7]. These structural and catalytic characteristics make NDM-1 an important molecular target for the structure-based investigation of potential inhibitors aimed at counteracting enzyme-mediated antibiotic resistance [6,7].

Despite considerable advances in β-lactamase inhibitor development, the identification of effective inhibitors targeting NDM-1 remains a significant challenge. Conventional inhibitors primarily developed against serine β-lactamases generally lack

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activity against Metallo-β-lactamases because of fundamental differences in their catalytic mechanisms [8]. Furthermore, the zinc-dependent active site of NDM-1 present additional challenges for inhibitor design, as compounds capable of coordinating catalytic metal ions may also interact with other biologically important metalloenzymes, raising concerns regarding selectivity and potential off-target effects [9]. The structural flexibility of the NDM-1 active site and the emergence of enzyme variants with altered inhibitor susceptibility further complicate the development of broadly effective inhibitors [8,9]. Consequently, structure-based computational approaches, including molecular docking, provide a useful preliminary framework for investigating ligand-binding orientations, identifying potential molecular interactions, and prioritizing candidate compounds for subsequent experimental evaluation.

The identification of small molecules capable of interacting with the zinc-dependent catalytic site of NDM-1 has attracted considerable interest as a potential strategy for overcoming Metallo-β-lactamase-mediated antibiotic resistance. Among previously investigated compounds, D-captopril, a stereoisomer of the angiotensin-converting enzyme inhibitor captopril, has demonstrated inhibitory activity against NDM-1, highlighting the importance of ligand stereochemistry and metal coordination in molecular recognition [10]. The thiol functionality of captopril is particularly relevant its interaction with the zinc-containing active site, providing a structural basis for its investigation as a reference inhibitor.

In the present study, PMIT was selected as a second compound for comparative structure-based assessment against NDM-1. Evaluating these compounds within a common molecular docking framework may help identify differences in their predicted binding affinities, binding orientations, and interactions with the enzyme. Such comparisons can provide preliminary insights into their molecular recognition characteristics without establishing inhibitory potency or biological efficacy.

Motivated by the growing need to identify new molecular candidates targeting NDM-1, the present study aimed to design and the evaluation of a thiosemicarbazone-derived compound, designated PMIT, using a comparative structure-based molecular docking approach. The design of PMIT was based on structural modification within the thiosemicarbazone chemical framework, with the aim of exploring its potential molecular recognition and binding interactions with the zinc-dependent NDM-1 enzyme. D-captopril was binding characteristic of PMIT. The crystallographic structure of NDM-1 was employed as the molecular target, and the investigation focused on

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comparing docking affinity scores, examining ligand-binding orientations, and identifying potential interactions within the predicted binding pockets. Through this approach, the study sought to provide preliminary structural insights into the binding behavior of the designed PMIT compound relative to D-captopril and to assess its suitability for further investigation as a potential NDM-1 inhibitor. Nevertheless, the in silico finding require experimental validation before any conclusions regarding enzyme inhibition or antibacterial activity can be established.

2. Materials and Methods

2.1 Study Design and Clinical Specimen Collection

A cross-sectional investigation was undertaken in several hospitals in Najaf, Iraq between February 2024 and April 2025 to examine carbapenem-resistant *Klebsiella pneumoniae* among adult patients with clinically suspected lower respiratory tract infections. Drugs this period, 314 sputum specimens were collected using sterile containers under aseptic conditions. The specimens were transferred promptly to the microbiology laboratory for processing, with collection and transportation procedures conducted in accordance with established biosafety and infection prevention practices. Relevant demographic and clinical information was documented at the time of specimen collection. Only sputum samples satisfying standard acceptability criteria were included in the microbiological investigation to minimize the influence of specimen contamination on subsequent analysis.

2.2 Bacterial Isolation and Species Identification

Sputum specimens were cultured on MacConkey agar and incubated aerobically at 37°C for 18-24 h. Colonies exhibiting characteristics consistent with *Klebsiella* spp. Were subjected to preliminary identification based on their cultural appearance, lactose-fermenting properties, Gram-staining characteristics, and conventional biochemical reactions. A total of 54 isolates were subsequently identified as *K. pneumoniae*. Identification was further assesses using CHROMagar Orientation medium, followed by species-level confirmation with the VITEK 2 Compact automated identification system using GN identification cards (bioMerieux, France), according to the manufacturer's.

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2.3 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility profiles of the bacterial isolates were determined by the Kirby-Bauer disk diffusion technique using Mueller Hinton agar, following standardized laboratory procedures [11]. The antibiotic panel comprised imipenem and meropenem (carbapenems), gentamicin and amikacin (aminoglycosides), ciprofloxacin (fluoroquinolones), trimethoprim/sulfamethoxazole (folate pathway inhibitors), and chloramphenicol and (amphenicols). Following incubation, the diameters of the inhibition zones were recorded and interpreted using the Clinical and Laboratory Standards Institute (CLSI) interpreted criteria published in 2025 [12]. Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

