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ORIGINAL ARTICLE

Evaluation of The Efficacy of Ceftaroline and Ceftobiprole Against PBP2a in Methicillin-Resistant *Staphylococcus aureus*: New Insights from Molecular Modeling

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KEYWORDS

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ABSTRACT: Penicillin-Binding Protein 2a (PBP2a) is the primary cause of bacterial resistance to beta-lactam antibiotics such as methicillin. PBP2a mutations produce structural alterations that reduce the antibiotic's efficacy. Fifth-generation antibiotics, such as ceftaroline and ceftobizole, have a high affinity for binding to PBP2a, making them effective treatments for resistant Staphylococcus aureus. The purpose of this study was to assess the influence of PBP2a mutations and the efficacy of ceftaroline fosamil and ceftobiprole when administered in combination as a novel therapeutic approach utilizing current molecular models**.** RCSB PDB data were utilized to select the wild-type strain 1VQQ and the mutant 4CJN, as well as to examine their mutations (E246G, N146K, N204K) in three dimensions using PyMol. The effect of the changes on binding energy was assessed using mCSM-PPI2 and mCSM-AB methods, and molecular docking simulations of ceftaroline and ceftobiprole were carried out using PyRx and AutodockVina to determine binding energy and active sites. The findings revealed that the bulk of mutations, including E246G, E239K, and N146K, decreased protein affinity, whereas mutation N204K increased affinity. Molecular research revealed that coadministration of ceftaroline and ceftobifole considerably reduced ceftaroline efficacy, while ceftobiprole efficacy was only slightly raised or unaffected in both wild-type and mutant strains. These findings point to an overlapping action between the two antagonists at the active and inactive locations. The combination of ceftaroline and ceftobiprol has adverse effects on antibiotic binding to PBP2a, confirming that they cannot be administered effectively together.

INTRODUCTION

The penicillin-binding protein 2a named PBP2a emerges from *Staphylococcus aureus* bacterial strain. The protein plays an essential role in developing resistance against all beta-lactam antibiotics including methicillin. The main role of PBP2a is to accelerate peptidoglycan linkage synthesis in order to generate bacterial cell walls. PBP2a distinguishes itself from other PBP proteins by having poor affinity with beta-lactam antibiotics so bacteria remain viable when exposed to these medications [1, 2].

Bacterium PBP2a obtains its genetic sequence from the mobile element SCCmec where it carries the mecA gene. Horizontal gene exchange through this genetic element

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enables the transmission of resistance within MRSA bacterial strains [3]. Altered PBP2a structures cause changes in its tertiary configuration leading to weakened beta-lactam binding sites that maintain enzymatic wall-building capabilities [4, 5].

MRSA cases that are resistant to methicillin often produce severe diseases such as pneumonia together with sepsis and skin infections [6]. Laboratories employ two analytical methods to detect PBP2a which helps identify MRSA cases through polymerase chain reaction (PCR) to detect mecA and latex agglutination assays to identify the protein [7, 8].

Therapeutic options are limited for MRSA because its resistance includes an ability to evade penicillins and cephalosporin beta-lactam antibiotics's effect on PBP2a. The rise of VRSA makes treatment more challenging because healthcare providers use alternative medicines including vancomycin and daptomycin. New fifth-generation cephalosporins including ceftaroline were developed to attach better to PBP2a [9,10].

Researchers are now working on developing compounds that disrupt PBP2a activity, such as allosteric inhibitors, which modify the enzyme structure and make it sensitive to beta-lactam antibiotics. One interesting technique is to mix beta-lactams with enzyme inhibitors (such as avibactam) to increase efficacy. X-ray crystallography is also being utilized to better understand PBP2a-drug interactions [11, 12].

The interaction of PBP2a with beta-lactam antibiotics receives different effects from mutations based on where the changes occur in its structure. The active site mutations modify the structural arrangement which binds antibiotics to bacteria proteins until the bonds become too weak or too strong. The E246K mutation creates an alteration between glutamic acid at position 246 and lysine that produces disruptions to active site conformation thus affecting beta-lactam inhibitory functions [1, 4].

