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STRUCTURAL CHARACTERIZATION AND COMPARATIVE *IN SILICO* SCREENING OF POTENTIAL LIGANDS AGAINST ANTIMICROBIAL RESISTANCE PROTEINS IDENTIFIED FROM THE RIVER VARUNA

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ABSTRACT:
Antimicrobial resistance (AMR) in aquatic environments represent an important environmental dimension of the global AMR load, specifically in the polluted river systems receiving diverse anthropogenic inputs. The present study investigated selected abundant AMR proteins identified at four sites (VR1-VR4) of the river Varuna using a whole-genome shotgun metagenomic approach. Further, a computational screening-ligand-associated study was performed. AMR protein sequences were subjected to BLASTp analysis for sequence-level identification, followed by conserved domain analysis to confirm their functional properties. The proteins identified were Sul1, Sul4, blaOXA, and blaVEB-9 from sites VR1, VR2, VR3, and VR4. Conserved domain analysis identified dihydropteroate synthase domain in Sul1 and Sul4, and a class D ß-lactamase domain in blaOXA and a class A ß-lactamase related serine hydrolase domain in blaVEB-9. Experimentally determined 3D protein structures were retrieved from the Protein Data Bank termed as 7S2I, 1TWS, 1M6K, and 6NVT. Comparative cavity-guided molecular docking was performed using CB-Dock2 with sulfamethoxazole (FDA-approved drug) and thymol (natural compound). Thymol showed predicted docking scores ranging from -6.3 to -8.1kcal/mol. The most favorable predicted interaction for both ligands was observed with blaVEB-9. This finding provides preliminary structural insights into ligand-binding patterns of environment-derived AMR (Antimicrobial resistance) proteins and identifies protein-ligand combinations to analyze further biochemical and microbiological validation.

KEYWORDS: Antimicrobial resistance, Varuan River, Molecular Docking, Thymol, Sulfamethoxazole

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as a major global health challenge because the progressive loss of antimicrobial efficacy compromises the treatment of bacterial infections and increases the risk of persistent, recurrent and difficult-to-treat diseases (Salam et al., 2023). Although AMR has traditionally been considered predominantly a clinical challenge, increasing evidence demonstrates that resistance is distributed across interconnected human, animal and environmental compartments (Al-Khalaifah et al., 2025). The "One Health Framework" therefore recognizes the environment as an important component of AMR emergence, persistence, dissemination and transmission (Naznine et al., 2025). Aquatic ecosystems are particularly relevant because they receive complex mixtures of domestic sewage, hospital effluents, agricultural run-off, livestock waste, pharmaceutical residues and industrial discharges, creating ecological conditions that can facilitate the maintenance and dissemination of antimicrobial resistance genes (ARGs) (Gaur et al., 2022). Environmental microbial communities can consequently function as reservoirs from which resistance determinants may potentially reach clinically relevant bacterial populations through horizontal gene transfer and mobile genetic elements (Kushwaha et al., 2023). Rivers are dynamic interfaces connecting terrestrial, agricultural, urban and human-associated microbial systems and therefore represent important environments for resistome surveillance (Rizvi et al., 2025). Metagenomic investigations of aquatic ecosystems, including rivers, lakes, oceans and marine environments, have established considerable diversity of ARGs even where direct antibiotic contamination has not been quantified (Gupta et al., 2020). For example, Genome-resolved metagenomic analysis of the Caspian Sea, identified ARGs associated with multiple bacterial lineages and resistance mechanisms, including the prevention of antibiotic access, target modification or protection and direct antibiotic modification (Goodarzi et al., 2022). The study further demonstrated the presence of beta-lactamases, aminoglycoside-modifying enzymes, macrolide resistance genes, and multidrug efflux systems in environmental microbial genomes. Such findings focus on the environmental resistomes contain diverse molecular markers with potential relevance to antimicrobial treatment failure and justify their characterization at the protein level (Goodarzi et al., 2022). ARG detection does not provide sufficient information regarding the structural characteristics of their encoded proteins or their suitability as molecular targets for inhibitor development (Kaprou et al., 2021). Translation of mutagenic AMR information into structural biology can bridge this gap by examining protein sequence characteristics, three-

