IN-SILICO IDENTIFICATION OF STRUCTURAL CHANGES AND MOLECULAR INTERACTIONS OF MUTATIONS IN MULTIPLE DRUG RESISTANT (MDR) BACTERIA

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Abstract

Introduction: Multiple Drug Resistance (MDR) in bacteria is a significant global health challenge, reducing the effectiveness of antibiotics and necessitating the discovery of alternative therapeutic strategies. Understanding the molecular basis of resistance at a structural and functional level is crucial for developing novel drug targets. Computational approaches provide efficient methods for analysing bacterial resistance proteins, identifying mutational impacts.

Methodology: This study utilizes an in-silico approach to analyse structural and functional changes in multiple drug-resistant (MDR) bacterial proteins. ***UniProt*** *and* ***PDB*** *were used to retrieve protein sequences and structures, while* ***PubChem*** *provided 3D antibiotic ligand structures.* ***ClustalW*** *performed sequence alignment, and* ***Chimera*** *enabled structural visualization.* ***AutoDock Vina*** *was used for molecular docking to assess protein-ligand interactions.* ***Protein Plus*** *calculated surface area and volume, while* ***I-Mutant*** *predicted stability changes.* ***Ramachandran Plot*** *validated structural integrity,* ***ProtParam*** *analysed.*

Conclusion This research demonstrates how mutations in MDR bacterial proteins influence antibiotic binding, stability, and resistance. Some mutations promote thermostability, while others increase resistance significantly and decrease drug effectiveness, such as in the case of methicillin (D → G) and ceftazidime (L → N). These results underscore the intricate balance between structural change and drug interaction and demonstrate the necessity of pharmacogenomic strategies to combat resistance. Experimental validation and computer-aided drug design must be the focus of future research to produce precision antibiotics and personalized antimicrobial treatments.

Keywords: *Multi- Drug Resistance, Pharmacogenomics, Computational Tools, Thermostability.*

1. Introduction

Multiple Drug Resistance

After penicillin was discovered in 1928 several more antibiotics were found and brought into commercial production. We now take it for granted that antibiotics can treat any infectious illness. The use of antibiotics has greatly affected the bacterial life and approximately 100,000 tons per year are produced all over the world. Some pathogenic

strains are resistant to antibiotics while others are multidrug-resistant i.e., they are resistant to several antibiotics and chemotherapeutic agents. Some pathogen strains are resistant to antibiotics while others are multidrug-resistant, that is, they are resistant to multiple antibiotics and chemotherapeutic agents in fact, some strains have become resistant to almost all the agents that are used widely.

One common example is MRSA (Methicillin resistant Staphylococcus aureus) which was developed to combat penicillinase producing S. aureus and is also resistant to methicillin. but frequently also to lacosamide, aminoglycosides, macrolides, tetracycline, and chloramphenicol. Additionally, these strains are resistant to disinfectants, and MRSA can be a significant contributor to infections acquired in hospitals. Vancomycin was revived as an ancient antibiotic to treat MRSA infections.