A mutation within an allosteric domain works against signal transmission toward the active site resulting in imbalance between active and inactive protein states. Disease-associated resistance then develops with the T446I mutation (threonine substitution of isoleucine at position 446) because this variant maintains a "closed" protein state for reduced medication effectiveness [6, 11].

The stability and biochemical relationships formed by proteins might be altered by changes in their secondary binding regions. A PBP2a structural stability decline occurs because of the G246E mutation that replaces glutamic acid with glycine leading to shortened half-life and potential changes in catalytic activity. The combined mutations actively create bacterial resistance which presents challenges to treatment procedures [7, 5].

Ceftaroline fosamil makes up a fifth-generation cephalosporin antibiotic which targets MRSA and similar resistant microorganisms. The antibiotic shows weak binding properties with most β-lactam antibiotics thus reducing their antibiotic potential. The modified chemical design of Ceftaroline fosamil helps it identify PBP2a protein more effectively thus allowing it to stop this specific protein that damages bacterial cell walls regardless of MRSA resistance. Minimal resistance against ceftaroline fosamil emerged through rare mutations taking place inside the mecA gene. FDA approved this drug in 2010 using clinical trials to verify its effectiveness but healthcare providers should use it with caution under medical supervision to stop resistance from developing [13, 14].

Ceftobiprole belongs to the fifth-generation cephalosporins which show strong activity against both MRSA and specific Gram-negative bacterial strains. It functions through binding to bacterial protein-binding proteins (PBPs) that include PBP2a which prevents cell wall creation thus leading to bacterial death. Ceftobiprole physicians employ to treat both hospital-acquired pneumonia and medical device-associated infections which represent severe systemic conditions. This antibiotic proves useful for resistant bacterial infections alongside severe bacterial infections but doctors need to exercise caution when administering these medications. There is typically no need to combine ceftaroline with ceftobiprole for treatment [15, 16].

The current research investigated PBP2a mutant analysis and presented findings about combining ceftaroline fosamil

and ceftobiprole therapy through modern molecular modeling methods.

MATERIALS AND METHODS

The RCSB PDB data was used to pick the wild type of PBP2a, which has 646 amino acids with ID 1VQQ. The mutant strain of PBP2a was selected in the active site residue (E246G) in the strain ID 4CJN. For the allosteric domains region, the mutation residue (N١46K) was selected. The position mutations from the secondary binding region, N146K, and N204K were assigned.

Each mutation's placement within the full 3D crystal structure of PBP2a was identified after listing each strain individually using PyMol. To assess the effect of mutations on the whole protein, mCSM-PPI2 and mCSM-AB technologies were used [17]. It was created to reliably and efficiently study the influence of missense mutations on protein-protein affinity interactions by utilizing advanced approaches from the network of non-covalent interactions between protein residues (such as hydrogen and hydrophobic interactions). To quantify the structural changes caused by the mutation, we apply better graph-based signatures and graph kernel techniques. Furthermore, the server uses evolutionary information to identify important areas of the protein, as well as complicated network metrics to assess the importance of each amino acid in the interaction's stability.

The crystal structures of ceftaroline fosamil and ceftobiprole were extracted from PubChem using CIDs (9852981, 135413542), respectively. Both antibiotics are the most potent against mutant S. aureus strains. Accordingly, PyRx and AutodockVina were used in this study to determine their binding nature to the mutant strain (4CJN) compared to the wild-type strain (1VQQ), separately. The dual molecular docking of the two antibiotics with the wild-type and mutant strains was measured, the binding affinity was determined, and the free energy was calculated. The novel docking sites were identified, and the binding score was calculated using PyMol.

RESULTS

The findings revealed that the majority of the mutations tested lowered protein affinity. Specifically, the alterations E246G, E239K, and N146K lowered affinity. However, mutation N204K had the reverse effect: it increased affinity, Table 1.

Table 1. Analysis of Wild-type and mutant residue characteristics in PBP2a.

