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Sequence and Structure-Based Classification of PenA β-Lactamase from Burkholderia stabilis

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Abstract:

The process of hydrolysis of the bond between amides of the four-membered β-lactam ring in Gram-negative bacteria is the key mechanism of resistance in β-lactamase enzymes. Because Burkholderia multivorans' PenA β-lactamase has been selected as a prototype sequence because it is associated with conditions such as cystic fibrosis and meningitis. In this study, the PenA β-lactamase sequence from Burkholderia multivorans was retrieved to demonstrate how multiple sequence alignment and phylogenetic analysis enable relationships between an initial sequence and its progeny. Additionally, this work demonstrates the close relationship between Burkholderia stabilis and Burkholderia cepacia in phylogenetic tree analysis. Consensus and sequence conservation have been noted in this work based on pattern analysis. Based on the crystal structure (PDB ID: 7DOO) of Burkholderia multivorans PenA β-lactamase Avibactam Complex, the active site residues in Burkholderia stabilis were shown using PDB files. The POCASA server was utilized for the active site investigation, and BIOVIA Discovery Studio 2019 was also used to show the results. Burkholderia stabilis is a major contributor to wound infection and produces non-invasive infections. PenA β-lactamase protein can be a target for antibiotic screening and inhibitor selection. Prominent active binding site residues of PenA Burkholderia stabilis, were shown to be interacted with the Avibactam based on molecular docking analysis.

Keywords: PenA β-lactamase, Burkholderia multivorans, Burkholderia stabilis, Avibactam, Docking analysis

1. Introduction

Alexander Fleming's discovery of penicillin marked the beginning of a new era in medical research by making it possible to treat infectious diseases as well as safely provide life-saving therapies like chemotherapy and surgery. Resistance to penicillin and other advanced β-lactam antibiotics has rapidly emerged since these drugs were initially employed in clinical settings. [Gaynes, 2017] The primary root of this type of resistance is a specific category of extracellular proteins called β-lactamases, which are enzymes that hydrolyze proteins. [Dalia Azhar Ahmed, 2021]

β-lactams continue to be one of the most significant targets for antibiotics. As the primary mechanism of susceptibility, β-lactamase enzymes hydrolyze the amide bond of the four-membered β-lactam ring of Gram-negative bacteria. [Tooke et al., 2019] One of the results demonstrated that one species, *S. clavuligerus*, was able to generate β-lactamases at cephamycin C and the β-lactamase inhibitor clavulanic acid, a compound with a five-membered oxazolidinic ring and a β-lactam ring. [Martin et al., 2022] Several enzymes are dispersed on mobile genetic components in non-fermenting

microorganisms like Pseudomonas sp. and pathogenic opportunistic germs like Enterobacteriaceae, which includes *E. coli.*The four distinct variants of beta-lactamases consist of extra zinc-sensitive or metallo- β-lactamases (MBLs; class B) and active-site serine beta-lactamases (classes A, C, and D) [Tooke et al., 2019].

A homologous system is the cornerstone of complementary biology. A trait having a homology is often a homolog. The term "homolog" is used in biology to refer to both the family of proteins and the gene sets (DNA sequences) that code for them [Pearson, 2013]. Similar to physical features, the homogeneity of DNA or protein complexes is determined by their common origin. Protein, RNA, and DNA homology is often defined by how close their nucleotide or amino acid sequences are. Significant similarity is demonstrated by the strong evidence that two sequences are linked by evolutionary changes through a shared ancestor sequence. Alignments combining numerous sequences can be used to identify the identical segments from each sequence [Barzel et al., 2008]. Large-scale multigene samples used for phylogenomics and comparative genomics investigations sometimes contain sequence errors obtained from parent genomes and transcriptomes. Long segments of non-homologous sequences are often the result of these flaws, which can be brought on by errors in sequencing, assembly, or annotation. [Tørresen et al., 2019] The proliferation of sequencing errors in huge datasets is caused by the absence of automatic methods to find and correct them. The command-line program PREQUAL finds and masks segments with contiguous non-homologous characters in collections of unaligned homologous sequences [Irisarri et al., 2021].

To identify related features and evolutionary gene links, a technique known as multiple sequence alignment (MSA) is employed. The symmetrical organization of more than a few biological structures most likely DNA, RNA, or proteinis frequently described by this term. The alignments are produced and examined using computational techniques. [Warnow et al., 2021] Heuristic and dynamic approaches are used in the majority of MSA procedures. One of the main goals of MSA is to compare protein sequences in order to identify structural or functional connections between proteins. [Chatzou et al., 2016] Because of the intricacy of the computation, MSAs require more advanced techniques than alignment. Most MSA software uses heuristic methodologies instead of globally optimal operation. The major difficulty in MSA will pertain to how to manage the expanding data set lengths in order to handle nucleic acid alignments. [Kemena et al., 2009]

Phylogenetic study examines the evolutionary path of a species, a group of organisms, or a particular feature of an organism's genome based on how similar its genes are. To illustrate this, a diagram known as a phylogenetic tree is created. In 2018, Lewis et al. Similarities and differences between the genomes have been revealed using aligned sequencing within phylogenetic trees. Phylogenetic analysis enables the understanding of relationships between an initial sequence and its progeny.

