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Cefoperazone targets D-alanyl-D-alanine carboxypeptidase (DAC) to control *Morganella morganii*-mediated infection: a subtractive genomic and molecular dynamics approach

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ABSTRACT

Morganella morganii is a Gram-negative bacterial pathogen that causes bacteremia, urinary tract infections, intra-abdominal infections, chorioamnionitis, neonatal sepsis, and newborn meningitis. To control this bacterial pathogen a total of 3565 putative proteins targets in *Morganella morganii* were screened using comparative subtractive analysis of biochemical pathways annotated by the KEGG that did not share any similarities with human proteins. One of the targets, D-alanyl-D-alanine carboxypeptidase DacB [*Morganella*] was observed to be implicated in the majority of cell wall synthesis pathways, leading to its selection as a novel pharmacological target. The drug that interacted optimally with the identified target was observed to be Cefoperazone (DB01329) with the estimated free energy of binding −8.9 Kcal/mol. During molecular dynamics simulations; it was observed that DB01328-2exb and DB01329-2exb complexes showed similar values as the control FMX-2exb complex near 0.2 nm with better stability. Furthermore, MMPBSA total free energy calculation showed better binding energy than the control complex for DB01329-2exb interaction i.e. −31.50 (±0.93) kcal/mol. Our presented research suggested that D-alanyl-D-alanine carboxypeptidase DacB could be a therapeutic target and cefoperazone could be a promising ligand to inhibit the D-alanyl-D-alanine carboxypeptidase DacB protein of *Morganella morganii*. To identify prospective therapeutic and vaccine targets in *Morganella morganii*, this is the first computational and subtractive genomics investigation of various metabolic pathways exploring other therapeutic targets of *Morganella morganii*. *In vitro/in vivo* experimental validation of the identified target D-alanyl-D-alanine carboxypeptidase and the design of its inhibitors is suggested to figure out the best dose, the drug's effectiveness, and its toxicity.

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1. Introduction

Antibiotic resistance among the microbial population is commonly increasing, which has sparked a continuous search for creative replacements using different models and approaches. Zebrafish has established itself as a valuable vertebrate model for examining infectious diseases caused by bacteria, fungi, viruses, and protozoa. This is due to the large selection of research tools for these small freshwater fish, which allows for the study of host-microbe-immune interactions with remarkable precision. Researchers have utilized zebrafish models to investigate numerous pathogenic bacteria, providing *in vivo* insights into the cellular response to infection field (Torraca & Mostowy, 2018). One such study revealed that the pathogenesis of *Streptococcus iniae* infection in zebrafish closely mirrors something, which has been shown in several human *streptococcal* infections and populations of farmed fish. An original focal necrotic lesion, which is the infection's hallmark, quickly spreads to the body's primary systems of organs, especially the brain. Additionally, *Streptococcus pyogenes*, a human pathogen, was demonstrated to be capable of infecting zebrafish Field (Neely et al., 2002).

Morganella morganii can cause chorioamnionitis, neonatal sepsis, newborn meningitis, bacteremia, urinary tract infections, intra-abdominal infections, and meningitis (Liu et al., 2016). Moreover, the bacterium has been reported to be resistant to several antibiotics, including beta-lactam antibiotics. The epidemiology of severe illnesses caused by this bacterium is poorly understood. *Morganella morganii* is a Gram-negative, rod-shaped relatively uncommon bacterial opportunistic pathogen, and it can cause a variety of infections, including: urinary tract infections (UTIs), bacteremia, wound infections, Intra-abdominal infections, meningitis, septic arthritis, endocarditis. *M. morganii*

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is often resistant to first-generation cephalosporin and ampicillin-clavulanate, which is commonly, used antibiotics for UTIs. This resistance can make it difficult to treat *M. morganii* infections, and it can increase the risk of mortality (Neely et al., 2002; Kuki et al., 2011). Several medications, including beta-lactam antibiotics, have also been documented to be ineffective against the bacterium. Recent reports on antibiotic-resistant strains of *Morganella morganii* have highlighted the disease's significance as an emerging disease. Even though *Morganella morganii* is becoming more and more important as a human disease agent, not much research has been done on how it spreads major infections (Laupland et al., 2022; Yang et al., 2020; Luo et al., 2022). The discovery of a significant therapeutic molecule is a multi-step, long, and costly journey. Conducting high-throughput screening and computationally integrating with metabolomics, transcriptomics, and genomic approaches makes drug discovery more efficient, targeted, and cost-effective.