Index	Chain	Wild residue	Binding residues	Residue position	Mutant residue	Binding residues	RSA (%)	ΔΔG	Outcome
1	A	GLU	TYR243, PRO244, ALA248, THR249,	246	GLY	TYR243, PRO244, ALA248, THR249, SER250	79.9	-0.211	Reduced affinity
3	A	GLU	THR156, GLY166, LYS317	239	LYS	THR156, GLY166	46.9	-0.306	Reduced affinity
4	A	ASN	LYS148, ASP295	146	LYS	Null	68.4	-0.003	Reduced affinity
5	A	ASN	VAL206	204	LYS	VAL206	117.2	0.251	Increased affinity

RSA: Relative Surface Access, ΔΔG: Change in binding energy

The E246G mutation occurs at position 246, when glutamic acid is replaced by glycine. The wild-type residues were originally bound by polar, hydrogen, and clash bonds (TYR243, PRO244, ALA248, and THR249). The mutation preserved these linkers while adding a new Van der Waals (VDW) bond to SER250. This mutation has a relative surface accessibility (RSA) of 79.9%, indicating a high level of surface exposure. A ΔΔG value of -0.211 shows a decrease in binding energy, negatively impacting protein binding (lower affinity), Figure 1-A.

The E239K mutation involves replacing glutamic acid with lysine at position 239 of the protein. The wild-type residues were initially bound via polar, hydrogen, and ionic connections to THR156, GLY166, and LYS317, but the mutation limited the binding to only THR156 and GLY166. The RSA of 46.9% implies a low level of surface exposure. The ΔΔG value of -0.306 indicates a negative influence on protein binding, resulting in lower affinity, Figure 1-B.

Mutation N146K: At position 146, asparagine is replaced by lysine. The wild-type residues were initially bound by polar, hydrogen to LYS148 and ASP295, but the mutation completely eliminates the binding, indicating a dramatic shift in the environment surrounding these residues. The RSA is 68.4%, and the ΔΔG value of -0.003 suggests a minor effect on the binding energy, which results in a slightly lower affinity, Figure 1-C.

The N204K mutation is situated at position 204 and swaps the amino acid asparagine with lysine. The ASN204 residue was initially linked to polar and VDW in PBP2a wild-type with VAL206. The mutant residue retains the same interaction with VAL206 via polar and clash bonds. The extremely RSA of 117.2% indicates a high level of surface exposure. A ΔΔG value of +0.251 shows an increase in binding energy, resulting in a positive effect on binding (higher affinity), Figure 1-D.