In homology modeling, sometimes called comparative protein modeling, an atomic resolution model of a protein is created using the amino acid sequence of a "target" protein and the three-dimensional molecular structure of a comparable homologous protein that has been identified by experimentation (the "template"). In homology modeling, one or more protein structures that are likely to match the structure of the query sequence are chosen in order to align the query sequence's residues with those of the template sequence. [Fiser, 2010] Sequences that are 20% or less identical might, nevertheless, employ rather distinct architectures. Compared to protein sequences, protein structures are shown to be significantly more highly conserved among homologues. [Schwede et al., 2003]

Molecular docking becomes an essential technique when it comes to identifying molecular structures and computer-powered medication development. The objective of ligand-protein docking is to estimate the principal contact approaches of an element that binds with a protein together with a certain three-dimensional configuration [Subhaswaraj et al., 2022]. Effective docking techniques employ a scoring system that accurately rates potential dockings while investigating high-dimensional spaces. Molecular docking's ultimate goal is to use computer programs to anticipate the structure of the binding site-receptor complex [Wu et al., 2003]. Docking can be performed in two complimentary steps: first,

sampling the ligand's structures in the protein's highly active region; and second, using a scoring system to assess the resultant conformations [Meng et al., 2011]. Lead enhancement benefits tremendously from the use of docking through online screening on large chemical libraries, grading the results, and providing conformational insights into how ligands work to interact the target computationally [Torres et al., 2019].

2. Materials and Methods

2.1 Sequence Retrieval and Alignment

PenA β-lactamase sequences were retrieved from the UniProt database (e.g., UniProt ID: Q02940). *Burkholderia multivorans*' PenA β-lactamase sequence was obtained from the UniProt service in FASTA format. Initially, the PenA β-lactamase protein sequence from *Burkholderia multivorans* was stored in the "sequences.fasta" format. Because the sequence was examined by the UniPortKB (Swiss-Prot) server, the received sequence status displayed a star sign prior to its unique ID, indicating that it was legitimate. This site may also be used to study other aspects of this protein, such as its structure, domain, sequence, and comparable proteins. Homologous sequences from six Burkholderia species were aligned using ClustalO and MEGA software. To find conserved motifs throughout the sequences, all obtained FASTA sequences were entered into the MEME server. The MEME server detected the motif based on the provided criteria. The number of motifs required for the research project may also be indicated on the MEME server.

2.2 Phylogenetic Analysis

Neighbor-joining and UPGMA phylogenetic trees were constructed in MEGA. Evolutionary distances were estimated to identify closely related species. The previously received FASTA format "sequences.fasta" was first opened in MEGA program in order to visualize the alignment. ClustalW was then used to accomplish the alignment. CLC Sequence Viewer 8.0 was then used to visualize alignment. NJ/UPGMA of MEGA program was used to create an additional phylogenetic tree. Conserved sites, half conserved sites, and singleton sites were computed later.

2.3 Homology Modeling

The 3D structure of PenA from *B. stabilis* was modeled using SWISS-MODEL based on the template structure of B. multivorans PenA-Avibactam complex (PDB ID: 7DOO) [Papp-Wallace et al., 2017]. Model validation was performed using the SAVES server, including Ramachandran plot statistics.

2.4 Active Site Prediction

Active site residues were predicted using POCASA, followed by visualization in BIOVIA Discovery Studio.Based on the crystal structure of *Burkholderia multivorans* PenA β-lactamase Avibactam Complex (7DOO), the PDB dataset was utilized to view the active site residues in *Burkholderia stabilis*. Cavity search, which predicts binding locations and pockets of 3D proteins and their active site residues, was conducted using POCASA. All of the settings for pocket cavity and active site identification were manually selected in order to achieve the intended outcome of the experiment.

2.5 Molecular Docking

The interaction between PenA β-lactamase and Avibactam (Compound CID: 9835049) was studied using BIOVIA Discovery Studio's LibDock and CDOCKER modules. The protein site properties that LibDock uses are called HotSpots. There are two types of hotspots: polar and apolar. An apolar atom selects an apolar hotspot, while a polar ligand atom (which may be a hydrogen bond donor or receiver) favors a polar hotspot. The binding site HotSpot file is calculated before to the docking procedure. But if you'd like, you can use a predefined or user-adjusted HotSpot file. The protocol offers the user a variety of methods to generate ligand conformations that are suitable for docking. CDOCKER is a CHARMm-based docking tool that uses a rigid receptor [Wu et al. 2003-.CHARMm is used in the grid-based docking of molecules method known as CDOCKER. The ligands are allowed to bend throughout the refining process, but the receptor stays rigid. For anticipated ligands, the binding position does not need to be known beforehand. However, by figuring out where the ligand is located in the active site, the binding site domain may be used. Residue-ligand interactions were analyzed in 2D and 3D formats.