The *Enterobacteriaceae* has *Morganella morganii*, a member of the Proteeae tribe. It is a Gram-negative bacillus. Morgan et al. isolated facultative anaerobic bacteria from pediatric stool culture in 1906 (Köbel et al., 2007). *M. morganii* has a genome that is around four million base pairs (bp) in size, and many of its protein-coding sequences, also known as CDSs, is approximately four thousand. The bacterium is characterized by DNA-DNA hybridization, and G + C content was used to differentiate the species. The members of this family share 51% G + C content, which is much more than the other members of this family. The primary illnesses caused by this species include urinary tract infections and post-operative wound infections (Xiang et al., 2021; Hassan et al., 2022). It is thought to be a unique opportunistic pathogen, but certain clinical isolates of this species exhibit resistance to a variety of drugs. blaNDM-1 and qnrD1 genes, offering a significant obstacle for treatment by having a variety of resistance genes. *M. morganii* is a significant pathogen due to virulence evolution field (Laupland et al., 2022; Yang et al., 2020; Power et al., 2006). According to accumulated evidence, *M. morganii* can cause a variety of illnesses, including sepsis, abscess, cellulitis, purple urine bag syndrome, and chorioamnionitis. Frequently, this bacterium causes individuals with certain infections to have a high mortality rate. *M. morganii*, due to its enhanced virulence and resistance, is an opportunistic pathogen (Laupland et al., 2022; Luo et al., 2022; Al-Muhanna et al., 2016; Yang et al., 2015). Resistance has been observed against β-lactam class of antibiotics, and also with first-generation and second-generation classes of cephalosporins. The *M. morganii* strain has a high antimicrobial susceptibility profile, showing resistance to ampicillin (MIC >256 g/mL), cefotaxime, cephalothin, cefoxitin, ampicillin-sulbactam, cephazolin, cefuroxime, (64 g/mL), aztreonam, ceftazidime, erythron, amoxicillin-clavulanate (>256 g/mL). The inherent resistance of *M. morganii* to cefotaxime, ampicillin, amoxicillin-clavulanate, first- and second-generation cephalosporins, erythromycin, tetracycline, and polymyxin B antibiotics have been well reported (Stock & Wiedemann, 1998; Park et al., 2020). In 67 cases (9.5%), there was resistance to one or more, aminoglycosides, quinolones, co-cotrimoxazole, carbapenems was reported (Laupland et al., 2022). Numerous computational techniques have been created, some of which compare the genomes of the pathogen and host to identify therapeutic targets that are not human-homologous but are crucial for the survival of the microbial cell or infection (Jamal et al., 2022; Wadood et al., 2018). One methodology used to identify novel therapeutic targets and develop vaccine candidates against pathogens is in silico subtractive analytic approaches for proteins or genes. This method finds a protein that is thought to be needed for the pathogen to live but is not found in humans (Jamal et al., 2022). The adverse effects of drugs prescribed to treat diseases brought on by microorganisms vary in severity in people. Additionally, prolonged use has led to the emergence of drug resistance in infections, necessitating the discovery of new medications and pharmacological targets to effectively battle pathogens. It has been discovered that a unique method termed 'subtractive proteomics' can identify brand-new therapeutic targets in infections.