2.4. Genomic DNA Extraction and PCR primer Sequences with Amplification Conditions

Genomic DNA was isolated from the 54 confirmed *K. pneumoniae* isolates using a commercially available bacterial DNA extraction kit (Promega, USA), following the manufacturer's protocol. The concentration and purity of the recovered DNA were evaluated before the extracts were preserved at -20°C for subsequent molecular investigations. Conventional polymerase chain reaction (PCR) was employed to screen the isolates for selected genetic markers, Carbapenemase-associated genes (*blaNDM-1*, *blaOXA-48-like*, and *blaIMP*), extended-spectrum β-lactamase- associated genes [13,14]. Conventional PCR was performed to detect the *blaNDM-1*using gene-specific primer for *blaNDM-1*, the forward primer sequences was 5'-GGTTTGGCGATCTGGTTTTC-3' and the reverse primer sequence was, 5'-CGGAATGGCTCACGATC-3' with an annealing temperature of 52°C for 50sec, and an expected amplicon size of 621 bp. The *blaOXA-48-like* gene was amplified using the forward primer 5'-GCGTGGTTAAGGATGACAC-3', and reverse primer 5'-CATCAAGTTCAACCCAACCG-3' under an annealing condition of 52°C for 50sec, yielding an expected 438-bp product. For blaIMP, the forward and reverse primer sequences were 5'-ACCGCAGCAGAGTTTGCC-3'and 5'-ACAACCAGTTTTGCCTTACC-3', respectively, with an annealing temperature of 44°C for 50sec and an expected amplification product of 586-bp [15].

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Each PCR assay was performed in a final reaction volume of 25 µL comprising genomic DNA template, target-specific primers, PCR master mix, and nuclease-free water. The amplification products were separated by electrophoresis in 1.5% agarose gels and subsequently visualized under ultraviolet illumination.

2.5 Preparation of the NDM-1 Protein Structure

The experimentally determined three-dimensional structure of New Delhi Metallo-β-lactamase-1(NDM-1) was retrieved from the RCSB Protein Data Bank for structure-based computational analysis [6]. The selected structure represents the Apo form of NDM-1, resolved by X-ray crystallography at a resolution of 2.10Å. As a zinc-dependent Metallo-β-lactamase encoded by the *blaNDM-1* gene, NDM-1 was selected as the molecular target for investigating potential interactions with D-captopril and the designed thiosemicarbazone-derived compound PMIT. The crystallographic structure served as the receptor framework for subsequent molecular docking analysis, enabling the assessment of predicted ligand-binding orientations, affinity scores, and interactions within the enzymes binding regions.

2.6 Ligand Selection and Molecular Preparation

Two structurally distinct compounds were selected for comparative molecular docking against New Delhi Metallo-β-lactamase-1 (NDM-1): D-captopril, serving as the reference ligand, and PMIT, a newly designed compound based on the thiosemicarbazone chemical scaffold. D-captopril was demonstrating the inhibitory potential of captopril-based compounds against NDM-1 [16]. The selection of the thiosemicarbazone framework for PMIT [17]. PMIT was independently designed within the thiosemicarbazone framework and was included to explore alternative ligand-protein recognition patterns. The molecular structures of both compounds were used as inputs for subsequent docking calculations to examine their predicted affinity scores, preferred binding orientations, and potential interactions with the NDM-1 binding regions. The computational comparison was intended to provide preliminary structural evidence rather than establish experimental inhibitory activity for PMIT.

2.7 Molecular Docking Analysis

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Structure-based molecular docking was performed using the CB-dock2 to investigate the predicted binding interactions of D-captopril and designed thiosemicarbazone-derived compound PMIT with New Delhi Metallo-β-lactamase-1 (NDM-1). CB-Dock2 employs an integrated workflow combining automated binding-cavity detection, ligand docking, and structural template-based approaches to identify potential ligand-binding regions without requiring prior specification of a single binding site [18]. The molecular structures of D-captopril and PMIT were introduced separately as ligand inputs. Following docking, the predicted binding cavities and corresponding ligand conformations were examined for each compound.

2.8 Protein-Ligand Interaction Analysis and Visualization

The docking-generated protein-ligand complexes were analyzed and visualized using BIOVIA Discovery Studio Visualizer (Dassault Systemes BIOVIA, San Diego, CA, USA) [19]. The predicted binding conformations of D-captopril and PMIT were examined through two-dimensional (2D) interaction diagrams and three-dimensional (3D) molecular representations to characterize their interactions with NDM-1. The analysis focused on identifying amino acid residues surrounding each ligand, examining hydrogen bonds, hydrophobic contacts, and other noncovalent interactions, and evaluating the spatial arrangement of the ligands within their respective binding cavities. Particular attention was given to the proximity of the predicted binding poses to the zinc-dependent catalytic region of NDM-1. The interaction profiles of D-captopril and PMIT were compared in terms of residue involvement, interaction types, and binding-site occupancy. These observations were interpreted as evidence of potential ligand-protein interactions rather than confirmation of NDM-1 inhibition.

2.9 Statistical Analysis

Microbiological and molecular data were analyzed using Microsoft Excel 2021(Microsoft Corporation, USA) [20]. Antimicrobial resistance patterns and the prevalence of resistance-associated genes among *Klebsiella pneumoniae* isolates were summarized using descriptive statistical analysis and expressed as frequencies and percentages.