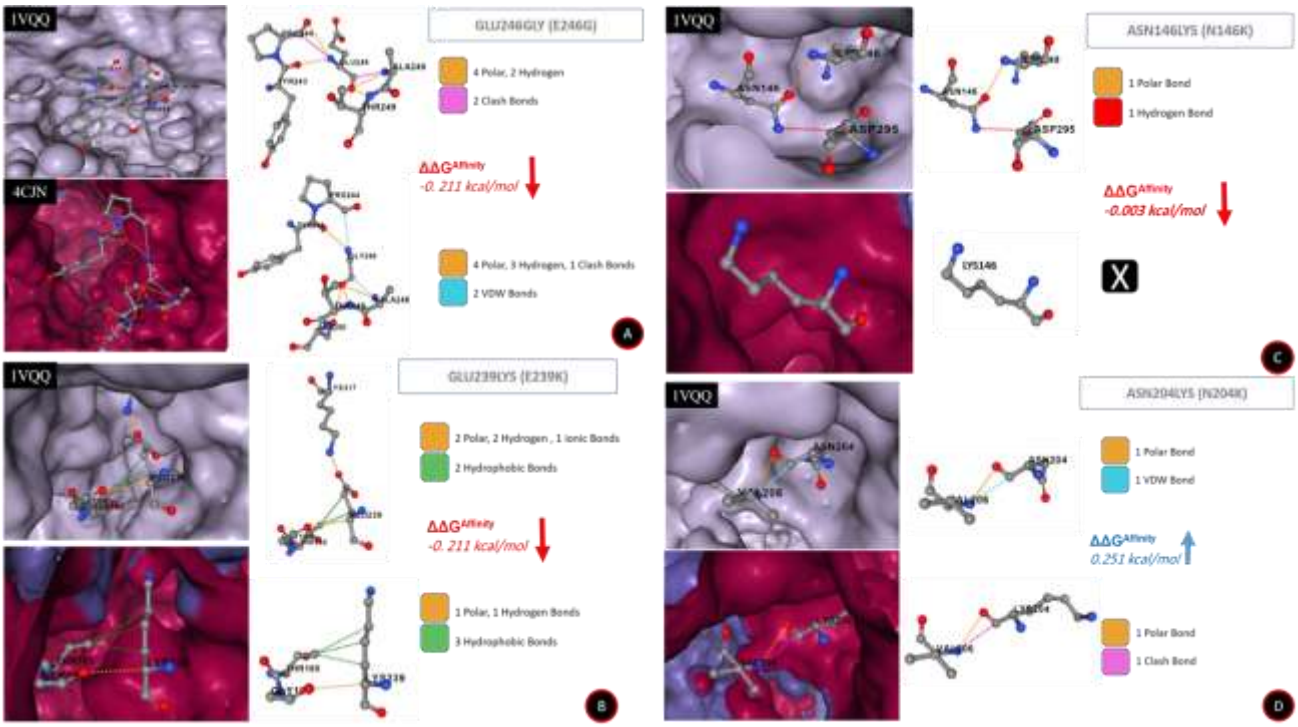

Figure 1. PPI interaction between PBP2a (1VQQ) wild type and mutant. A: Mutation E246G: The impact of structural alterations on protein binding and surface energy in 1VQQ and mutant starin (4CJN). B: E239K mutation disrupts key interactions and decreases protein binding affinity. C: Structural analysis of the N146K mutation resulted in complete elimination of binding interactions. D: N204K mutation preserves interaction with VAL206 and increases binding affinity.

This study looked at the molecular docking mechanism between the wild-type strain (1VQQ) and the mutant strain (4CJN) using ceftaroline. The results showed that the GLU239 residue is a key site for ceftaroline binding in the wild-type strain, with chemical interactions (such as charged bonds, hydrogen bonds, and pi-alkyl bonds) allowing for successful anchoring within the active site. However, the mutation in the 4CJN strain resulted in a loss of ability to attach to this specific location, with the binding site migrating to another residue and poor interactions (shown in red in Figure 2).

Figure 2-A shows that the wild-type strain's binding is characterized by the diversity and strength of chemical bonds, whereas Figure 2-B shows a significant decrease in binding energy (energy loss) and weakness in interactions with the mutant strain, as they were limited to weak charged bonds and a single carbon-hydrogen bond with Pi-Alkyl, which explains the antibiotic's decreased effectiveness.

The molecular docking data revealed a significant difference in ceftaroline binding between the wild-type (1VQQ) and mutant (4CJN) strains. In the wild-type strain, model M1 had the maximum binding affinity (-9.2 kcal mol^{-1})(-38.5 kJ mol^{-1} and zero RMSD (Upper/Lower bound = 0), indicating excellent stability in the active site. Other models (M2-M4) saw a steady decline in affinity (up to −35.514kJ mol^{-1}) and a significant increase in RMSD values (e.g., M2: ~50.91/48.17 Å), indicating crystallographic instability. The mutant strain (4CJN) had greater binding affinity values in models M1-M3 (−39.748 kJ mol^{-1}) and lower RMSDs in M2 (6.49/2.82 Å), but showed significant heterogeneity in the binding pattern (particularly in M3-M4, with RMSDs of ~36.25-24.36 Å). These discrepancies suggest that the mutation may cause structural changes that impair optimal ceftaroline stabilization, despite the apparent affinity improvement in some models, necessitating a more in-depth examination of the chemical interactions and the mutation's effect on binding thermodynamics.