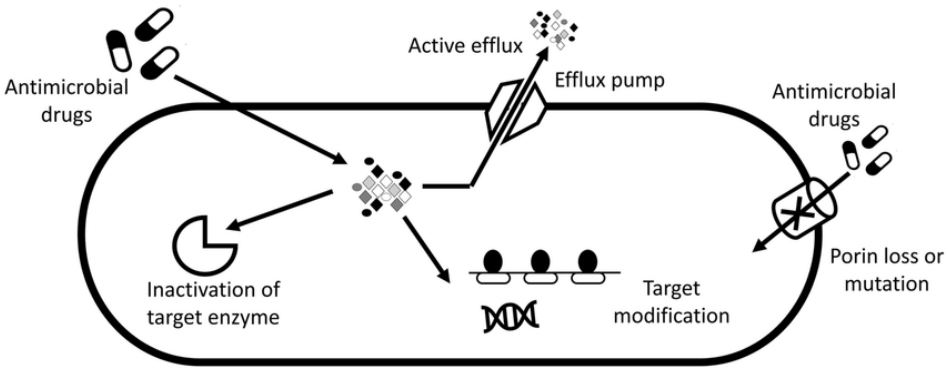

Figure 1.1: Multi-Drug Resistance Mechanism

MDR microorganisms were initially linked to diseases contracted in sanitariums. Due to their prevalence, MDR bacteria are currently the main source of illnesses that are acquired in the community. Antibiotic use, illness, mortality, and healthcare costs have all increased as a result of the rise of multidrug-resistant (MDR) microorganisms in society. Antimicrobial compound resistance is "one of the topmost pitfalls to mortal health worldwide," according to the IDSA (the Contagious Diseases Society of America). It has been noted that instances involving MDR bacterial strains have more serious outcomes than those involving other susceptible species. Therefore, this rise in the transmission of antibiotic resistance poses a significant threat to society and the available medications. It also has a detrimental effect on surgical procedures, transplants, and cancer treatments. The situation of specific MDR bacterial strains has been thought to be closely linked to the effectiveness of broad-spectrum antibiotics, which correspond to an empirical and conclusive treatment. The advanced prevalence of MDR is caused by similar overexploitation, which also creates a vicious cycle. [Nikaido Hiroshi (2009)]

Due to the limited number of effective antimicrobial medicines available moment, the frequence of these medicine- resistant bacteria is adding. According to estimates, by 2050, there will not be any feasible antibiotics accessible to treat these murderous resistant bacteria if new, innovative specifics are not set up or developed. thus, to treat similar multidrug-

resistant bacteria(MDR), we must formulate some new novel specifics or other options or backups. The elaboration of the most current multidrug- resistant bacteria is the main content of this paper, which also covers how these bacteria come resistant to numerous medicines by escaping the goods of specific antibiotics. likewise, we also go over a many other ways to stop them from getting infected.[Alok Bharadwaj, et al 2022].

The World Health Organization claims that these resistant microorganisms, such as bacteria, fungi, viruses, and parasites can fend off attacks from antimicrobial medications, which results in inefficient treatment that causes diseases to continue and spread. Even though MDR is a natural phenomenon, the widespread increase in immunocompromised conditions, such as HIV infection, diabetes, organ transplant recipients, and severe burn patients, makes the body a prime target for infectious diseases acquired in hospitals, which further spreads MDR.

Bacteria like Escherichia coli have very high rates of resistance to antibiotics like cephalosporin and fluoroquinolones, Klebsiella pneumoniae has resistance to cephalosporin and carbapenems, Staphylococcus aureus has resistance to methicillin, Streptococcus pneumoniae has resistance to penicillin, and nontyphoidal Salmonella has resistance to fluoroquinolones, Shigella species against fluoroquinolones, *Neisseria gonorrhoeae* against cephalosporin, and *Mycobacterium tuberculosis* against rifampicin, isoniazid, and fluoroquinolone causing common infections (such as bloodstream infections, pneumonia, and urinary tract infections) and a high proportion of hospital-acquired infections are caused by *Shigella* species, *Neisseria gonorrhoeae*, and *Mycobacterium tuberculosis,* which are resistant to fluoroquinolones, rifampicin, and isoniazid. Chronic fungal infections can be treated with a small number of antifungal medications. Isolates of *Candida spp., Aspergillus spp., Cryptococcus neoformans, Trichosporon beigelii, Scopulariopsis spp., and Pseudallescheria boydii* exhibit resistance to medications like polyene macrolides (amphotericin B), azole derivatives (ketoconazole, fluconazole, itraconazole, and voriconazole), DNA and RNA synthesis inhibitors (flucytosine), and 1,3-β-glucan synthase inhibitors (echinocandins). The emergence of several resistant strains and the persistence of infections despite treatment are caused by prolonged medication exposure and unabated viral reproduction. Plasmodium-caused malaria is one example of an illness that is susceptible to MDR. [Jyoti Tanwar et al, 2014]