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2.10 Ethical Approval

The present study constitutes a continuation of previously published investigation conducted by the same researcher [15], using the same clinical *Klebsiella pneumoniae* isolates for further molecular genetic investigation and protein-level structural assessment of NDM-1. Ethical approval for the original study was obtained from the relevant institutional ethical committee, as documented in the previous publication [15]. Patient confidentiality was maintained throughout the investigation

3. Results

3.1 Bacterial Isolation and Antimicrobial Resistance Profiles

The 314 clinical specimens examined during the study period, 54 (17.2%) yielded confirmed *Klebsiella pneumoniae* isolates. Species identification was established using conventional microbiological methods, culture, and the VITEK 2 Compact identification system. Antimicrobial testing revealed markedly elevated resistance rates across the investigated antibiotics (Figure 1). All 54 isolates (100%) exhibited resistance to imipenem, gentamicin, amikacin, and trimethoprim/sulfamethoxazole. Resistance to meropenem was detected in 49 isolates (90.7%), followed by chloramphenicol in 42 isolates (77.8%) and ciprofloxacin in 38 isolates (70.4%). Overall, the susceptibility profiles demonstrated widespread antimicrobial resistance among the recovered *K. pneumoniae* isolates.

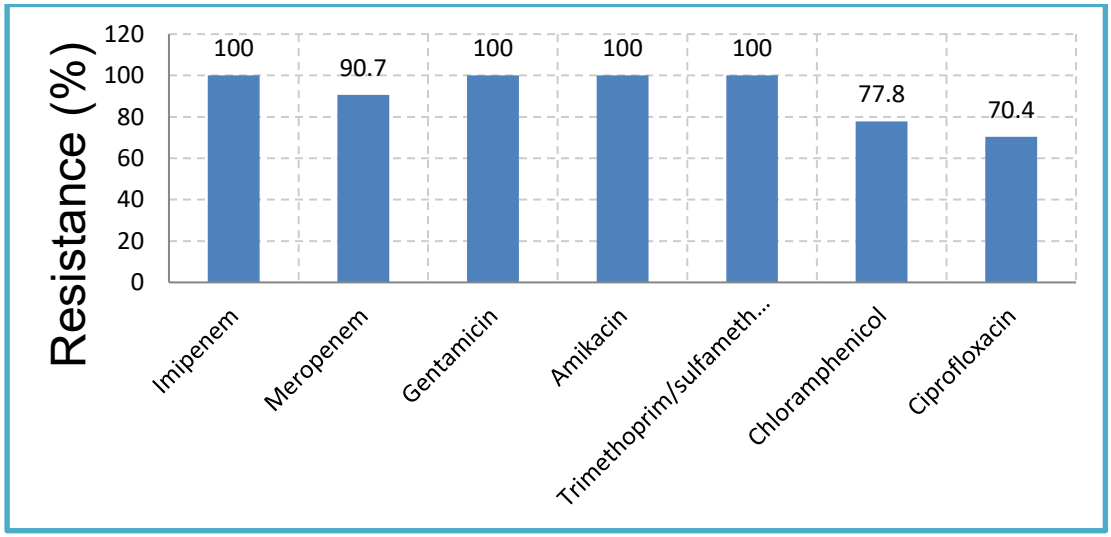

Figure 1. Antibiotic resistance rates in clinical *Klebsiella pneumoniae* isolates (n=54)

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3.2 Molecular Dectection of Carbapenemase –Encoding Genes

PCR-based screening of 41 selected *Klebsiella pneumoniae* isolates revealed distinct detection frequencies of the investigated Carbapenemase-encoding genes (Figure 2). The blaIMP gene was identified in all examined isolates (41/41; 100%), making it the most frequently detected gene. In contrast, *blaNDM-1* was detected in 15 isolates (36.6%), while *blaOXA-48-like* was identified in 11 isolates (26.8%). These results demonstrated a predominance of *blaIMP* among the screened isolates, followed by *blaNDM-1* and *blaOXA-48-like.*

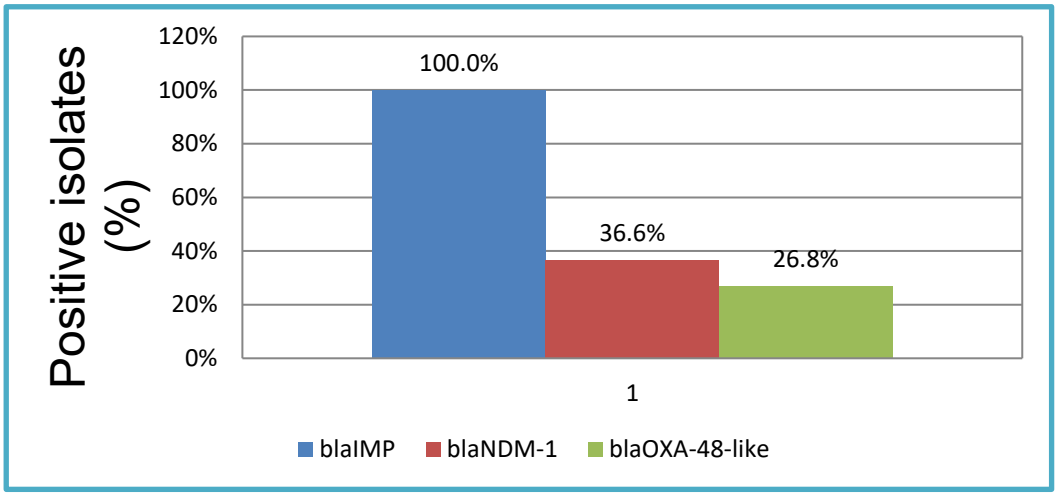

Figure 2. Prevalence of Carbapenemase-encoding genes *Klebsiella pneumoniae* isolates (n=41)