3. Results

3.1 Sequence Similarity and Phylogeny

Multiple sequence alignment revealed high conservation in regions such as ALERAAGGRLGVCAID and PGLLQQRVT across Burkholderia species. Phylogenetic analysis showed *B. stabilis* clustering closely with *B. cepacia*.

```
CLUSTAL O(1.2.4) multiple sequence alignment

WP_012216561.1      MTHSSQRRILLLAAATAPLALSLGACAARDATVSDAASPVGAAPASFAALERAAGGRLGV       60
WP_069263816.1      MTYSSQRRTLLLAAASAPLVVTVGACAA-PS----AAAPDIASAATFAALERAAGGRLGV       55
WP_129515490.1      MTYSSQRRTLLLAAATAPLVLAVTACAS-PR----AGAPDVSSTTSFDALERAAGGRLGV       55
WP_048245795.1      MTYSSQRRTLLLAAATAPLVLTVTACAS-PR----AGAPDVSSTTSFDALERAAGGRLGV       55
WP_060190872.1      MTYSSQRRTLLLAAATAPLVLTVAACAS-PP----AAGQDVSSATSFAALERAAGGRLGV       55
WP_175690122.1      MTYSSQRRTLLLAAATAPLVLTVTACASSPP----AAAPGIASATSFAALERAAGGRLGV       56
                    **:***** ******:***.::: ***:       *..   :: ::* ***********

WP_012216561.1      CAIDTATGRRALHRADERFPFCSTFKAMLGAAVLAQSVAHPGLLQQRVTYGRSDLVNYSP       120
WP_069263816.1      CAIDTASGRRAQHRADERFPFCSTFKAMLGAAVLARSVEHPGLLQQRVTYAQADLVNYSP       115
WP_129515490.1      CAIDTASGRRALYRADERFPFCSTFKAMLSAAVLAQSVERPGLLQQRVTYGKADLVKYSP       115
WP_048245795.1      CAIDTASGRRALYRADERFPFCSTFKAMLSAAVLAQSVERPGLLQQRVTYGKADLVKYSP       115
WP_060190872.1      CAIDTASGRQARHRADERFPFCSTFKAMLSAAVLAQSVEHPGLLQQRVTIAQSDLVNYSP       115
WP_175690122.1      CAIDAASGRRAQHRADERFPFCSTFKTMLSAAVLAQSVERPGLLQQRVTYGQGDLVNYSP       116
                    ****:*:**:* :*************:**.*****:** :******** .:.***:***

WP_012216561.1      VTERHVDTGMTVAELCAATIQYSDNTAANELMKRIGGPAAVTAYARSIGDDTFRLDRWET       180
WP_069263816.1      VSGRHVDTGMTVAQLCEAAIQYSDNTAANLAMKLIGGPAAVTAYARSIGDDTFRLDRWET       175
WP_129515490.1      VSEKHVDTGMTVADLCAAAIQYSDNSAANLLMKLLGGPSAVTAYARSIGDDTFRLDRWET       175
WP_048245795.1      VSEKHVDTGMTVADLCAAAIQYSDNSAANLLMKLLGGPSAVTAYARSIGDDAFRLDRWET       175
WP_060190872.1      VSGKHVGAGMTVAELCEATIQYSDNSAANLAMKLIGGPAAVTAYARSIGDDTFRLDRWET       175
WP_175690122.1      VSSKHVGTGMTVAALCEAAIQYSDNSAANLLMKMIGGPSAVTAYARSIGDDTFRLDRWET       176
                    *: :**.:***** ** *:******:***  ** :***:************:********

WP_012216561.1      ELNTALPGDLRDTTTPAAMAANLRVLVLGDALPPAQRAQLIEWLRGNKVGDKRIRAGVPT       240
WP_069263816.1      ELNTALPGDLRDTTTPAAMAASIRALTLGDALPPAQRAQLVAWMRGNKVGDKRIRAGVPA       235
WP_129515490.1      ELNTALPGDLRDTTTPAAMAASLRVLTLGEALPAAQRAQLVTWLRGNKVGDKRIRAGVPA       235
WP_048245795.1      ELNTALPGDLRDTTTPAAMAASLRVLTLGDALPAAQRAQLVAWLRGNKVGDKRIRAGVPA       235
WP_060190872.1      ELNTALPGDPRDTTTPSAMAASMRVLTLGDALPPAQRAQLVAWLRGNKVGDKRIRAGVPA       235
WP_175690122.1      ELNTALPGDLRDTTTPAAMAASLRVLTLGDALPGAQRAQLVAWLRGNKVGDKRIRAGVPA       236
                    ******** ******:****.:*.*.**:*** ******: *:***************:

WP_012216561.1      GWRVGDKTGTGDYGTTNDVGVLWPPSRAPIVLAVYYTQTRADAKAKDDVIAAATRIASAT       300
WP_069263816.1      GSQVADKTGTGNYGTTNDAGVVWLPSRAPIVLTVYYTQARADAKAKENVIAAAARIAVAT       295
WP_129515490.1      GWTVGDKTGTGDYGTTNDAGVVWPPSRAPIVLTVYYTQTRADARAKEDVIAAATRIAVAT       295
WP_048245795.1      GWTVGDKTGTGDYGTTNDAGVVWPPSRAPIVLTVYYTQTRADARAKEDVIAAATRIAVAT       295
WP_060190872.1      GWQVADKTGTGDYGTTNDAGVIWPPSRAPIVLAVYYTQTRADAKAKDDVIAAAARIAVAT       295
WP_175690122.1      GWQVADKTGTGDYGTTNDAGVMWPPSGAPIVLAVYYTQARADAKAKDDVIAAAARIAVTT       296
                    *  *.******:******.**:* ** *****:*****:****:**::*****:*** :*

WP_012216561.1      LA  302
WP_069263816.1      FA  297
WP_129515490.1      FG  297
WP_048245795.1      FG  297
WP_060190872.1      FA  297
WP_175690122.1      FG  298
                    :.
```

Figure 1: Alignment visualization of selected multiple sequences with *B. stabilis* PenA by using Clustal O