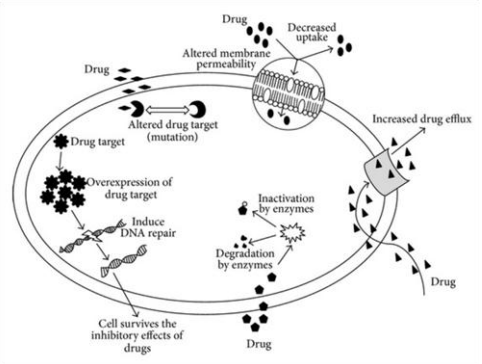

Figure 1.2: Mechanism of Resistance

"The silent tsunami facing modern medicine," has been said of antibiotic resistance. Numerous worldwide health and political summits have addressed it.

To address the problems posed by antibiotic resistance, numerous thorough reports, guidelines, and recommendations have been produced at both the national and international levels. Antibiotic resistance is still rising globally despite this knowledge in politics and science as well as the media's more recent focus. Under these circumstances, the emergence of multidrug resistance in Gram-negative bacteria (MDR-GNB) has emerged as a significant obstacle for medical practitioners. An expert panel of well-known infection preventionists from Germany, Austria, India, and the United States discussed current viewpoints on preventing the emergence and spread of multidrug-resistant Gram-negative bacteria (MDR-GNB) during a two-day symposium hosted by the Rudolf-Schulke-Stiftung in Hamburg. Key components of a One Health approach are outlined in the consensus report below, which takes into account a number of factors, including the unique characteristics of Gram-negative bacteria and their resistance to antibiotics, the global epidemiological situation, reservoirs, transmission pathways, risk groups, available treatments, and the efficacy of current preventative measures. [Martin Exner et al, 2017]

Biochemical Mechanisms of Antibiotic Resistance

Antibiotic resistance in bacteria is caused by multiple important mechanisms:

1. Target Protein Mutations

By altering the proteins that antibiotics typically target, certain bacteria become resistant.

2. Inactivation of Drugs by Enzymes

Antibiotics are chemically modified and neutralized by enzymes produced by a variety of microorganisms. β-lactamases break down β-lactam antibiotics (penicillin, cephalosporins), whereas phosphorylation, acetylation, or adenylation inactivates aminoglycosides like kanamycin. Resistance is transferred among bacteria by these resistance genes, which are frequently carried on plasmids.

3. Limiting Access to Drugs: By actively pumping the medicine out or preventing entrance, bacteria reduce its effectiveness. While some use **efflux pumps** to expel medications, as in tetracycline resistance, others generate proteins that shield their targets from antibiotics. [Nikaido H. (2009)]

Efflux Pumps

Antibiotic resistance, especially the emergence of multidrug resistance (MDR), is significantly influenced by efflux pumps. These pumps aid in the removal of medications from the cell, which lowers the internal concentration of antibiotics and increases resistance. Overexpression of these drug transporters has been linked to clinically significant drug resistance, especially against tetracyclines, erythromycin, and fluoroquinolones. Efflux pumps are also central to resistance in cancer chemotherapy.

Although prokaryotic and eukaryotic organisms have different efflux pump systems, several of their classes can mediate resistance in both. There are six classes for prokaryotic efflux pumps and five classes for eukaryotic pumps. One important factor in multidrug

resistance is the ATP-binding cassette (ABC) transporters. ABCA to ABCG are the seven subfamilies that comprise the 48 ABC transporter genes that are present in humans.

Known by many as ABCB1 or MDR1, the ABC transporter P-glycoprotein is a member of the ABCB (MDR/TAP) family and is linked to drug resistance. Two cytoplasmic nucleotide-binding domains (NBDs) that bind and hydrolyse ATP and two transmembrane domains (TMDs) that recognize, and transport substrates make up the structural makeup of ABC transporters. To help regulate the amounts of hormones, lipids, ions, and xenobiotics under typical physiological conditions, these transporters carry substances across cell membranes. They're highly expressed in organs that remove these compounds, such as liver, kidneys, and epithelial tissues. Some ABC transporters' capacity to bind a range of substances results in drug resistance.