3.3 Structural Characterization of NDM-1

Structural examination of New Delhi metallo-βlactamase-1 (NDM-1) was performed using its experimentlly determined crystal structure, resolved by X-ray crystallography at a resolution of 2.10 Å. The three-dimensional ribbon representation illustrated the overallfolding architecture of the enzyme, comprising α-helical segments, β-strands arranged into β-sheets, and interconnected loop regions (Figure 3). These scondary structural elements collectively form the characteristic αβ/βα fold associated with subclass B1 metallo-β-lactamases.

The structural organization of NDM-1provides the framework for a catalytic region located within a cleft formed by adjacent structural elements. As a zinc-dependent metallo-β-lactamase, NDM-1 possesses a metal-associated catalytic site that

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is essential for β-lactam hydrolysis. The arrangement of the surrounding helices, β-sheets, and flexible loops contributes to the architecture of the substrate-binding region and provides potential sites for ligand recognition.

The crystallographic model was therefore used as structural for subsequent molecular docking analysis of D-captopril and the independently designed thiosemicabazone derivative PMIT. This approach enabled further examination of predicted ligand-binding cavities, molecular orientations, and interactions with the NDM-1protein structure.



Figure 3. The crystal structure of the NDM-1 is shown in three dimensions as a ribbon.

3.4 Chemical Structures of D-Captopril and PMIT

Two structurally distinct compounds were investigated: D-Captopril, selected as the reference ligand, and N4-(pyridine-3-yl)-5-methoxyisatin-β-thiosemicarbazone (PMIT), an independently designed thiosemicarbazone derivative (Figure 4). D-Captopril possesses a thiol-containing side chain and a proline-derived ring system, with the stereochemically defined SMILES representation C[C@H](CS)C(=O)N1CCC[C@@H]1C(=O)O. In contrast, PMIT was designed around a methoxy-substituted isatin framework bearing a pyridinyl-containing thiosemicarbazone moiety. Its supplied SMILES representation is COC1=CC2=C(C=C1)NC(=C2O)/N=N/C(=S)Nc1cccnc1. The structural features of both compounds were considered in the subsequent molecular docking analysis to compare their predicted binding orientations and interactions with NDM-1.

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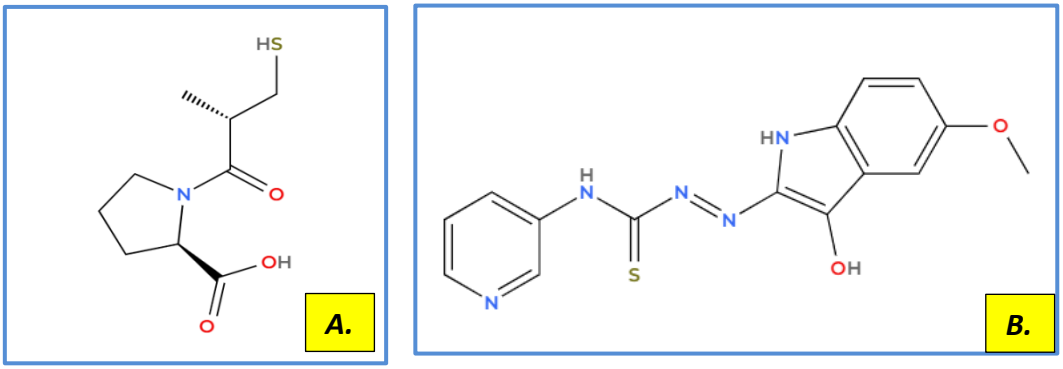

Figure 4. Chemical structures of (A) D-Captopril and (B) PMIT

3.5 Molecular Docking Results of NDM-1 with Reference and Candidate Compounds

3.5.1 Molecular Docking Results of Reference Ligand D-Captopril

Molecular docking of D-captopril against the NDM-1 crystal structure was performed using CB-Dock3. Five potential binding cavities (C1-C5) were identified, with predicted Vina score ranging from -4.6 to -8.4 kcal/mol (Table.1). Among these, cavity C5 exhibited the most favorable docking score (-8.4 kcal/mol), followed by C3 (-6.4 kcal/mol), C1 (-6.0 kcal/mol), C4 (-5.7kcal/mol) and C2 (-4.6 kcal/mol). The highest-ranked cavity, C5, had a calculated volume of 324Å^3 and was centered at coordinates (30, 15, 48), using a docking grid of 18x 18 x 18Å. Analysis of the highest-ranked docking pose revealed that D-captopril occupied a predicted binding region involving residues from chains A and C of the crystallographic NDM-1 model (Figure 5). The CB-Dock3 cavity report identified Asn142, Ala143, Leu144, Gln147, Leu161, Thr162, Phe163, Ala164, Gly167, Try184, Ser191, and Asp192 in chain A, together with the corresponding residues and Glu170 in chain C.