dimensional conformation, conserved functional residues, ligand-binding regions and potential mechanisms of inhibition. This transition from metagenomic resistome profiling to protein level structural analysis is valuable for environmental AMR genes for which experimental structures and biochemical characterization may be unavailable (De Oliveira Teixeira et al., 2026). ß-lactamases compromise structural distinct molecular classes, with Metallo-ß-lactamases presenting substantial challenges for inhibitor development (Nusrat et al., 2025). Advances in structural bioinformatics and computer-aided drug discovery provide an efficient strategy for investigating such targets (Brown & Bishop, 2017). Protein structure prediction, homology-based analysis, binding-pocket identification, virtual screening and molecular docking calculation can together provide mechanistic hypotheses regarding protein-ligand recognition (Yoon et al., 2026). Molecular docking predicts energetically favorable ligand orientation and interactions within a receptor and can therefore be used to prioritize compounds from large chemical libraries before experimental testing (Meng et al., 2011). Blind molecular docking is relevant to environmental derived or non-characterized AMR proteins because it permits exploration of the complete protein surface without assuming a predefined binding pocket, thereby allowing potential catalytic, regulatory or allosteric binding regions to be identified (Asim, 2024). Recent computational studies against clinically important resistance enzymes demonstrates the value of integrating virtual screening with the structural validation, ADMET assessment, molecular docking and binding free energy calculations (Ferreira et al., 2015). For example, a recently CTX-M-14 ß-lactamase study combined pharmacophore-based screening, docking, ADMET filtering, MD simulation and MM/GBSA analysis to identify a potential inhibitor scaffold, while focusing that experimental biochemical and microbiological validation remains essential (Haque et al., 2025). This integrated framework provides a rational model for extending environmental AMR research beyond surveillance towards target oriented ligand screening (Baroja et al., 2026).

In the present study, structure based computational investigation of AMR protein identified from the polluted river Varuna, indicating a disturbed and anthropogenically stressed ecosystem. AMR protein sequences derived from the river metagenome dataset, published earlier shown beta-lactam and sulfonamide resistant antimicrobial proteins were abundant among four major regions of river Varuna at site VR1 (Rameshwar Ghat), VR2 (Andhrapul), VR3 (Chaukaghat) and VR4 (Adikeshav Ghat) as given in table 1. The AMR dataset subjected to the sequence and structural characterization, target analysis, three-dimensional structure and blind molecular docking analysis. The study is designed to establish a computational link between environmental metagenomic AMR surveillance and structure-based ligand screening, generating molecular hypothesis for biochemical and microbiological validation rather than proving antimicrobial efficacy from computational predictions only (Lakhanpal et al., 2024).

Table 1. Investigational sites of the river Varuna in the Varanasi region

Sample Sites	Name of Sites	Geo-Coordinate Locations
VR1	Rameshwar Ghat	25°23'26.1"N 82°51'21.6"E
VR2	Andhrapul Region	25°20'08.4"N 82°59'31.1"E
VR3	Chaukaghat Region	25°20'05.8"N 82°59'45.3"E
VR4	Adikeshav Ghat	25°19'46.7"N 83°02'38.3"E

2. METHODOLOGY

2.1 Antimicrobial Resistance Protein Identification

After metagenomic sequencing using whole genome shotgun method, multiple antimicrobial resistance (AMR) proteins (FASTA format) were derived from each site of the river Varuna and most abundant sequences were taken for 'BLASTp' analysis against non-redundant protein database to identify homologous protein and infer the probable function identity of the unknown sequences. The best supported homologous were evaluated based on the sequence identity, query coverage, E-value and bit score, followed by confirmation of conserved functional domains and AMR associated characteristics preceding to further structural analysis (Mazumder et al., 2022).

2.2 Conserved domain search for AMR proteins

NCBI (National Center for Biotechnology Information) based conserved domain search to confirm the presence of characteristic functional domains. The annotated AMR proteins were considered for structural and molecular docking analysis (Yang et al., 2019).

2.3 Three Dimensional (3D) structure prediction and validation

Experimentally determined three-dimensional structures corresponding to the identified AMR proteins were retrieved from the protein databank based on sequence similarity and query coverage obtained through BLASTp analysis (Altschul et al., 1990).