ABC transporters work by using ATP hydrolysis to pump substrates against a chemical gradient. The drug efflux pumps usually only work in one way, but in some situations, they can work in both directions. The operation of these transporters has been described by a number of models, including the alternating site, switch, and constant contact models. Addressing drug resistance and creating more potent treatments require a deeper comprehension of the mechanics underlying drug transporters and the creation of the efflux pump inhibitors. [Alessia Catalano et al, 2022]

Bacterial Proteins and their Mutations

Ceftriaxone

Ceftriaxone**,** is a third-generation cephalosporin antibiotic widely used to treat various bacterial infections, including respiratory tract infections, skin infections, urinary tract infections, and sexually transmitted infections like gonorrhoea. It works by inhibiting bacterial cell wall synthesis, leading to cell death. ***Neisseria gonorrhoeae*** is found to be resistant to ceftriaxone due to mutation in PBP2 protein These mutations often occur, Specifically, at position 502, A is replaced by V (valine) leading to resistance. [Fenton et al, 2021]

Ceftazidime

Ceftazidime is an injected broad-spectrum third-generation cephalosporin beta-lactam antibiotic used to treat or prevent a variety of bacterial infections, including pneumonia, gynaecological infections, bone and joint infections, and septicaemia, among others. ***Acinetobacter baumannii*** is found to be resistant to ceftazidime due to mutation in the protein OXA-23, **Lys73** to **Asn**: One of the significant mutations associated with increased resistance in OXA-23 beta-lactamase is the change at position **73** in the OXA-23 enzyme, where lysine (Lys) is replaced by asparagine (Asn).

Ser206 to **Ala**: Another mutation found in certain OXA-23 variants, specifically at position **206**, is a change from serine (Ser) to alanine (Ala), which further enhances the enzyme's ability to hydrolyse beta-lactam antibiotics. [Jennifer M Colquhoun et al, 2021]

Amoxicillin

Amoxicillin is a widely used penicillin-type antibiotic effective against various bacterial infections. It works by inhibiting the growth of bacteria, thereby treating the infection**.**

Staphylococcus aureus shows resistance to Amoxicillin due to mutation in Position 542: The mutation Ser542 to Phe in PBP2a is known to significantly contribute to resistance. Position 554: The mutation Val554 to Glu in PBP2a also plays a significant role in resistance to beta-lactam antibiotics, including amoxicillin. Position 529: The Asp529 to Gly mutation is associated with reduced affinity for beta-lactams, contributing to resistance. Penicillin, which inhibit cell-wall synthesis, were the first-line treatment of infections by *S. aureus*. But within a few years of penicillin use, 50% of *S. aureus* infections were resistant to the drug due to the catalytic function of β-lactamase, a resistance enzyme that hydrolytically turns over these antibiotics, rendering them inactive. This resistance phenotype was due to the acquisition of a gene that encodes for a protein that overcomes the challenge by a broad range of β-lactam antibiotics (including penicillin, cephalosporins and carbapenems) [Jennifer Fishovitz et al, 2014]

Bactrim

Bactrim is a widely used antibiotic that combines two active ingredients, sulfamethoxazole and trimethoprim. It belongs to the class of sulphonamide antibiotics and works by blocking bacterial DNA and protein synthesis, effectively stopping the growth and spread of bacteria. ***Escherichia coli*** shows resistance to Bactrim due to A common mutation associated with trimethoprim resistance is the substitution of leucine (L) with arginine (R) at position 28 of the DHFR protein. 4′-desmethyltrimethoprim (4′-DTMP) inhibits both bacterial dihydrofolate reductase (DHFR) and its L28R mutant, while preventing the emergence of TMP-resistant bacteria carrying the L28R mutation in lab settings. Additionally, antibiotic-sensitive E. coli develop resistance much more slowly when exposed to 4′-DTMP compared to TMP. 4′-DTMP inhibits resistance evolution by selecting against the L28R mutation and guiding genetic changes toward other DHFR mutations with reduced catalytic activity. [Madhu Sudan Manna et al, 2021]