Table 1. Docking results of NDM-1 and D-Captopril complex cavities

CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
C5	-8.4	324	30, 15, 48	18, 18, 18
C3	-6.4	976	15, 23, 32	18, 18, 18
C1	-6.0	6137	17, 16, 22	35, 18, 18
C4	-5.7	860	39, 15, 29	18, 18, 18

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C2	-4.6	996	29, -1, 20	18, 18, 18

Pocket: C5 & Score: -8.4

Chain A: ASN142 ALA143 LEU144 GLN147 LEU161 THR162 PHE163 ALA164 GLY167 TYR184 SER191 ASP192

Chain C: ASN142 ALA143 LEU144 GLN147 LEU161 THR162 PHE163 ALA164 GLY167 GLU170 TYR184 SER191 ASP192

Two-dimensional interaction analysis indicated conventional hydrogen-bond contacts involving Gln147 and Phe163 of chain C, as well as Thr162 chain A. Additional noncovalent interactions included hydrophobic contacts classified as alkyl, π-alky1, and π-sigma interactions, involving residues in the surrounding binding region. Several neighboring residues contributed van der Waals contacts.

The three-dimensional protein-ligand visualization showed D-captopril positioned within the predicted cavity and surrounded by amino acid residues from the adjacent protein chains. Collectively, these docking results identified C5 as the highest-scoring predicted binding cavity for D-captopril under the applied computational conditions. However, the docking score represents a computational estimate rather than an experimentally measured binding affinity or confirmation of NDM-1 inhibition.

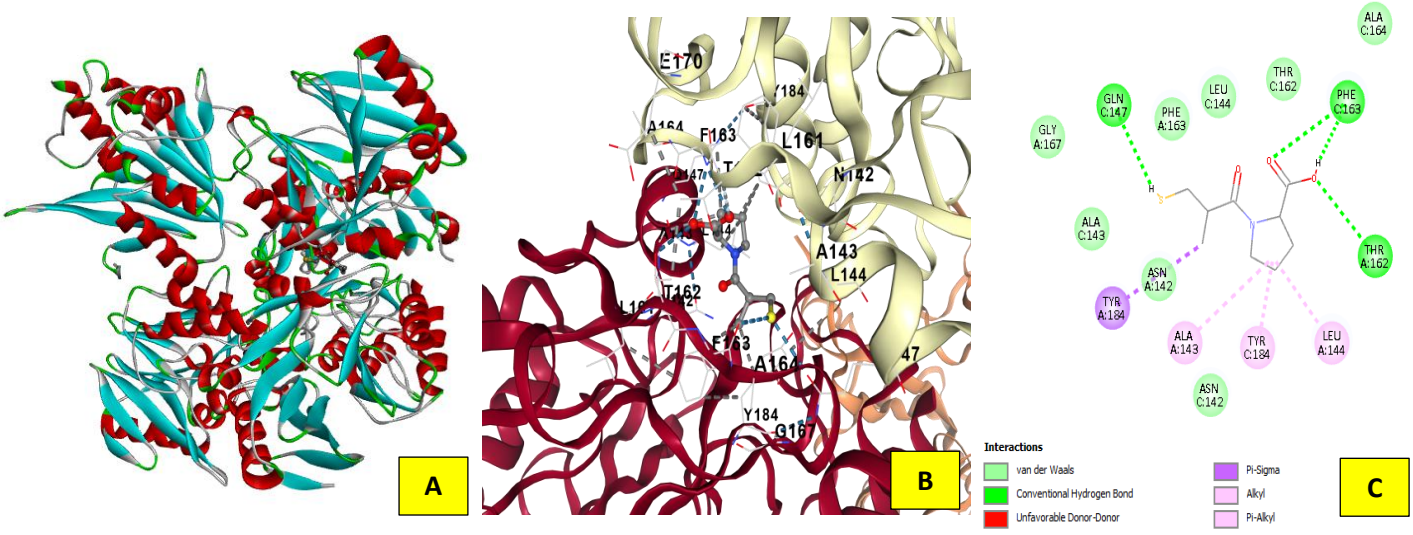

Figure 5. Predicted binding of D-captopril to NDM-1.

(A) Three-dimensional visualization of highest-ranked docking pose within the protein structure. (B) Ribbon structure of NDM-1 model. (C) 2D-dimensional interaction

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diagram showing the predicted hydrogen-bonding, hydrophobic and van der Waals contacts of D-captopril in cavity C5.

3.5.2 3.5.2 Molecular Docking Results of NDM-1 and PMIT

Molecular docking of the designed compound PMIT against the NDM-1 crystal structure identified five potential binding cavities, with predicted Vina scores ranging from -5.0 to -8.5 kcal/mol (Table 2). The most favorable docking score was obtained for cavity C3 (-8.5 kcal/mol), followed by C1 (-7.9kcal/mol), C4 (-6.9kcal/mol), C2 (-6.6kcal/mol), and C5 (-5.0kcal/mol). Cavity C3 had a calculated volume of 976 $Å^3$, with its center located at coordinates (15,23,32). The docking search box for this cavity measured 22 x 22 x 22 Å.