2.4 Cavity Identification and Molecular Docking

Cavity detected guided blind molecular docking was performed by allowing selected ligands to explore the protein surface, and the resulting complexes were evaluated based on binding affinity and protein–ligand interactions (Liu et al., 2022).

3. RESULTS

3.1 Interpretation of AMR Proteins identified from the sites of River Varuna

Table 2. BLASTp identification of AMR proteins from VR1-VR4 based on sequence identity, query coverage, E-value and functional annotation

Varuna river site	BLASTp identification	Identity	Coverage	E-value	Accession No.
VR1	Sul1	100%	100%	0.0	EKQ5508431.1
VR2	Sul4	100%	100%	0.0	WP_206434233.1
VR3	blaOXA	100%	100%	0.0	WP_032490448.1
VR4	blaVEB-9	100%	100%	0.0	WP_032494864.1

The BLASTp analysis provided sequence level identification of the four most abundant AMR proteins recovered from the Varuna River sites. The VR1 and VR2 proteins showed 100% identity and 100% query coverage with Sul1 (EKQ5508431.1) and Sul 4 (WP_206434233.1), respectively, while VR3 and VR4 showed complete sequence identity and coverage with blaOXA (WP_032490448.1) and blaVEB-9 (WP_032494864.1) as observed. All four alignments produced an E-value of 0.0, indicating highly significant sequence matches and providing strong sequence-based evidence for the assignment of these proteins to their respective AMR categories, as given in table 2. These four site specific AMR proteins were therefore selected for conserved-domain and structural analysis.

3.2 Identification of Conserved Domains for AMR proteins

Table 3. Conserved domain analysis of the most abundant antimicrobial resistance proteins identified from the four sites of the river Varuna (VR1-VR4), showing domain description, accession numbers and corresponding amino acid intervals

Varuna river site	BLASTp identification	Conserved Domain Description	Conserved Domain Accession No.	Conserved Domain E-value	Conserved Domain Interval
VR1	Sul1	Dihydropteroate synthase	PRK13753	0.0	2-279
VR2	Sul4	Dihydropteroate synthase [Coenzyme transport and metabolism]	COG0294	4.26×10^{-138}	12-287
VR3	blaOXA	Class D beta-lactamase	COG2602	3.59×10^{-86}	1-276
VR4	blaVEB-9	class A beta-lactamase-related serine hydrolase	COG2367	2.11×10^{-76}	11-296

The conserved domain analysis of all four AMR proteins from the river Varuna River sites (VR1-VR4) shown that VR1 (Sul1) showed a dihydropteroate synthase domain (PRK13753) with an E-value of 0.0, having residues 2–279; VR2 (Sul4) showed a dihydropteroate synthase domain (COG0294) with an E-value of 4.26×10^{-138} having 12–287 residues. VR3 (blaOXA) contained a Class D β-lactamase domain (COG2602; E-value 3.59×10^{-86}) across residues 1–276, whereas VR4 (blaVEB-9) exhibited a Class A β-lactamase-related serine hydrolase domain (COG2367; E-value 2.11×10^{-76}) having 11–296 residues, as in table 3. The highly significant E-values and extensive domain intervals provide strong domain-level support for the respective AMR protein annotations and justify their progression to structural analysis.

3.3 AMR proteins structural analysis

BLASTp searches against the PDB database identified suitable structural matches for all four AMR proteins. VR1 (Sul1) and VR2 (Sul4) showed 100% identity and 100% query coverage with PDB structures 7S2I and 1TWS, respectively, indicating exact sequence-level structural matches. VR3 (blaOXA) showed 97.21% identity with 91% coverage to 1M6K, whereas VR4 (blaVEB-9) showed 91.28% identity with 91% coverage to 6NVT, as shown in table 4; all matches had an E-value of 0.0, supporting their statistical significance and suitability for structural assessment.