Methicillin

Methicillin is a narrow-spectrum β-lactam antibiotic belonging to the penicillin class, ***Staphylococcus aureus***, Asp338 to Gly mutation is one of the changes in PBP2a that leads to methicillin resistance. The first methicillin-resistant *Staphylococcus aureus* (MRSA), strain was identified in the UK in 1961. It became a global problem with two years. This resistance phenotype was due to the acquisition of a gene that encodes for a protein that overcomes the challenge by a broad range of β-lactam antibiotics (including penicillin, cephalosporins and carbapenems. Currently, the drugs of choice for treatment of infections by MRSA are vancomycin, daptomycin, ceftaroline and linezolid. Even with controlled use, strains that show reduced susceptibility, or outright resistance, to these drugs have arisen. [Jennifer Fishovitz et al, 2014]

2. Materials and Methods

2.1 Homology Modelling

Identification of Antibiotic Resistance-Associated Mutations

Mutations connected to antibiotic resistance were established on the basis of an extensive literature search, mainly taking advantage of PubMed Central. The database contained published papers in peer-reviewed journals on bacterial resistance mechanism. Some of the applicable keywords included "antibiotic resistance," "mutation," and "bacterial protein" for retrieving associated articles.

Selection of Resistant Proteins

Here, resistant proteins were identified in the UniProt database, which contains protein sequences and functional annotations. The below steps were used:

1. Search for UniProt and access (https://www.uniprot.org/).
2. Enter the protein name and bacterial species in the search bar.
3. Select the entry ID, retrieve the sequence, and download in FASTA format.

Retrieving Protein Structures

3D structures of resistant proteins were obtained via the Protein Data Bank (PDB). The steps followed include:

1. Access OHBP (https://www.rcsb.org/).
2. Search for the protein structure.
3. Select those structures in complex with ligands and download the file.

Retrieving 3D Structures of Antibiotics

Second- and third-generation antibiotics' 3D structures were obtained from PubChem. The process followed was:

1. Access from PubChem (https://pubchem.ncbi.nlm.nih.gov/).
2. Search for the antibiotic in question.
3. Download the 3D conformer in .sdf format.

Multiple Sequence Alignment

Multiple sequence alignments of the retrieved protein sequences were performed using ClustalW. The process was based on:

1. Access ClustalW (https://www.genome.jp/tools-bin/clustalw).
2. Paste or upload FASTA sequences.
3. Run the alignment and analyse the mutation sites.

Docking of Proteins and Ligands

Molecular docking was performed with the help of UCSF Chimera and Auto Dock Vina: Preparation of wild-type proteins**: Retrieved from the PDB, removed chains, and selected ligand binding sites.

- Mutation process: Amino acid substitution performed

- Docking process: The protein-ligand structures were opened.
- using AutoDock Vina, docking parameters were defined. The Binding affinity and interactions were analysed.

2.2 Model Validation

Ramachandran Plot Analysis

Validation was performed using SAVES v6.1 software for checking protein stability:

1. Upload protein model.
2. Run PROCHECK for Ramachandran plot analysis.
3. Interpret torsion angle distributions.

Binding Site Prediction

Protein Plus was used for prediction of ligand binding site:

1. Upload receptor and ligand files;
2. Run DoGSiteScorer to detect the sites;
3. Evaluate binding pocket characteristics.

Mutational Stability Analysis

The i-Mutant was used to give predictions about mutations:

1. The protein sequence was entered.
2. The mutated residue was specified.
3. Finally, the ΔG change was evaluated.

ProtParam

It is a server that predicts the physicochemical characteristics of a protein from its amino acid sequence. It offers valuable information such as molecular weight, isoelectric point (Pi), extinction coefficient, stability index, aliphatic index. It aids in interpreting a protein's stability, solubility, and general biochemical properties, hence suitable for studies and experimental design.

Steps Involved:

1. Search for ProtParam.
2. Paste the protein sequence into the input box and press Compute Parameters to analyse its characteristics.