Table 2. docking of NDM-1 and PMIT complex cavities

CurPocket ID	Vina score	Cavity volume ($Å^3$)	Center (x, y, z)	Docking size (x, y, z)
C3	-8.5	976	15, 23, 32	22, 22, 22
C1	-7.9	6137	17, 16, 22	35, 22, 22
C4	-6.9	860	39, 15, 29	22, 22, 22
C2	-6.6	996	29, -1, 20	22, 22, 22
C5	-5.0	324	30, 15, 48	22, 22, 22

Pocket: C3 & Score: -8.5

Chain A: PRO185 GLY186 ALA231 SER232 ALA233 ARG234 ALA235 GLY237 ALA238 ALA239 PRO241 ARG256 ILE259 THR260 ALA263 ARG264 ASP267 LYS268 ARG270

Chain B: PRO150 GLN151 GLU152 GLY153

Chain C: GLN147 LEU148 GLN151

Chain D: ARG270

Chain E: GLY83 GLY84 ARG85 LEU102 ASN103 ILE105 LYS106 ASN110 LEU111 PRO112 VAL113 ALA114 ALA135 GLY136 ILE137 ALA138 ASN176

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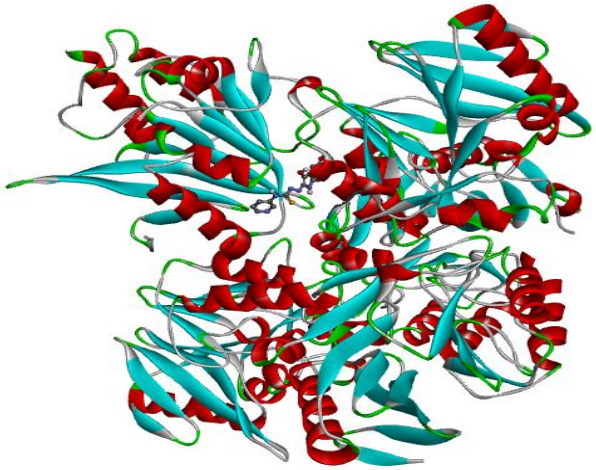
The highest-ranked docking pose placed PMIT within a binding region involving residues from multiple chains of the crystallographic NDM-1 model (Figure 6). The CB-Dock3 cavity report identified neighboring residues from chains A,B,C,D and E. Two-dimensional interaction analysis indicated conventional hydrogen-bond interactions involving Thr260 of chain A, together with Ala135 and Pro112 of chain E. The interaction diagram also displayed additional contacts classified as carbon hydrogen bonds, π-donor hydrogen bonds, π-sigma interactions, alkyl interactions, and π-alkyl interactions. Residues surrounding the ligand included Arg234, Ala238, Ile259, and Arg256 of chain A, together with Arg85, Leu102, Leu111, Val113, Gly136, Ile137 and Ala138 of chain E. Three-dimensional visualization further illustrated the orientation of PMIT with in the predicted cavity and its proximity to amino acid residues from e lowest predicted docking score nformation is the cavity that is p nd in a number of crystallograph g with its possible biological sign



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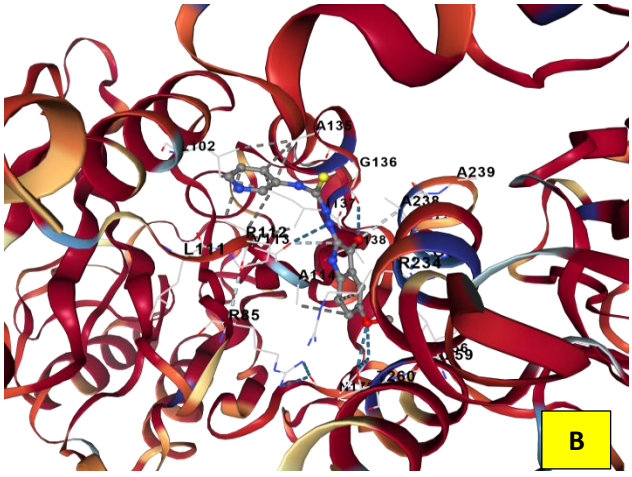

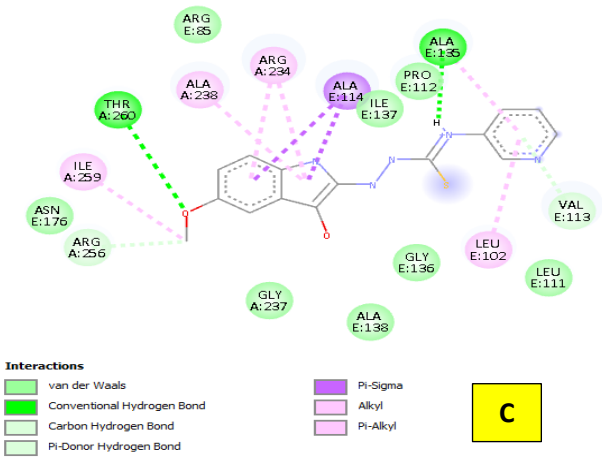

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Figure 6. Predicted molecular docking interactions of PMIT with NDM-1.

(A) Three dimensional of PMIT with C3 cavity. (B) Ribbon structure of NDM-1 model with PMIT docked ligand. (C) 2D-dimensional interaction diagram illustrating the predicted hydrogen bonding hydrophobic and other noncovalent contacts between PMIT and amino acid residues.