Table 4. PDB based structural identification of the most abundant antimicrobial resistant proteins from the four Varuna River sites (VR1-VR4) using BLASTp against PDB (Protein Databank) database

Varuna river site	BLASTp identification	Percent Identity	Query Coverage	E-value	Relevant Structure PDB ID

VR1	Sul1	100%	100%	0.0	7S2I
VR2	Sul4	100%	100%	0.0	1TWS
VR3	blaOXA	97.21%	91%	0.0	1M6K
VR4	blaVEB-9	91.28%	91%	0.0	6NVT

3.4 Ligand Selection and Molecular Docking Analysis

For comparative molecular docking, sulfamethoxazole, an FDA-approved sulfonamide antibiotic (Adzah et al., 2026), was selected as the reference ligand because of its direct relevance to the Sul1/Sul4-associated sulfonamide resistance mechanism (Venkatesan et al., 2023), while thymol, a naturally occurring monoterpenoid phenol with reported antibacterial activity (Ugras et al., 2026). Thymol is a natural chemical substance ($C_{10}H_{14}O$) derived mostly from thyme oil, ajwain (*Trachyspermum ammi*), and other plants (Ugras et al., 2026). It appears as white crystals with a pleasant aroma and potent antiseptic, antibacterial, and antifungal properties was selected as the natural antibacterial candidate and ADMET properties of thymol was calculated using SwissADME and ADMET lab 3.0 (Qureshi & Parvez, 2026), as given in table 5. Both compounds were considered for comparative ligand-binding screening across the four environmentally derived AMR proteins (VR1–VR4) to evaluate their predicted binding affinities and interaction profiles. Molecular docking was performed using the CB-Dock2 web server, which automatically identifies potential ligand-binding cavities in the receptor structure and performs docking within the predicted binding regions (Liu & Cao, 2023). The docked complexes were evaluated based on predicted binding affinity, docking pose, cavity location, and protein–ligand interactions (Liu et al., 2019), with more favorable (more negative) binding scores indicating stronger predicted ligand-binding potential (Castro-Alvarez et al., 2017).

Table 5. Swiss ADME and ADMET lab 3.0 associated analysis of natural compound Thymol

Parameter	Results
Molecule Name	Thymol
Chemical Formula	$C_{10}H_{14}O$
Molecular Weight	150.22 g/mol
Gastrointestinal Absorption	High
Blood Brain Barrier Permeability	Yes
Lipinski Violation	0, No
Bioavailability Score	0.55
Aquatic Toxicity (toxicity to water)	0
Acute Toxicity Rule	0

The SwissADME and ADMETlab 3.0 (Qureshi & Parvez, 2026) analysis indicated that Thymol possesses favorable predicted drug-like characteristics, with a low molecular weight (150.22g/mol), high predicted gastrointestinal absorption, and predicted blood–brain barrier permeability. The compound showed no Lipinski rule violations and a bioavailability score of 0.55, supporting its potential for further pharmacological investigation. Moreover, the observed aquatic toxicity and acute toxicity rule values were 0, according to the prediction outputs. Overall, these results support the selection of Thymol as a natural compound for preliminary comparative molecular docking against the identified AMR proteins.

Table 6. CB-Dock2 based molecular docking analysis of Thymol against AMR proteins identified from the four sites of the river Varuna.

Varuna river sites	AMR proteins identified	AMR relevant Protein (PDB)	Ligand selected	CB Dock2 score (kcal/mol)	Cavity Volume (Å3)	Docking size (x, y, z)
VR1	Sul1	7S2I	Thymol	-5.8	9124	35,35,17
VR2	Sul4	1TWS	Thymol	-5.4	205	17,17,17
VR3	blaOXA	1M6K	Thymol	-5.5	165	17,17,17
VR4	blaVEB-9	6NVT	Thymol	-6.0	750	17,17,17

Table 7. CB-Dock2 based molecular docking analysis of Sulfamethoxazole against AMR proteins identified from the four sites of the river Varuna.