The comparison of docking results and crystallographic deviations (RMSD) for both the wild-type and mutant PBP2a with ceftaroline is presented in Table 2 using the models M1–M4. The findings suggest that PBP2a mutants exhibit lower flexibility which may explain their different interactions with antimicrobial drugs.

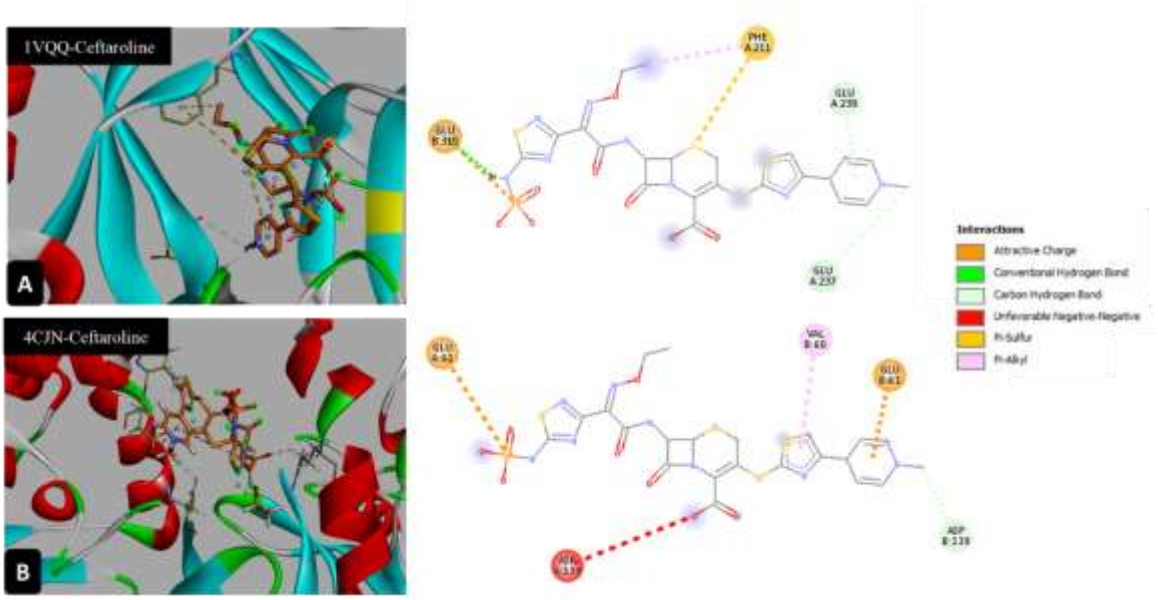

Figure 2. Detailed analysis of the molecular docking interactions between Ceftaroline and 1VQQ/4CJN, with emphasis on key bonds.: A: 1VQQ-Ceftaroline. B: 4CJN-Ceftaroline.

Table 2. Comparison of molecular docking affinity and crystallographic position deviations (RMSD) between the wild-type (1VQQ) and mutant (4CJN) PBP2a with ceftaroline across Multiple Models (M1-M4).

Docking: PBP2a-Ceftaroline		Binding affinity	RMSD/upper bound	RMSD/ lower bound
1VQQ-Ceftaroline	M1	-9.2	0	0
	M2	-9.1	50.91	48.17
	M3	-8.5	14.31	10.91
	M4	-8.5	49.15	45.85
4CJN-Ceftaroline	M1	-9.5	0	0
	M2	-9.5	6.49	2.82
	M3	-9.5	36.25	32.34
	M4	-9.2	26.01	24.36

The best docking models between the 1VQQ and the mutant strain (4CJN) with Ceftobiprole have an RMSD value of zero, as shown in Table 3. Model M1 has the maximum binding affinity to the 1VQQ at -8.3 kcal mol^{-1}, while model M4 has the lowest binding affinity at -8.0 kcal mol^{-1}. Similarly, for the mutant strain (4CJN), the model M1 has a binding affinity of -8.0 kcal mol^{-1}, while model M4 has a lower affinity of -7.8 kcal mol^{-1}.