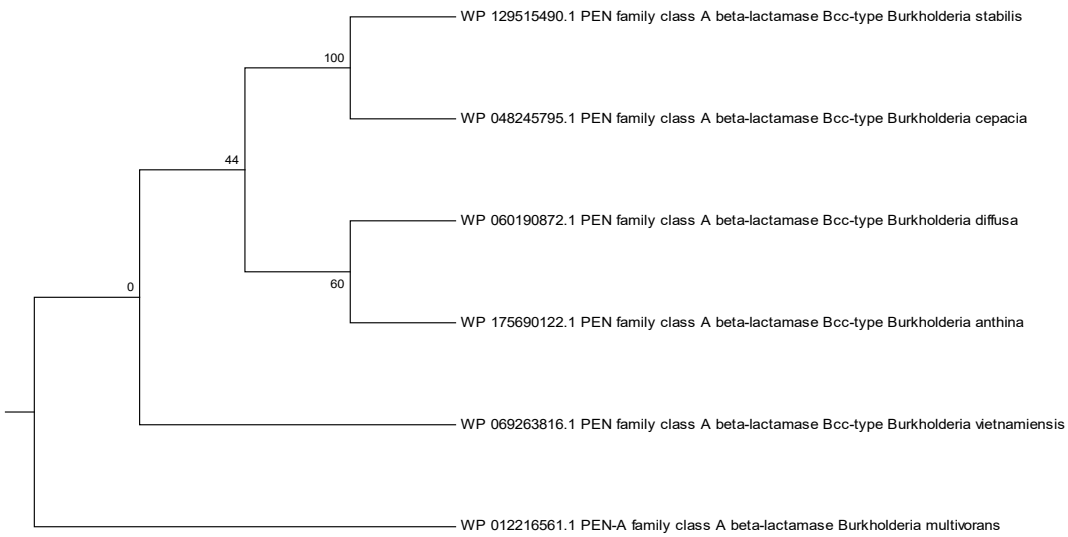

Figure 2: UPGMA based phylogenetic tree generation by using MEGA tool

3.2 Conserved Motif Analysis

MEME analysis identified motifs shared across all species except minor variations in motifs 28–33. These variations aligned with observed phylogenetic branching.

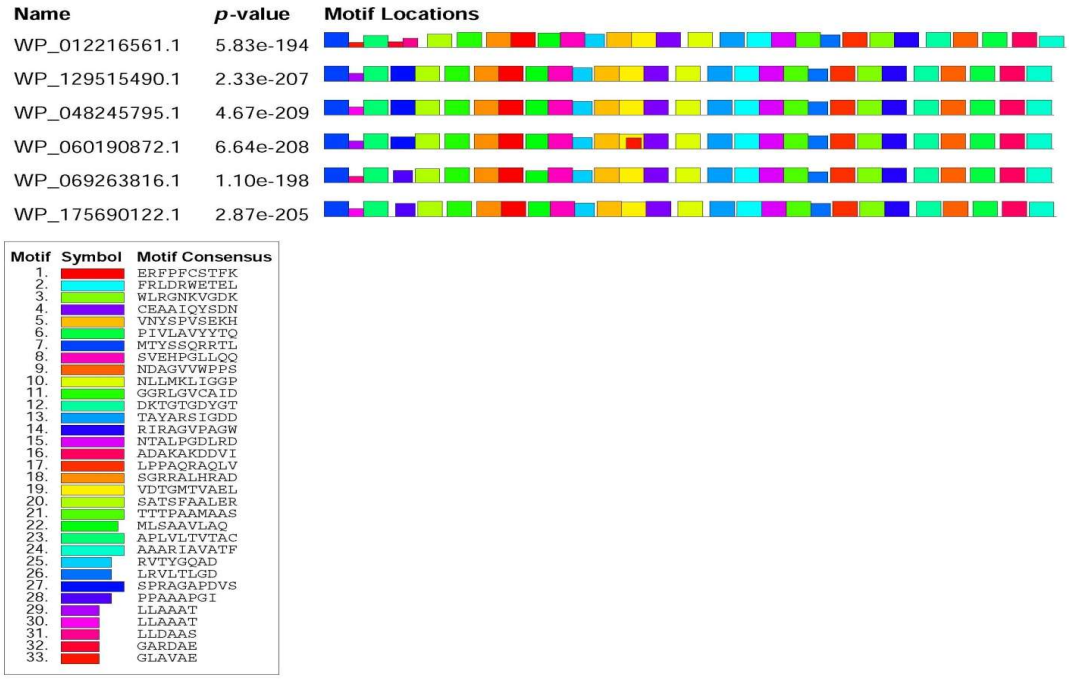

Figure 3: Colorimetric observation of multiple motifs by using MEME server

3.3 Structural Modeling and Validation

The modeled structure had 93.4% of residues in the most favored regions of the Ramachandran plot, indicating a high-quality structure.