Varuna river sites	AMR proteins identified	AMR relevant Protein (PDB)	Ligand selected	CB Dock2 score (kcal/mol)	Cavity Volume (Å3)	Docking size (x, y, z)
VR1	Sul1	7S2I	Sulfamethoxazole	-7.4	9124	33,35,19

VR2	Sul4	1TWS	Sulfamethoxazole	-6.4	1948	19,19,19
VR3	blaOXA	1M6K	Sulfamethoxazole	-6.3	165	19,19,19
VR4	blaVEB-9	6NVT	Sulfamethoxazole	-8.1	443	19,19,19

CB-Dock2 analysis of Thymol against the four AMR proteins derived from the WGS analysis of the river Varuna showed predicted binding score ranging from -5.4 to -6.0kcal/mol. Thymol exhibits the most favorable predicted binding with VR4 (blaVEB-9; 6NVT) at -6.0kcal/mol, followed by VR1 (Sul1; 7S2I) at -5.8kcal/mol. VR3 (blaOXA; 1M6K) at -5.5kcal/mol and VR2 (Sul4; 1TWS) at -5.4kcal/mol as mentioned in table 6. These results represent computational binding predictions and require further interaction and experimental validation. Similarly, CB-Dock2 analysis showed favorable predicted binding of Sulfamethoxazole with all four AMR proteins with docking scores ranging from -6.3 to -8.1kcal/mol, as in table 7. The strongest prediction binding was observed for VR4 (blaVEB-9, 6NVT) with a score of -6.0kcal/mol, the docking figure is mentioned as fig 1 and another strongest docking observed with sulfamethoxazole against 6NVT with a score of -8.1kcal/mol, mentioned in table 6 and 7. Sulfamethoxazole generally showed more favorable docking score than thymol, specifically against blaVEB-9 identified at the VR4 region of the river Varuna. These finding indicates differential ligand-binding potential among the AMR proteins; however, docking scores represent computational predictions.

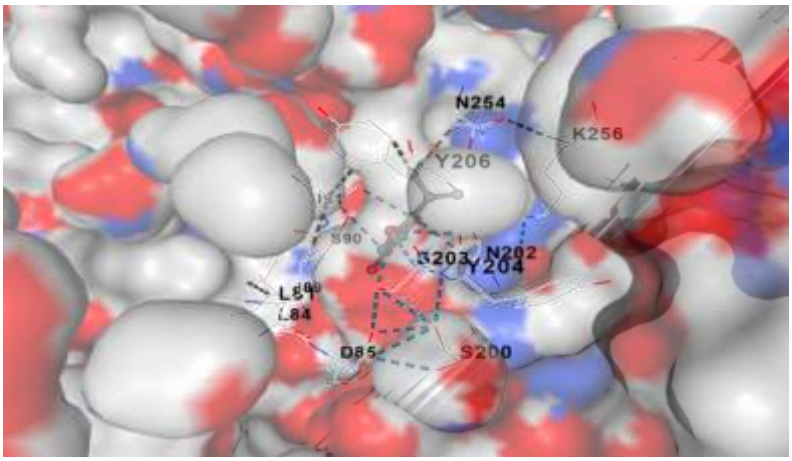

Fig 1. Molecular Docking pose of Thymol within the predicted binding cavity of blaVEB-9 protein (6NVT), showing the ligand docked within the protein binding pocket and its surrounding amino acid residues on the molecular surface.