Biovia Discovery Studio

BIOVIA Discovery Studio is software utilized in molecular structure and interaction study, specifically drug discovery and protein research. It visualizes molecular models, binds to analyse sites, and predicts how ligands bind to proteins. Scientists employ it in order to enhance drug design, gain insights into biomolecular structures, and refine prospective drugs.

Steps Involved:

1. Open BIOVIA Discovery Studio.
2. File → Open, choose "All Files" from the file type, and open the docked .pdb file.
3. Click on Receptor-Ligand Interactions from the top menu.
4. Under Tools from the side panel, choose Show 2D Diagram in order to see the protein-ligand interactions in 2D.

3. Results and Discussion

The comparative study of the wild-type and mutant variant, incorporating tools such as the Ramachandran plot, I-mutant, Protein Plus, docking simulations, ProtParam, and Discovery Studio, provides a comprehensive evaluation of structural, functional, and stability differences

Docking Energy Values

S. No	Antibiotic	Binding Affinity Score- WT	Binding Affinity Score-Mutant
1.	Ceftriaxone	-6.3	-6.7
2.	Methicillin	-7.5	-6
3.	Bactrim	-6.5	-6.1
4.	Ceftazidime	-7	mutant 1- -7 mutant 2-6.7
5.	Amoxicillin	-7.2	mutant 1- -6.3 mutant 2- -6.7

Protparam

S.No	Antibiotic	Aliphatic Index	Half Life
1.	Ceftriaxone (PBP2)	WT-87.64 Mutant- 87.97	WT-30 HRS Mutant-30 HRS
2.	Ceftazidime (OXA 23)	WT-92.45 Mutant-92.82	WT-30 HRS Mutant-30 HRS
3.	Amoxicillin (PBP2a)	WT-82.43 Mutant-81.99	WT-30 HRS Mutant-30 HRS

4.	BACTRIM (DHFR)	WT- 84.65 MUTANT-82.20	WT-30 HRS MUTANT-30 HRS
5.	METHICILLIN (PBP2a)	WT-82.43 MUTANT-82.43	WT-30 HRS MUTANT-30 HRS

Discovery Studios (Protein-Ligand Interaction)