Molecular docking research of ceftobiprole with both 1VQQ and 4CJN strains indicates intricate interactions involving several types of bonds, which contribute to the drug-protein combination's stability. Ceftobiprole interacts with residues PHE B:211 and HIS B:232 via van der Waals bonds in the wild-type strain, while THR A:312 and ASN A:111 form conventional hydrogen bonds, ARG A:110 and ASN B:177 form carbon-hydrogen links, and pi-alkyl bonds. Residues GLN A:353, THR A:354, ASN A:540, VAL A:557, GLU A:356, HG A:351, and LE A:666 interact with ceftobiprole via van der Waals bonds, while they form conventional hydrogen bonds with ASN B:665, THR B:354, ASN B:555, ASP A:665, and ASP A:667, as well as carbon-hydrogen bonds with ASP B:665. These various interactions contribute to ceftobiprole's binding strength to both strains, while there are minor changes in the types and distribution of bonds between the two strains, which may have an impact on the drug's effectiveness, Figure 3.

Table 3. Comparison of molecular docking affinity and crystallographic position deviations (RMSD) between the 1VQQ and 4CJN PBP2a with ceftobiprole across multiple models (M1-M4).

Docking: PBP2a-Ceftobiprole		Binding affinity	RMSD/upper bound	RMSD/ lower bound
1VQQ-Ceftobiprole	M1	-8.3	0	0
	M2	-8.2	39.61	35.39
	M3	-8.1	39.32	22.94
	M4	-8	24.17	21.76
4CJN-Ceftobiprole	M1	-8	0	0
	M2	-7.9	36.98	34.04
	M3	-7.9	11.4	37.68
	M4	-7.8	11.46	39.86

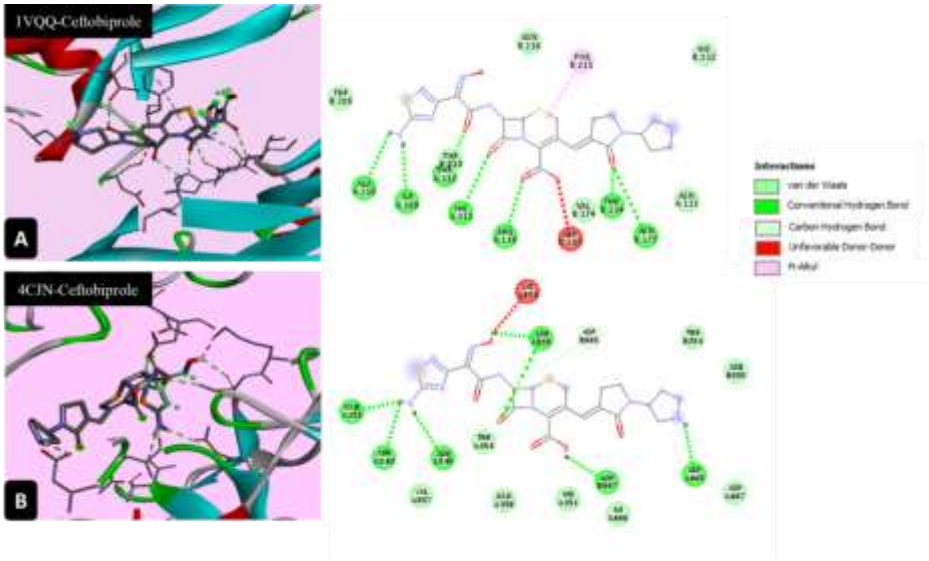

Figure 3. Detailed analysis of the molecular docking interactions between Ceftobiprole and 1VQQ/4CJN, with emphasis on key bonds.: A: 1VQQ-Ceftobiprole. B: 4CJN-Ceftaroline.

Analysis of dual docking combination

The data 1VQQ strain demonstrate that the binding energy of ceftaroline decreased when supplied with ceftobiprole, as evidenced by a fall in the binding affinity from -9.2 (when administered alone) to -8.7 in the M1 model. Ceftobiprole's binding energy increased significantly when combined, from -8.3 to -8.5. This shows that providing the two antibiotics concurrently may unintentionally impair the response to ceftaroline while having a limited favorable effect on ceftobiprole.