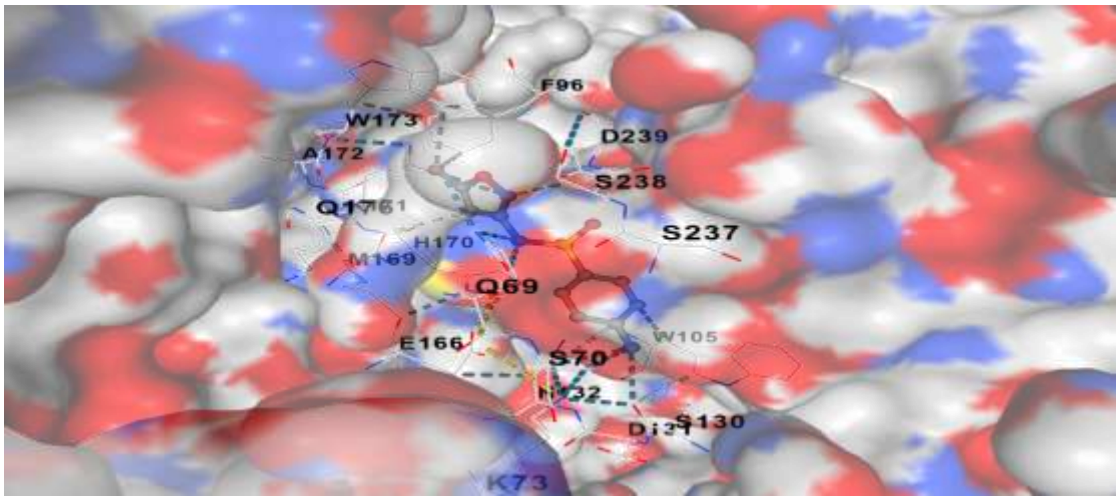

Fig 2. Molecular Docking pose of Sulfamethoxazole within the predicted binding cavity of blaVEB-9 protein (6NVT), showing the ligand docked within the protein binding pocket and its surrounding amino acid residues on the molecular surface.

4. CONCLUSION

The present investigation establishes the utility of a metagenome-guided structural bioinformatics framework for characterizing antimicrobial resistance proteins occurring in a polluted river ecosystem and for conducting preliminary comparative ligand-binding analysis. Four abundant AMR protein sequences representing the VR1-VR4 sampling sites of the river Varuna River were investigated through sequential sequence, domain structural and docking analysis. BLASTp analysis provided clear sequence-level identification of the VR1, VR2, VR3 and VR4 as Sul1, Sul4, blaOXA and blaVEB-9, with the reported results showing 100% sequence identity and query

coverage and E-values of 0.0. These results provide strong evidence for study of the site-specific sequences to their specific AMR protein groups. Comparative molecular docking using CB-Dock2 demonstrated predicted interactions of both sulfamethoxazole and thymol with all four AMR proteins. Thymol showed docking scores ranging from -5.4 to -6.0 kcal/mol, with the most favorable predicted interaction observed against VR4/blaVEB-9 (-6.0kcal/mol), followed by VR1/Sul1 (-5.8kcal/mol), VR3/blaOXA (-5.5kcal/mol) and VR2/Sul4 (-5.4kcal/mol). Sulfamethoxazole produced comparatively more favorable predicted scores, ranging from -6.3 to -8.1kcal/mol, with the strongest predicted interaction again observed for VR4/blaVEB-9 (-8.1kcal/mol). Aquatic environments receiving fecal, domestic, agricultural and wastewater inputs can act as reservoirs for antimicrobial resistance genes, with ß-lactam, sulfonamide, tetracycline, aminoglycoside, quinolone and multidrug-resistance genes frequently associated with the polluted water. The persistence and dissemination of these genes may be influenced by the environmental conditions and mobile genetic elements that facilitate horizontal gene transfer. Sulfamethoxazole and thymol were selected as major ligands for comparative molecular docking against the identified AMR proteins. Sulfamethoxazole is a clinically established sulfonamide antibiotic that targets the folate biosynthetic pathway through the inhibition of dihydropteroate synthase making it relevant to Sul1 and Sul4 resistance associated proteins. Thymol, a naturally occurring monoterpenoid phenolic compound, possesses reported antimicrobial properties and was selected as a structural distinct natural ligand for comparative assessment. The study covers SDG 3 and 6 (clean water sanitation, good health and well-being) because the study investigates antimicrobial resistance genes within a polluted river as environment-based AMR represents a potential component of wide AMR threat to effective antimicrobial treatment. Overall, the study provides a preliminary structure-based framework for prioritizing ligand interactions with environment derived AMR proteins.

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Conflict of Interest
The author declares there is no conflict of interest.