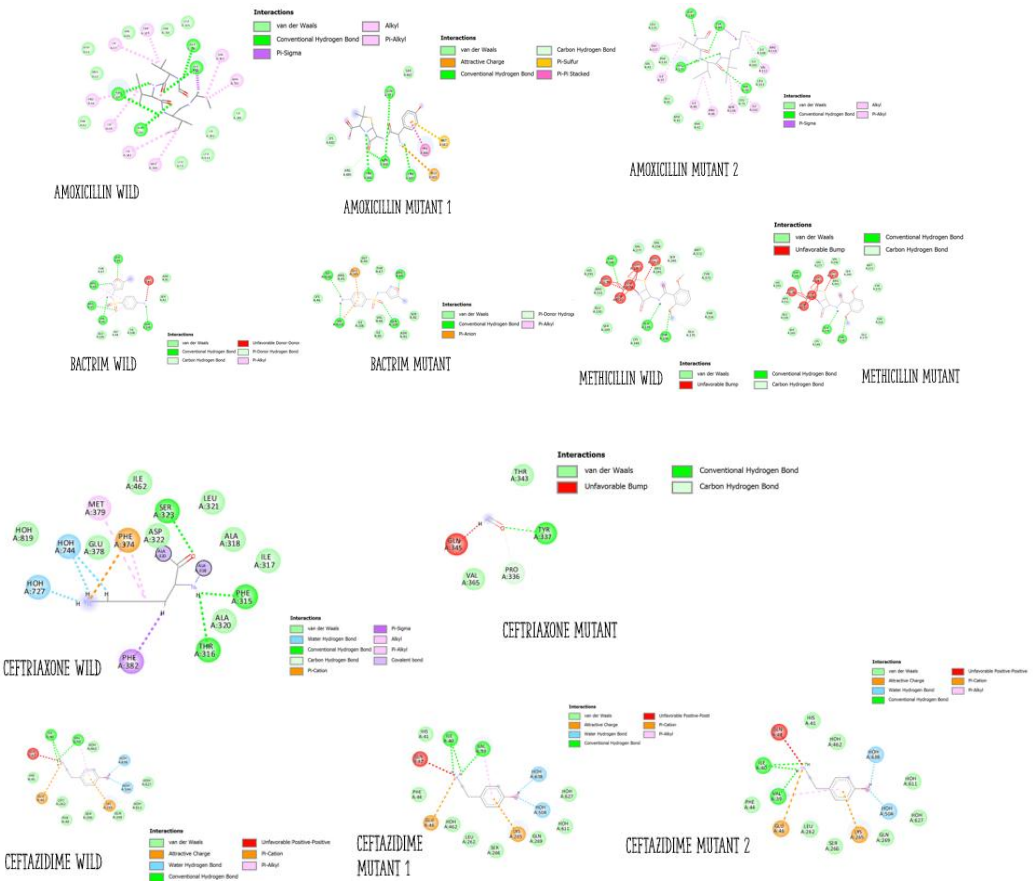

Figure 3.1 Protein – Ligand Interaction

I-mutant

Antibiotic	WT	NEW	Stability	RI
Ceftriaxone (PBP2)	A	V	Decrease	1
Ceftazidime (OXA23) Mutant	K	N	Decrease	5

Ceftazidime Mutated 2	**S**	**A**	**Decrease**	**7**
Bactrim(DHFR)	**L**	**R**	**Decrease**	**6**
Amoxicillin (PBP2a) Mutation 1	**S**	**F**	**Increase**	**1**
Amoxicillin Mutation 2	**V**	**E**	**Decrease**	**3**
Methicillin (PBP2a)	**D**	**G**	**Decrease**	**7**

Protein Plus

Ceftriaxone's wild type has a surface area of 1028.32 and a volume of 868.71, while its mutant has a surface area of 871.81 and a volume of 1030.58. Ceftazidime's wild type has a surface area of 745.32 and a volume of 614.72, while its mutants have surface areas of 834.35 and 870.31, with volumes of 771.33 and 634.84, respectively. Bactrim's wild type has a surface area of 1606.16 and a volume of 1392.32, while its mutant has a surface area of 1690.23 and a volume of 1384.43. Methicillin's wild type has a surface area of 1237.94 and a volume of 978.06, while its mutant has a surface area of 831.56 and a volume of 609.14. amoxicillin wild type has a surface area 1042.2 and volume 738, while mutant has surface area 1038.1 and volume 765.

Discussion: The mutations investigated have different effects on drug resistance and structural stability. Ceftriaxone has minor resistance development with minimal structural adaptation, whereas ceftazidime Mutation 2 has dramatic resistance increase through weakening drug interaction. Amoxicillin mutations have decreased binding affinity, reducing the effectiveness of drugs despite structural integrity. Bactrim has intermediate resistance potential with minimal destabilization but still has effectiveness. Methicillin has the greatest resistance with a tight structure and significant loss in binding affinity. Generally, structural modifications affect drug interaction, where certain mutations increase resistance, but others ensure stability with least effect.

Conclusion

This study brings into perspective how mutations in various drug-resistant (MDR) bacterial proteins affect antibiotic binding, structural stability, and overall mechanisms of resistance. Some mutations promote thermostability and structural stability, whereas others markedly elevate bacterial resistance, making drugs less effective. For example, methicillin (D → G) and ceftazidime (K→ N) mutations caused significant development of resistance, where structural changes compromised drug interaction.The results highlight the delicate balance between drug binding affinity and structural alterations.

A few mutations created more stability but affected drug effectiveness, while others radically changed protein structure, making the antibiotic less effective. These further stresses the significance of elucidating molecular adaptation mechanisms in MDR bacteria for devising effective targeted therapies.

To check the rising tide of antibiotic resistance, molecular modelling strategies and pharmacogenomic approaches need to be given priority. Computer-aided drug design, as well as experimental validation, will become pivotal in the future to design precision antibiotics and tailor-made antimicrobial treatments for effective action against resistant bacterial pathogens.

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