For the mutant strain (4CJN), providing both antibiotics resulted in a considerable drop in ceftaroline binding energy (from -9.5 alone to -8.6 in the M1 model). Ceftobiprole's binding energy remained practically unchanged (-8.0 both alone and in combination with ceftaroline). This shows that combining the two antibiotics may lower the efficacy of ceftaroline while maintaining the efficacy of ceftobiprole in the mutant strain, Table 4.

Figure 4 compares the binding of the antibiotics ceftaroline and ceftobiprole to both 1VQQ and 4CJN when administered together. The interaction occurs at the non-active site, as suggested by important binding amino acid residues such as ARG110, ASN204, and GLU246. The varying colors in the molecular depiction show atom locations and bond types, such as hydrogen bonds and hydrophobic interactions.

Table 4. Estimation of the binding affinity of antibiotics when administered alone or in combination with the targets 1VQQ and 4CJN.

Strain	Model	Binding affinity (Ceftaroline)	Binding affinity (Ceftaroline + Ceftobiprole)	Binding affinity (Ceftobiprole)	Binding affinity (Ceftaroline + Ceftobiprol)
1VQQ	1	-9.2	**-8.7**	-8.3	**-8.5**
	2	-9.1	**-8.7**	-8.2	**-8.4**
	3	-8.5	**-8.5**	-8.1	**-8.3**
	4	-8.5	**-8.4**	-8	**-8.3**
4CJN	1	-9.5	**-8.6**	-8	-8
	2	-9.5	**-8.3**	-7.9	-7.9
	3	-9.5	**-8.2**	-7.9	-7.9
	4	-9.2	**-8.1**	-7.8	-7.8

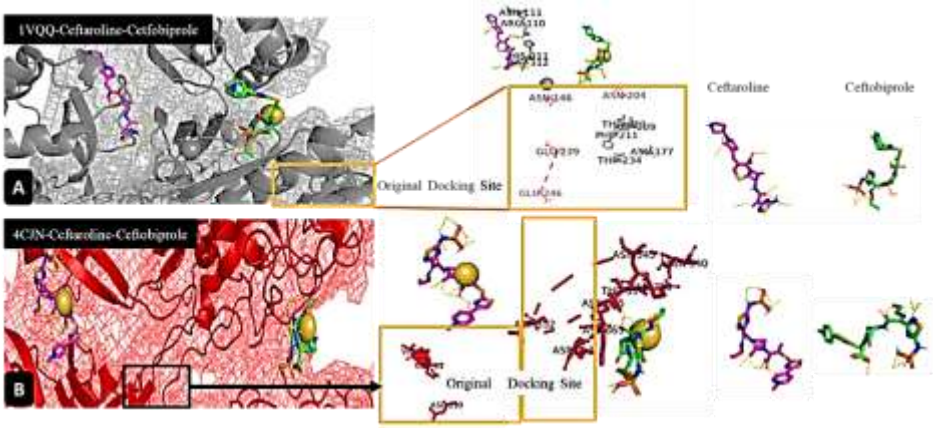

Figure 4. Dual molecular docking shows differences in the binding of ceftaroline and ceftobiprol to wild and mutant strains.

DISCUSSION

Protein affinity decreases as a result of most examined mutations according to the findings. The E246G and E239K mutations altered the active site chemical bonds in protein structure thus decreasing protein binding strength and structural integrity. The N146K mutation caused protein affinity reduction as it broke critical bonds yet it did not change the overall energetic binding.

The N204K mutation produced an improved binding affinity through the combination of maintained earlier bonds with newly formed ones. The N204K form delivered a distinctive beneficial change that stood out from other mutations because of how it affected binding relationships through its particular surface location. Analysis results show that mutations produce major changes in both damaged bonds structure and the local protein active region environment.

According to a previous study, mutations in protein active-binding regions alter the shape of molecular bonds such as hydrogen bonds and polar interactions, influencing protein stability and binding affinity. Mutations can cause the loss of critical bonds or the formation of new bonds, enhancing or weakening drug interaction [18].