3.5.3 Comparative Molecular Docking Analysis of D-Captopril and PMIT

Comparative analysis of the molecular docking results revealed distinct binding preferences for D-captopril and PMIT within the NDM-1 crystallographic structure. Although both ligands were evaluated against the same protein model, their highest-ranked docking poses occupied different predicted cavities (Table 3). D-captopril exhibited its most favorable Vina score of -8.4kcal/mol in cavity C5, whereas PMIT achieved a slightly more negative score of -8.5kcal/mol in cavity C3. The numerical difference between these highest-ranked scores was 0.1 kcal/mol.

Table 3. Comparative molecular docking between D-captopril and PMIT scores

Cavity	Volume Å^3	D-captopril score	PMIT score
C1	6,137	-6.0	-7.9
C2	996	-4.6	-6.6
C3	976	-6.4	-8.5 *
C4	860	-5.7	-6.9
C5	324	-8.4 *	-5.0

The two ligands also differed in their predicted interaction patterns. In its highest-ranked pose, D-captopril formed conventional hydrogen-bond contacts involving Glin147 and Phe163 of chain C and Thr162 of chain A, alongside hydrophobic and van der Waals interactions with neighboring residues. By comparison, PMIT displayed conventional hydrogen-bond interactions involving Thr260 of chain A and Pro112 and Ala135 of chain E. Its interaction profile additionally included carbon hydrogen bonds, π-donor hydrogen bonds, π-sigma contacts, and alkyl and π-alkyl interactions, consistent with the presence of aromatic and heteroaromatic structural features in the designed ligand.

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The predicted binding environments were also structurally distinct. The highest-ranked D-captopril cavity (C5) had a volume of 324Å^3 and involved residues from chains A and C, whereas the highest-ranked PMIT cavity (C3) had a larger volume of 976 Å^3 and included neighboring residues from multiple crystallographic chains. These findings indicate that the two ligands adopted different predicted binding orientations and contact networks within the multi-chain NDM-1 model. Across the five investigated cavities, PMIT yielded more negative docking scores than D-captopril in C1,C2,C3 and C4 whereas D-captopril showed the more negative score in C5. However, differences in docking-box dimensions and binding-site locations limit direct quantitative interpretation of these scores.

4. Discussion

The present study integrated phenotypic antimicrobial susceptibility testing, molecular detection of Carbapenemase encoding genes, and structure-based molecular docking to investigate carbapenem resistance in clinical *Klebsiella pneumoniae* isolates and explore the predicted interactions of D-captopril and the designed compound PMIT with NDM-1. This combined approach provided complementary information on the resistance characteristics of the recovered isolates and the molecular behavior of the investigated ligands [21,22].

The antimicrobial susceptibility profiles demonstrated extensive resistance among the recovered *K. pneumoniae* isolates. All 54 isolates were resistant to imipenem, gentamicin, amikacin, and trimethoprim/sulfamethoxazole, while resistance to meropenem, chloramphenicol, and ciprofloxacin reached 90.7%, 77.8% and 70.4%, respectively. The simultaneous resistance to agents belonging to different antimicrobial classes indicates a substantial multidrug-resistance burden within the investigated collection. Particularly concerning was the high frequency of resistance to carbapenems, which are important therapeutic agent for severe infections caused by Gram-negative bacteria. The difference between imipenem and meropenem resistance frequencies also emphasizes that susceptibility to individual carbapenems should not be assumed to be identical, even within the same bacterial population [21,22].

Molecular screening of 41 selected isolates demonstrated the presence of several clinically relevant Carbapenemase encoding genes. The *blaIMP* gene was detected in all screened isolates (100%), whereas *blaNDM-1* and *blaOXA-48-like* were identified in 36.6% and 26.8%, respectively. The high blaIMP prevalence indicated that blaIMP

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determinant might be a significant part of the resistance background in this sampled collection. However, the reported detection frequencies cannot be taken as a representation of general prevalence, as a subset of the recovered isolates were screened for the presence of molecular types of the Carbapenemase families. A considerable geographical and bacterial population variation in the distribution of Carbapenemase families has also been previously documented [22].

The detection of *blaNDM-1* is particularly relevant because NDM-1 is a zinc-dependent class B Metallo-β-lactamase capable of hydrolyzing a broad range of β-lactam antibiotics, including carbapenems. Its catalytic activity depends on a metal-associated active site situated within the characteristic αβ/βα structural fold. The presence of *blaNDM-1* in 15 screened isolates therefore identifies a clinically important resistance determinant and provides a rationale for examining NDM-1 as a potential molecular target. Nevertheless, PCR detection establishes the presence of the target genetic sequence and does not, by itself, quantify enzyme expression or demonstrate its individual contribution to the observed resistance phenotype [23,24].

The structural examination of NDM-1 using the crystallographic model the arrangement of α-helices, β-sheets and connecting loops that form the enzyme overall architecture. These structural features are relevant to substrate recognition and catalytic function, particularly in the vicinity of the metal-dependent active site. Structural studies have established that the NDM-1 catalytic region contains conserved residues involved in coordinating divalent ions. Accordingly, evaluation of candidate inhibitors should consider not only their predicted docking scores but also whether their binding poses are located near functionally important catalytic residues and metal-binding regions [23,24].