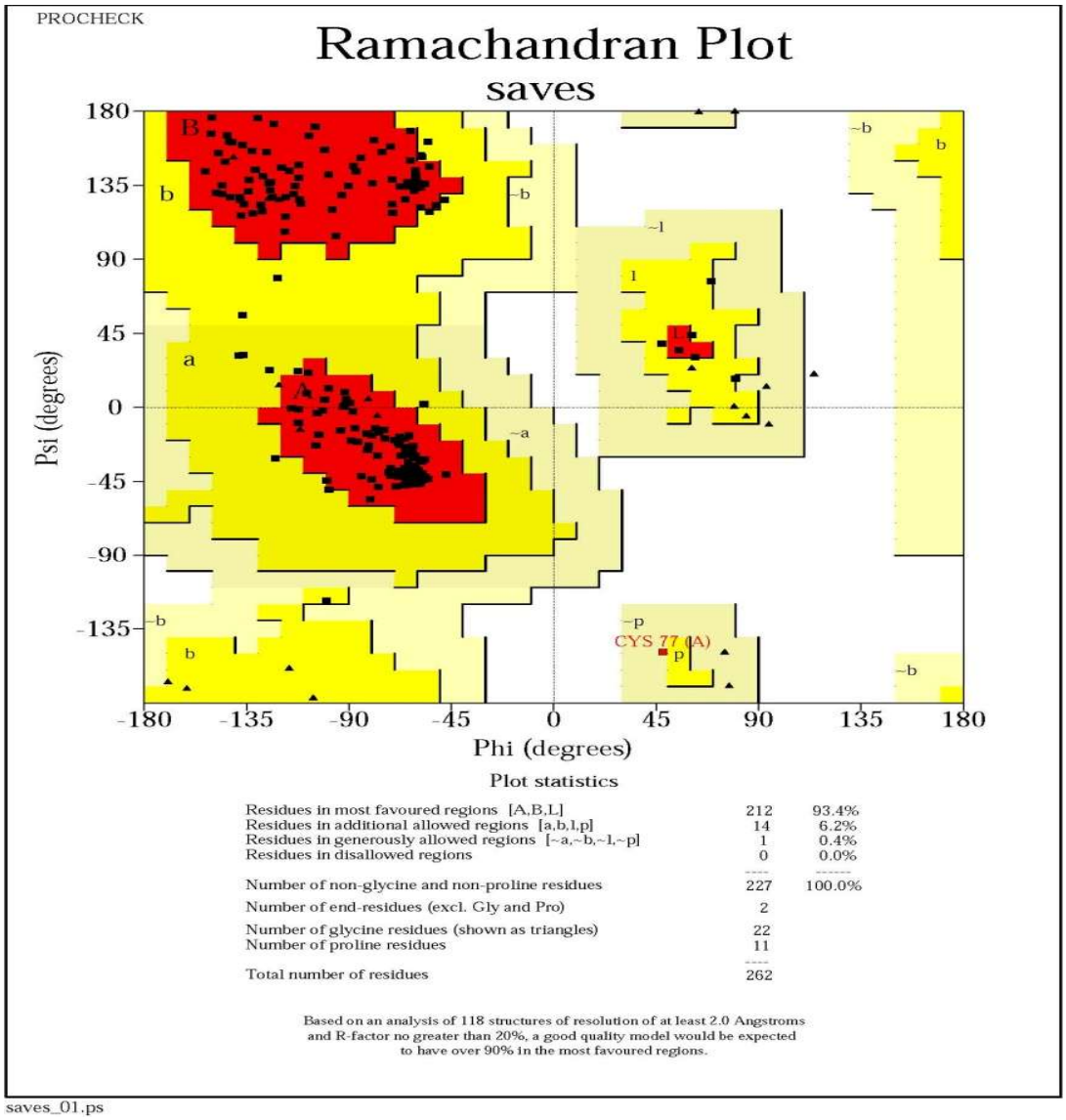

Figure 4: Visualization of Ramachandran Plot Statistics done by SAVES server PROCHECK source information

3.4 Active Site Characterization

Predicted active sites in *B. stabilis* corresponded well with those observed in the template structure (7DOO), particularly residues like Lys^{81}, Ser^{78}, and Tyr^{113}.

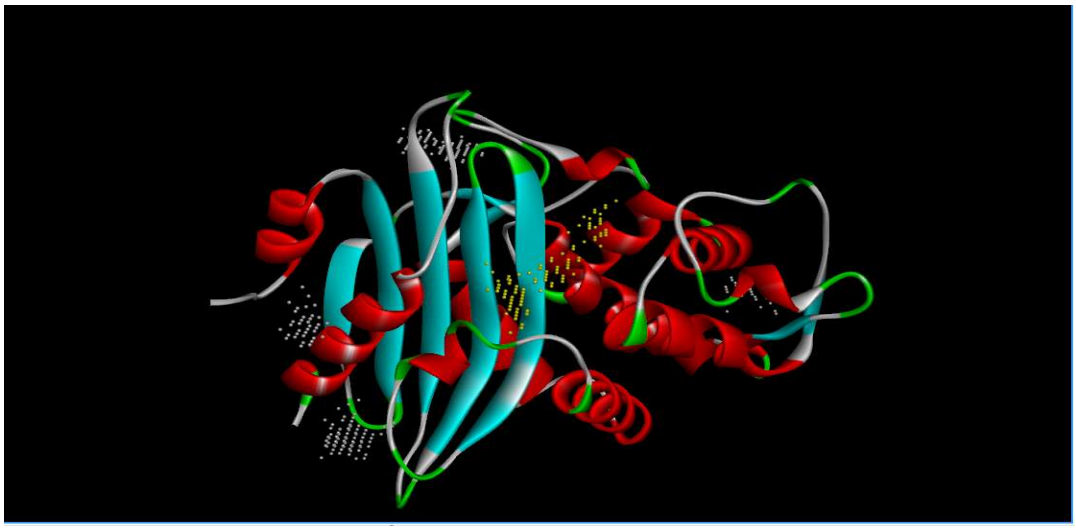

Figure 5: POCASA generated pockets visualized using BIOVIA Discovery Studio 2019

3.5 Docking Analysis

Avibactam docked effectively at the predicted active site. Sixteen residues were involved in binding, 12 of which overlapped with predicted active sites, supporting the functional relevance of the modeled structure.

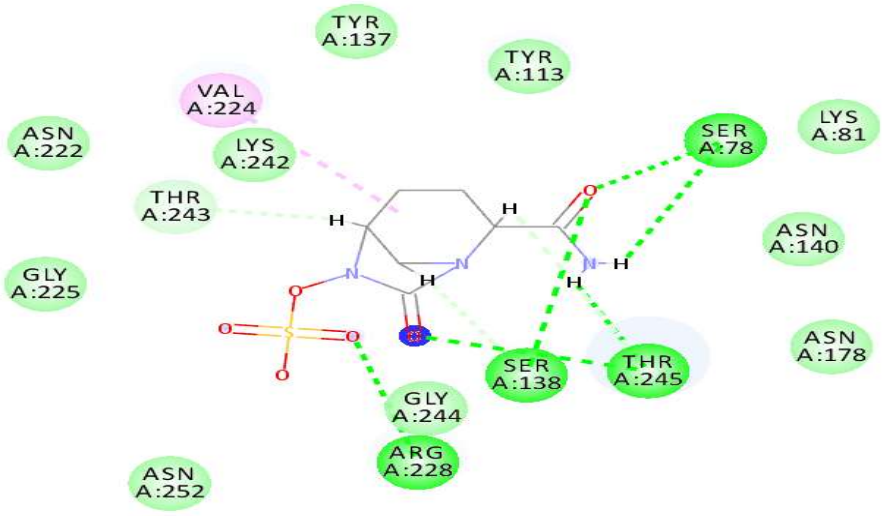

Figure 6: Visualization of 2D interaction of residues with drug Avibactam in BIOVIA Discovery Studio 2019