REFERENCES
1. Adzah, B. Y., Biniyam, P. D., Ohene-Adu, V., Osei, M., Adu-Adjei, K., Elmes, V., Goodall, I., Faustina, V., Agboyibor, Y. D., Gyan, P., Quansah, P., Amengor, C. M., Amengor, C. M., Boateng, J., Zhang, E., Ampomah-Wireko, M., Oyeh, E., Amengor, C. D. K., & Amengor, C. D. K. (2026). Dual-targeting sulfamethoxazole derivatives overcome multidrug resistance in ESKAPE pathogens by inhibiting quorum sensing and efflux pumps. RSC Advances, 16(10), 8921–8944. https://doi.org/10.1039/d5ra08024j
2. Al-Khalaifah, H., Rahman, M. H., Al-Surrayai, T., Al-Dhumair, A., & Al-Hasan, M. (2025). A One-Health Perspective of Antimicrobial Resistance (AMR): Human, Animals and Environmental health. Life, 15(10), 1598. https://doi.org/10.3390/life15101598
3. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. Journal of Molecular Biology, 215(3), 403–410. https://doi.org/10.1016/s0022-2836(05)80360-2
4. Asim, A. (2024). Approaches to backbone flexibility in Protein–Protein docking. In Methods in molecular biology (Vol. 2780, pp. 45–68). https://doi.org/10.1007/978-1-0716-3985-6_4
5. Baroja, E., Batalla, I., Sanz, M. J., & Chiabai, A. (2026). An integrated framework for antimicrobial resistance: links with climate change and vulnerability. Frontiers in Public Health, 13, 1679189. https://doi.org/10.3389/fpubh.2025.1679189
6. Brown, D. K., & Bishop, Ö. T. (2017). Role of structural bioinformatics in drug discovery by computational SNP analysis. Global Heart, 12(2), 151. https://doi.org/10.1016/j.gheart.2017.01.009
7. Castro-Alvarez, A., Costa, A., & Vilarrasa, J. (2017). The performance of several docking programs at reproducing Protein–Macrolide-Like crystal structures. Molecules, 22(1), 136. https://doi.org/10.3390/molecules22010136
8. De Oliveira Teixeira, T., Da Silva, R. C. C., Alves, M. C. S., De Araujo Silva, R., Echeverria, J. E. S., & Simionatto, S. (2026). Innovative approaches to therapeutic target discovery amid the global challenge of antimicrobial resistance. Journal of Computer-Aided Molecular Design, 40(1). https://doi.org/10.1007/s10822-026-00905-3
9. Ferreira, L., Santos, R. D., Oliva, G., & Andricopulo, A. (2015). Molecular Docking and Structure-Based drug design Strategies. Molecules, 20(7), 13384–13421. https://doi.org/10.3390/molecules200713384
10. Gaur, V. K., Sirohi, R., Bhat, M. I., Gautam, K., Sharma, P., Srivastava, J. K., & Pandey, A. (2022). A review on the effect of micro- and nano-plastics pollution on the emergence of antimicrobial resistance. Chemosphere, 311(Pt 1), 136877. https://doi.org/10.1016/j.chemosphere.2022.136877
11. Goodarzi, Z., Asad, S., & Mehrshad, M. (2022). Genome-resolved insight into the reservoir of antibiotic resistance genes in aquatic microbial community. Scientific Reports, 12(1), 21047. https://doi.org/10.1038/s41598-022-25026-3