The molecular docking analysis revealed distinct predicted binding preferences for D-captopril and PMIT. D-captopril achieved its most favorable Vina score of -8.4 kcal/mol in cavity C5, whereas PMIT produced a score of -8.5 kcal/mol in cavity C3. Although PMIT exhibited a slightly more negative highest-ranked score, the difference of 0.1 kcal/mol is too small to support a meaningful conclusion regarding relative binding affinity or inhibitory potency. Furthermore, these values were obtained for different predicted cavities and docking-box dimensions, limiting their direct quantitative comparability. Such limitations are consistent with previous evaluations

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demonstrating that docking scoring functions cannot reliably substitute for experimental measurements of binding affinity [25].

The two compound also had different predicted interaction profile. The hydrogen-bond contacts of D-captopril are typical for the class of compounds, featuring contacts with Glin147, Phe163, and Thr162, as well as a few hydrophobic and van der Waals contacts. In contrast, PMIT predicted hydrogen-bond interactions between Thr260, Pro112, and Ala135, as well as a number of aromatic and hydrophobic contacts.

These differences may reflect the distinct molecular architectures of the ligands, including the relatively compact thiol-containing structure of D-captopril and the larger heteroaromatic framework of PMIT. However, the number or diversity of predicted contacts should not be interpreted independently as evidence of stronger biological activity. Previous investigations of D-captopril derivatives and thiosemicarbazone-based compounds support the relevance of these chemical frameworks to NDM-1 inhibitor research, although their reported experimental activities cannot be directly attributed to PMIT [16,17,25].

An important consideration is that highest-ranked docking cavities involved residues from more than one chain of crystallographic NDM-1 model. The D-captopril binding region involved chains A and C, whereas the PMIT binding region included residues from several crystallographic chains. Such multi-chain binding environments may reflect interfaces associated with crystal packing rather than physiological relevant ligand-binding sites. Consequently, the predicted poses cannot be assumed to represent direct interactions with the NDM-1 catalytic center. This distinction is particularly important for a Metallo-enzyme whose catalytic mechanism depends on metal coordination and specific active-site residues [23,24, 25].

Further studies should focus on docking onto a biologically relevant NDM-1 monomer, taking into account the catalytic metal ions, and subsequently binding-site validation and experimental testing of the potential of the inhibitors to inhibit the enzyme. Whether the observed interactions are a result of biochemical inhibition and lead to measurable biological activity would require biochemical inhibition assays (where applicable) and antimicrobial susceptibility testing (AMST) in bacterial strains producing NDM. Experiments with NDM-1 inhibitors previously have shown the usefulness of using biochemical inhibitor measurements along with microbiological testing [16,17,26].

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Overall, the finding highlight a substantial antimicrobial resistance burden among the investigated *K. pneumoniae* isolates and demonstrate the occurrence of clinically relevant Carbapenemase genes, including *blaNDM-1*. The docking results further reveal distinct predicted binding patterns for D-captopril and PMIT with the NDM-1 crystallographic model. Although these observations provide a preliminary structural basis for further investigation, they do not establish PMIT as a superior NDM-1 inhibitor [26-28].

5. Conclusions

This study showed a significant problem with antimicrobial resistance with clinical Klebsiella pneumoniae (KKP) exhibiting high levels of resistance to carbapenems and other antimicrobial classes. blaIMP was the predominant Carbapenemase determinant detected, with blaNDM-1 and blaOXA-48-like also being detected in the molecular screening of selected isolates comprising multiple clinically important Carbapenemase determinants. Molecular docking using the crystallographic model of the NDM-1 enzyme also showed different predicted binding modes of D-captopril and designed compound PMIT. PMIT exhibited a highest-ranked Vina score of -8.5 kcal/mol, closely comparable to the -8.4 kcal/mol obtained for D-captopril, although the corresponding poses occupied different predicted cavities. These findings suggest that PMIT warrants further investigation as a in silico identified ligand rather than an experimentally validated NDM-1 inhibitor. Importantly, the multi-chain binding environments and differences in docking conditions limit conclusions regarding relative inhibitory potency. Overall, the integrates clinical resistance surveillance with preliminary structure-based ligand assessment and provides a foundation for future active-site-focused in silico studies and evaluation of NDM-1 inhibition.

Highlights

- Clinical *Klebsiella pneumoniae* isolates exhibited extensive antimicrobial resistance.
- *blaIMP* predominated among screened isolates, with additional detection of *blaNDM-1* and *blaOXA-48-like*.

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- D-captopril and the designed PMIT compound displayed distinct predicted binding patterns with NDM-1.
- PMIT and D-captopril showed closely comparable highest-ranked docking scores (-8.5 and -8.4 kcal/mol, respectively).
- Experimental Validation is required to establish the inhibitory potential of PMIT against NDM-1.

Declaration

Author Contributions

The sole author was responsible for the conceptualization and design of the study, methodology, investigation, experimental work, data collection, formal analysis, molecular docking, visualization, manuscript preparation, and critical revision.

Acknowledgments

The author wish to express their sincere appreciation to Department of Medical Microbiology, the College of Medicine, University of Kufa, Najaf, Iraq, for its valuable cooperation and for facilitating the experimental work at the Postgraduate Molecular Biology Laboratory.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to applicable ethical and institutional restrictions.

Conflicts of interest

The author declare that they have no conflicts of interest.

Funding

This research received no external funding.

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