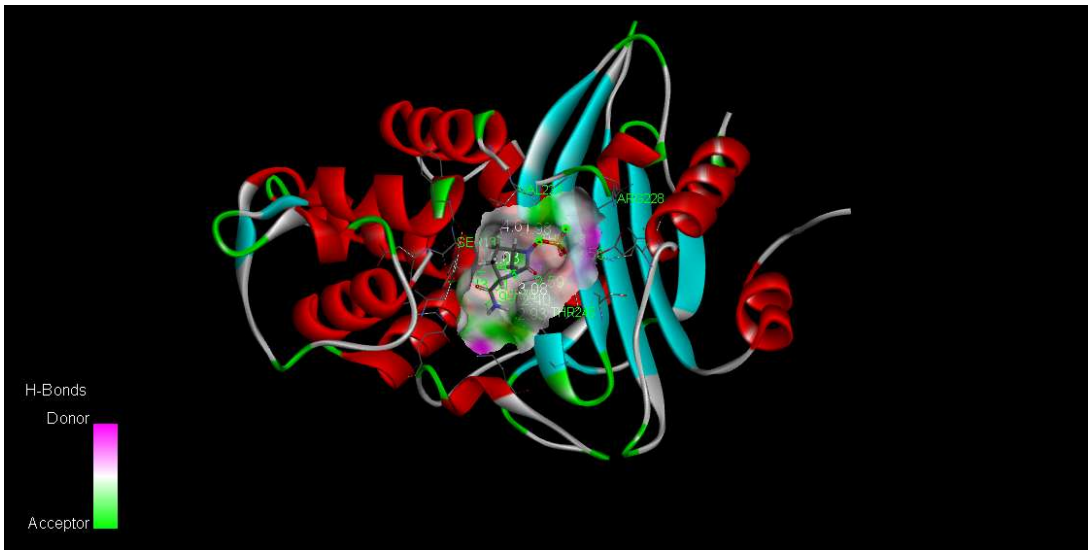

Figure 7: Full 3D representation of docked PenA β-lactamase Avibactam complex with showing the acceptor & donor regions

4. Discussion

This study provides the first detailed sequence and structure-based classification of PenA β-lactamase from *B. stabilis*. The conserved motifs and active site residues align with known resistance mechanisms, reinforcing the model's accuracy. Docking analysis demonstrated Avibactam's potential as a viable inhibitor. Similarity to other clinically significant Burkholderia species further validates these findings and encourages broader therapeutic screening.

Table 1: Active site residues details with interacted residues

Predicted Active Site Residues BIOVIA	**Interacted Residues by 2D**	**Common Residues**
Lys^{81}	Tyr^{137}	Lys^{81}
Ser^{78}	Asn^{252}	Ser^{78}
Cys^{77}	Asn^{222}	Tyr^{113}
Tyr^{113}	Gly^{225}	Ser^{138}
Ser^{138}	Lys^{81}	Asn^{140}
Asn^{140}	Ser^{78}	Asn^{178}
Glu^{174}	Tyr^{113}	Val^{224}
Asn^{178}	Ser^{138}	Arg^{228}
Val^{224}	Asn^{140}	Lys^{242}
Arg^{228}	Asn^{178}	Thr^{243}
Lys^{242}	Val^{224}	Gly^{244}
Thr^{243}	Arg^{228}	Thr^{245}
Gly^{244}	Lys^{242}	
Thr^{245}	Thr^{243}	
Gly^{246}	Gly^{244}	
	Thr^{245}	

5. Conclusion

Burkholderia stabilis is a major contributor to wound infection and produces non-invasive infections. PenA protein can be a target for antibiotic screening and inhibitor selection. Docking analysis revealed that residues Tyr^{137}, Asn^{252}, Asn^{222}, Gly^{225}, Ser^{78}, Lys^{81}, Tyr^{113}, Ser^{138}, Asn^{140}, Asn^{178}, Val^{224}, Arg^{228}, Lys^{242}, Thr^{243}, Gly^{244}, and Thr^{245} of *Burkholderia stabilis* PenA were interacting with Avibactam. Of these, 12 residues Ser^{78}, Lys^{81}, Tyr^{113}, Ser^{138}, Asn^{140}, Asn^{178}, Val^{224}, Arg^{228}, Lys^{242}, Thr^{243}, Gly^{244}, and Thr^{245} were shared by the identified active site residues (PDB ID: 7DOO). According to *in-silico* research, Avibactam may also be utilized as a well-known antibiotic to treat illnesses linked to *Burkholderia stabilis*.