Relative surface area analysis (RSA) has showed that regions with high surface exposure are more prone to alterations. Changes in RSA can help to improve or decrease accessibility to the active site, influencing drug action [19, 20].

The binding principles for ceftaroline between the wild-type strain (1VQQ) and mutant strain (4CJN) demonstrate opposite mechanisms. Drug binding stability in the wild-type strain occurs at GLU239 where various chemical interactions including charged particles along with hydrogen bonds and Pi-Alkyl bonds maintain the antibiotic position in the active site. The genetic mutation in the mutant strain destroyed binding potential at GLU239 so the active site relocated to adjacent residues with diminished and less useful binding connections. The wild-type strain presented stronger binding interactions through Figure 2-A but the mutant strain demonstrated weaker bond strength and decreased interaction efficiency according to Figure 2-B.

The binding patterns of ceftobiprole remained consistent across both wild-type and mutant strains then the M1 form demonstrated the strongest binding strength. Strong binding stability results from different bonds between compounds which incorporate van der Waals forces together with hydrogen bonds and pi-alkyl bonds. The differences in bond distribution between the strains produced minor changes in drug efficacy levels compared to ceftaroline binding. Drug binding stability shows sensitivity to mutations which lead to structural changes thereby requiring more investigation for understanding molecular interactions and thermodynamic effects resulting from these mutations.

The medical effectiveness and stability of a compound can be substantially influenced by protein active binding region mutations as they modify both hydrogen bonds and van der Waals bonds. Placing or losing specific bonds in the active site through mutations could lower binding strength but adding new bonds would enhance structural stabilization according to study findings. The influence of mutations depends on their nature along with their position according to the protein's active site binding region [21, 22].

Scientific data reveals that the joint use of ceftaroline and ceftobiprole exhibits divergent effects on binding energy analysis of wild-type (1VQQ) and mutant (4CJN) strains. The dual antibiotic treatment weakened ceftaroline action yet it produced a minimal enhancement in ceftobiprole binding capability in the wild-type bacterial strain. Distribution of binding energy in cefaroline treatment decreased at a higher rate than ceftobiprole concentrations in mutated bacteria. The chemical interactions as shown in the image demonstrate both hydrogen bonds and hydrophobic interactions relocating toward inactive areas which causes decreased activity of the primary antibiotic when taken with other antibiotics thus suggesting an unfavorable communication.

Research studies show that combined usage of antibiotics ceftaroline with ceftobiprole disrupts active protein areas which decreases medication potency. Research demonstrates that PBPs mutations alter the bonds between penicillin and its proteins mainly through affecting hydrogen and van der Waals bonds which ultimately decreases antibiotic joint effectiveness. The improvement of one antibiotic's binding stability by certain modifications can lead to reduced effectiveness of the other antibiotic mainly in vital active areas. Molecular interaction research requires deeper investigation because it helps prevent adverse drug effects on treatment performance [23-26].

The joint administration of these antibiotics created substantial alterations to the enzyme active site for wild-type and mutant strains of the protein. Computational molecular research revealed that antibiotic affinity toward the active site decreased while a substantial shift occurred in its site of binding [27, 28].

The study shows genetic mutations alone do not lead to antibiotic binding site modifications because two antibiotic exposures can produce equivalent effects that may become problematic for treatment. The present study verifies previous research by demonstrating beyond doubt that administering ceftaroline and ceftobiprole together would be unsuitable.

CONCLUSIONS

PBP2a represents a major challenge for antibacterial treatment yet understanding its mechanism produces potential opportunities for developing intelligent medication solutions. The joint cooperation between researchers and medical staff represents a fundamental requirement for resolving the antibiotic resistance threat. The alteration made to the antibiotic binding site results not just from genetic mutations but possibly emerges from combining different antibiotics without desired outcomes. The findings of this study together with past research establish that both ceftaroline and ceftobiprole should not be administered simultaneously.

Conflict of interest

The author declares no conflict of interest in this study.

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