12. Gupta, C. L., Tiwari, R. K., & Cytryn, E. (2020). Platforms for elucidating antibiotic resistance in single genomes and complex metagenomes. Environment International, 138, 105667. https://doi.org/10.1016/j.envint.2020.105667
13. Haque, S., Bantun, F., Jalal, N. A., Faidah, H., Babalghith, A. O., Aldairi, A. F., & Rehman, M. T. (2025). Pharmacophore-based virtual screening and molecular dynamics simulations for identifying potential enamine inhibitors of bacterial CTX-M-14 β-lactamases. Journal of Taibah University for Science, 19(1). https://doi.org/10.1080/16583655.2025.2557037
14. Kaprou, G. D., Bergšpica, I., Alexa, E. A., Alvarez-Ordóñez, A., & Prieto, M. (2021). Rapid methods for antimicrobial resistance diagnostics. Antibiotics, 10(2), 209. https://doi.org/10.3390/antibiotics10020209
15. Kushwaha, S. P., Hasan, S. M., Kumar, A., Arif, M., & Varshney, M. M. (2023). Human health hazards due to antimicrobial resistance spread. In Elsevier eBooks (pp. 225–240). https://doi.org/10.1016/b978-0-323-96124-0.00007-6
16. Lakhanpal, G., Tiwari, H., Shukla, M. K., & Kumar, D. (2024). In silico exploration of hypothetical proteins in Neisseria gonorrhoeae for identification of therapeutic targets. In Silico Pharmacology, 12(1), 10. https://doi.org/10.1007/s40203-023-00186-w
17. Liu, Y., & Cao, Y. (2023). Protein–Ligand blind docking using CB-Dock2. In Methods in molecular biology (Vol. 2714, pp. 113–125). https://doi.org/10.1007/978-1-0716-3441-7_6
18. Liu, Y., Grimm, M., Dai, W., Hou, M., Xiao, Z., & Cao, Y. (2019). CB-Dock: a web server for cavity detection-guided protein–ligand blind docking. Acta Pharmacologica Sinica, 41(1), 138–144. https://doi.org/10.1038/s41401-019-0228-6
19. Liu, Y., Yang, X., Gan, J., Chen, S., Xiao, Z., & Cao, Y. (2022). CB-Dock2: improved protein–ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic Acids Research, 50(W1), W159–W164. https://doi.org/10.1093/nar/gkac394
20. Mazumder, L., Hasan, M. R., Fatema, K., Islam, M. Z., & Tamanna, S. K. (2022). Structural and Functional Annotation and Molecular Docking Analysis of a Hypothetical Protein from Neisseria gonorrhoeae: An In-Silico Approach. BioMed Research International, 2022(1), 4302625. https://doi.org/10.1155/2022/4302625
21. Meng, X., Zhang, H., 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. Naznine, F., Shafi, Z., Aafreen, U., Shahid, M., Parveen, S., & Ansari, M. I. (2025). Tracking antimicrobial resistance in river waters: Sources, key microbes, and detection techniques. The Microbe, 7, 100386. https://doi.org/10.1016/j.microb.2025.100386
23. Nusrat, S., Aliyu, M., & Zohora, F. T. (2025). Mechanisms of antimicrobial resistance: From genetic evolution to clinical manifestations. AIMS Microbiology, 11(4), 1007–1034. https://doi.org/10.3934/microbiol.2025045
24. Qureshi, K. A., & Parvez, A. (2026). Antimicrobial and antibiofilm evaluation of thymol, sodium azide, and sodium lauryl sulfate against multidrug-resistant pathogens: An integrated experimental and computational study. PLoS ONE, 21(4), e0345977. https://doi.org/10.1371/journal.pone.0345977
25. Rizvi, M., Khan, M., Al-Jardani, A., Al-Abri, S. S., Ghoshal, U., Jabri, Z. A., Ahanjan, M., Mamari, A. A., Shizawi, N. A., Sami, H., Balkhair, A., Shaukat, A., Alkharusi, A. N. S., Almahrouqi, A., Hassan, A., Kumar, A., Malehi, A. S., Gul, A., Singh, A., . . . Abri, R. A. (2025). Mapping antimicrobial susceptibility of community-acquired uropathogenic Escherichia coli across low, middle and high-income countries highlights significant differences: insights for empiric treatment. IJID Regions, 16, 100706. https://doi.org/10.1016/j.ijregi.2025.100706
26. Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. a. A. (2023). Antimicrobial resistance: a growing serious threat for global public health. Healthcare, 11(13), 1946. https://doi.org/10.3390/healthcare11131946
27. Ugras, S., Rasgele, P. G., Karagul, P., Sipahi, N., & Ugras, H. I. (2026). Thymol as a natural monoterpenoid antibacterial agent: a comprehensive review of applications and current advances. Archives of Microbiology, 208(11). https://doi.org/10.1007/s00203-026-05086-z
28. Venkatesan, M., Fruci, M., Verellen, L. A., Skarina, T., Mesa, N., Flick, R., Pham, C., Mahadevan, R., Stogios, P. J., & Savchenko, A. (2023). Molecular mechanism of plasmid-borne resistance to sulfonamide antibiotics. Nature Communications, 14(1), 4031. https://doi.org/10.1038/s41467-023-39778-7
29. Yang, M., Derbyshire, M. K., Yamashita, R. A., & Marchler-Bauer, A. (2019). NCBI's conserved domain database and tools for protein domain analysis. Current Protocols in Bioinformatics, 69(1), e90. https://doi.org/10.1002/cpbi.90