References

1. Barzel, A., & Kupiec, M. (2008). Finding a match: how do homologous sequences get together for recombination? Nature reviews. Genetics, 9(1), 27–37. https://doi.org/10.1038/nrg2224
2. Bush K. (2010). Bench-to-bedside review: The role of beta-lactamases in antibiotic-resistant Gram-negative infections. *Critical care (London, England)*, *14*(3), 224. https://doi.org/10.1186/cc8892
3. Chatzou, M., Magis, C., Chang, J. M., Kemena, C., Bussotti, G., Erb, I., &Notredame, C. (2016). Multiple sequence alignment modeling: methods and applications. Briefings in bioinformatics, 17(6), 1009–1023. https://doi.org/10.1093/bib/bbv099
4. Chen, J., Shang, X., Hu, F., Lao, X., Gao, X., Zheng, H., & Yao, W. (2013). β-Lactamase inhibitors: an update. Mini reviews in medicinal chemistry, 13(13), 1846–1861. https://doi.org/10.2174/13895575113139990074
5. Depoorter, E., Bull, M. J., Peeters, C., Coenye, T., Vandamme, P., &Mahenthiralingam, E. (2016). Burkholderia: an update on taxonomy and biotechnological potential as antibiotic producers. *Applied microbiology and biotechnology*, *100*(12), 5215–5229. https://doi.org/10.1007/s00253-016-7520-x
6. Diller, D.J.; Merz, M.L. III., High Throughput Docking for Library Design and Library Prioritization. Proteins: Structure, Function, and Genetics 2001, 43, 113-124.
7. Dalia Azhar Ahmed (2021) Beta-Lactamases enzymes : Mechanism and classification. Biochem. Cell. Arch. 21,1903-1916. DocID: https://connectjournals.com/03896.2021.21.1903
8. Fiser A. (2010). Template-based protein structure modeling. Methods in molecular biology (Clifton, N.J.), 673, 73–94. https://doi.org/10.1007/978-1-60761-842-3_6
9. Flanagan, J. N., Kavanaugh, L., &Steck, T. R. (2020). *Burkholderia multivorans* Exhibits Antibiotic Collateral Sensitivity. *Microbial drug resistance (Larchmont, N.Y.)*, *26*(1), 1–8. https://doi.org/10.1089/mdr.2019.0202
10. Gaynes R. (2017). The Discovery of PenicillinNew Insights After More Than 75 Years of Clinical Use. Emerging Infectious Diseases, 23(5), 849–853. https://doi.org/10.3201/eid2305.161556
11. Hirvonen, V. H. A., Hammond, K., Chudyk, E. I., Limb, M. A. L., Spencer, J., Mulholland, A. J., & van der Kamp, M. W. (2019). An Efficient Computational Assay for β-Lactam Antibiotic Breakdown by Class A β-Lactamases. *Journal of chemical information and modeling*, *59*(8), 3365–3369. https://doi.org/10.1021/acs.jcim.9b00442
12. Hudson, M. J., Park, S. C., Mathers, A., Parikh, H., Glowicz, J., Dar, D., Nabili, M., LiPuma, J. J., Bumford, A., Pettengill, M. A., Sterner, M. R., Jr, Paoline, J., Tressler, S., Peritz, T., Gould, J.,

Hutter, S. R., Moulton-Meissner, H., & Perkins, K. M. (2022). Outbreak of Burkholderia stabilis Infections Associated with Contaminated Nonsterile, Multiuse Ultrasound Gel - 10 States, May-September 2021. MMWR. Morbidity and mortality weekly report, 71(48), 1517–1521. https://doi.org/10.15585/mmwr.mm7148a3

13. Irisarri, I., Burki, F., & Whelan, S. (2021). Automated Removal of Non-homologous Sequence Stretches with PREQUAL. Methods in molecular biology (Clifton, N.J.), 2231, 147–162. https://doi.org/10.1007/978-1-0716-1036-7_10
14. Ivanov, A. S., Dubanov, A. V., Skvortsov, V. S., & Archakov, A. I. (2002). Komp'iuternoekonstruirovanielekarstvnaosnoveanalizastrukturymakromolekuly-misheni: I. Poisk i opisaniemestasviazyvaniialiganda v belke-misheni [Computer drug design based on analysis of a target macromolecule structure. I. Search and description of a ligand binding site in a target molecule]. Voprosymeditsinskoikhimii, 48(3), 304–315.
15. Kaderabkova, N., Bharathwaj, M., Furniss, R. C. D., Gonzalez, D., Palmer, T., & Mavridou, D. A. I. (2022). The biogenesis of β-lactamase enzymes. Microbiology (Reading, England), 168(8), 10.1099/mic.0.001217. https://doi.org/10.1099/mic.0.001217
16. Kemena, C., &Notredame, C. (2009). Upcoming challenges for multiple sequence alignment methods in the high-throughput era. Bioinformatics (Oxford, England), 25(19), 2455–2465. https://doi.org/10.1093/bioinformatics/btp452
17. Kroemer R. T. (2007). Structure-based drug design: docking and scoring. *Current protein & peptide science*, *8*(4), 312–328. https://doi.org/10.2174/138920307781369382
18. Lees, J. A., Kendall, M., Parkhill, J., Colijn, C., Bentley, S. D., & Harris, S. R. (2018). Evaluation of phylogenetic reconstruction methods using bacterial whole genomes: a simulation based study. Wellcome open research, 3, 33. https://doi.org/10.12688/wellcomeopenres.14265.2
19. Lewis, E. R., & Torres, A. G. (2016). The art of persistence-the secrets to Burkholderia chronic infections. *Pathogens and disease*, *74*(6), ftw070. https://doi.org/10.1093/femspd/ftw070
20. Martin, J. F., Alvarez-Alvarez, R., & Liras, P. (2022). Penicillin-Binding Proteins, β-Lactamases, and β-Lactamase Inhibitors in β-Lactam-Producing Actinobacteria: Self-Resistance Mechanisms. International journal of molecular sciences, 23(10), 5662. https://doi.org/10.3390/ijms23105662
21. Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M. (2011). Molecular docking: a powerful approach for structure-based drug discovery. *Current computer-aided drug design*, *7*(2), 146–157. https://doi.org/10.2174/157340911795677602
22. Mohammad Yaseen Sofi, Afshana Shafi, Khalid Z. Masoodi, Chapter 6 - Multiple sequence alignment, Bioinformatics for Everyone, Academic Press, 2022, Pages 47-53, ISBN9780323911283, https://doi.org/10.1016/B978-0-323-91128-3.00011-2.
23. Morris, G. M., & Lim-Wilby, M. (2008). Molecular docking. *Methods in molecular biology (Clifton, N.J.)*, *443*, 365–382. https://doi.org/10.1007/978-1-59745-177-2_19
24. Papp-Wallace, K. M., Becka, S. A., Zeiser, E. T., Ohuchi, N., Mojica, M. F., Gatta, J. A., Falleni, M., Tosi, D., Borghi, E., Winkler, M. L., Wilson, B. M., LiPuma, J. J., Nukaga, M., & Bonomo, R. A. (2017). Overcoming an Extremely Drug Resistant (XDR) Pathogen: Avibactam Restores Susceptibility to Ceftazidime for Burkholderia cepacia Complex Isolates from Cystic Fibrosis Patients. ACS infectious diseases, 3(7), 502–511. https://doi.org/10.1021/acsinfecdis.7b00020
25. Pearson W. R. (2013). An introduction to sequence similarity ("homology") searching. Current protocols in bioinformatics, Chapter 3, 3.1.1–3.1.8. https://doi.org/10.1002/0471250953.bi0301s42
26. Peralta, D. P., Chang, A. Y., Ariza-Hutchinson, A., & Ho, C. A. (2018). *Burkholderia multivorans:* A rare yet emerging cause of bacterial meningitis. *IDCases*, *11*, 61–63. https://doi.org/10.1016/j.idcr.2018.01.002

27. Schwede, T., Kopp, J., Guex, N., & Peitsch, M. C. (2003). SWISS-MODEL: an automated protein homology-modeling server. Nucleic acids research, 31(13), 3381-3385.
28. Seth-Smith, H. M. B., Casanova, C., Sommerstein, R., Meinel, D. M., Abdelbary, M. M. H., Blanc, D. S., Droz, S., Führer, U., Lienhard, R., Lang, C., Dubuis, O., Schlegel, M., Widmer, A., Keller, P. M., Marschall, J., &Egli, A. (2019). Phenotypic and Genomic Analyses of Burkholderia stabilis Clinical Contamination, Switzerland. *Emerging infectious diseases*, *25*(6), 1084–1092. https://doi.org/10.3201/eid2506.172119
29. Sherrard, L. J., Tunney, M. M., & Elborn, J. S. (2014). Antimicrobial resistance in the respiratory microbiota of people with cystic fibrosis. *Lancet (London, England)*, *384*(9944), 703–713. https://doi.org/10.1016/S0140-6736(14)61137-5
30. Somboro, A. M., Osei Sekyere, J., Amoako, D. G., Essack, S. Y., & Bester, L. A. (2018). Diversity and Proliferation of Metallo-β-Lactamases: a Clarion Call for Clinically Effective Metallo-β-Lactamase Inhibitors. *Applied and environmental microbiology*, *84*(18), e00698-18. https://doi.org/10.1128/AEM.00698-18
31. Stokell, J. R., Gharaibeh, R. Z., &Steck, T. R. (2013). Rapid emergence of a ceftazidime-resistant Burkholderia multivorans strain in a cystic fibrosis patient. *Journal of cystic fibrosis: official journal of the European Cystic Fibrosis Society*, *12*(6), 812–816. https://doi.org/10.1016/j.jcf.2013.01.009
32. Subhaswaraj, P., &Siddhardha, B. (2022). Molecular docking and molecular dynamic simulation approaches for drug development and repurposing of drugs for severe acute respiratory syndrome-Coronavirus-2. Computational Approaches for Novel Therapeutic and Diagnostic Designing to Mitigate SARS-CoV-2 Infection, 207–246. https://doi.org/10.1016/B978-0-323-91172-6.00007-8
33. Tooke, C. L., Hinchliffe, P., Bragginton, E. C., Colenso, C. K., Hirvonen, V. H. A., Takebayashi, Y., & Spencer, J. (2019). β-Lactamases and β-Lactamase Inhibitors in the 21st Century. Journal of molecular biology, 431(18), 3472–3500. https://doi.org/10.1016/j.jmb.2019.04.002.
34. Torres, P. H. M., Sodero, A. C. R., Jofily, P., & Silva-Jr, F. P. (2019). Key Topics in Molecular Docking for Drug Design. International journal of molecular sciences, 20(18), 4574. https://doi.org/10.3390/ijms20184574
35. Tørresen, O. K., Star, B., Mier, P., Andrade-Navarro, M. A., Bateman, A., Jarnot, P., Gruca, A., Grynberg, M., Kajava, A. V., Promponas, V. J., Anisimova, M., Jakobsen, K. S., & Linke, D. (2019). Tandem repeats lead to sequence assembly errors and impose multi-level challenges for genome and protein databases. Nucleic acids research, 47(21), 10994–11006. https://doi.org/10.1093/nar/gkz841
36. Vandamme, P., Mahenthiralingam, E., Holmes, B., Coenye, T., Hoste, B., De Vos, P., Henry, D., &Speert, D. P. (2000). Identification and population structure of Burkholderia stabilis sp. nov. (formerly Burkholderia cepacia genomovar IV). *Journal of clinical microbiology*, *38*(3), 1042–1047. https://doi.org/10.1128/JCM.38.3.1042-1047.2000
37. Warnow T. (2021). Revisiting Evaluation of Multiple Sequence Alignment Methods. Methods in molecular biology (Clifton, N.J.), 2231, 299–317. https://doi.org/10.1007/978-1-0716-1036-7_17
38. Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F. T., de Beer, T. A. P., Rempfer, C., Bordoli, L., Lepore, R., & Schwede, T. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic acids research, 46(W1), W296–W303. https://doi.org/10.1093/nar/gky427
39. Wu, G.; Robertson, D.H.; Brooks, C.L. III; Vieth, M., Detailed Analysis of Grid-Based Molecular Docking: A Case Study of CDOCKER - A CHARMm-Based MD Docking Algorithm. J. Comp. Chem. 2003, 24, 1549. https://doi.org/10.1002/jcc.10306