To explore the efficacy of cefoperazone on D-alanyl-D-alanine carboxypeptidase (DAC), computational techniques could be utilized for a quick interaction assessment. A recent study used model-informed drug development (MIDD) approaches for the evaluation of the efficacy of the new cefoperazone/sulbactam combination. (3:1) for *Enterobacteriaceae* infection (Ji et al., 2022). Another, *In silico* study revealed that cefoperazone Iron complex (CFPF) and cefoperazone Cobalt complex (CFPC) have significant inhibitory actively quorum sensing signaling and virulence factors of *Pseudomonas aeruginosa* (Naga et al., 2021). Thus, in search for novel drug targets for *Morganella morganii* and searching effective interactive drug molecules will help to face the disease or histamine accumulation of food toxicity mediated by *Morganella morganii*.

2. Materials and methods

This conducted study's primary goal was to identify the novel drug targets for the identification and development of therapeutic molecules against *Morganella morganii* NZ_LR699007 MGYG-HGUT-02512 bacteria-mediated health problems. The screened target must be specific to the bacteria and non-functional for humans, and interacting therapeutic molecules with targets must adhere to the required drug ability standard. The complete protein sequence of *Morganella morganii* with was taken from NCBI. The CD-HIT was used at an 80% sequence identity threshold for the screening of duplicate protein sequences and also proteins with less than 100 amino acids were removed from the study. For proteins in *Morganella morganii* that are not similar to human proteins analyzed with BLASTp in conjunction with an e value of 10^{-3} threshold expectation rate, and proteins similar to human proteins were excluded from the study. The sequences that weren't homologous were subjected to a BLASTp search using the DEG database, and an e-value criterion of 10^{-5} was used to find out the essential proteins supported only by the *Morganella*. The KEGG database was used to analyze the significance of pathogen-essential proteins involved in various metabolic pathways.

The PSORTb was used to screen the localization of identified proteins (Stock & Wiedemann, 1998; Park et al., 2020). The study has been conducted with compression to humans and specific proteins that are only present in bacterial pathogens are considered significant target proteins for drug development and to minimize the toxicity in humans. The protein that is present in both bacteria and humans are not considered as drug targets, because of toxicity concerns in humans. The E score is a parameter of statistical significance used by the BLAST, KEGG etc. like programs to define the anticipated highest hits when comparing against a certain section of the human database. The association between the scores and the number of hits is inversely exponential. Moreover, Binding energy, or doc energy, was additionally employed to identify protein-binding interactions. The ranking of the binding interaction is done using statistical methods (The highest negative value was selected). The study used the binding location with the highest score. The KEGG database was searched to find the binding ligands for the proteins that made the shortlist. The KEGG database revealed that our protein's shortlist bind to different ligands. These ligands' structures were obtained from PubChem for docking and molecular dynamics and simulation studies.

The stepwise conduction of the study is summarized in Figure 1 and the following sections

2.1. Protein sequence retrieval of pathogen

The complete proteins sequence of *Morganella morganii* with the whole genome was taken from NCBI's Protein Database (https://www.ncbi.nlm.nih.gov/protein/) (Wadood et al., 2018), and non paralogous sequences were screened.

2.2. Finding non-paralogous sequences

The CD-HIT suite is frequently used to compare, and group proteins that meet the sequence identity. It is used to remove duplicate protein sequences as well as proteins that have less than 100 amino acids. The CD-HIT was used at an 80% sequence identity threshold for the screening of duplicate protein sequences or paralogous sequences. The non-analogous sequences were used for further study field (Li & Godzik, 2006).

2.3. Identification of protein sequences that are non-similar to the proteome of human

In order to identify specific sequences of proteins in *Morganella morganii* that are not similar to human proteins, BLASTp(www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=9606) was used in conjunction with an e-value of 10^{-3} threshold expectation rate. Sequences that were similar and those that weren't were both present in the resulting sequences. To be analyzed further, proteins. The host-adequately comparable sequences to the human were filtered out, while the non-homologous sequences were left in (Haag et al., 2012).

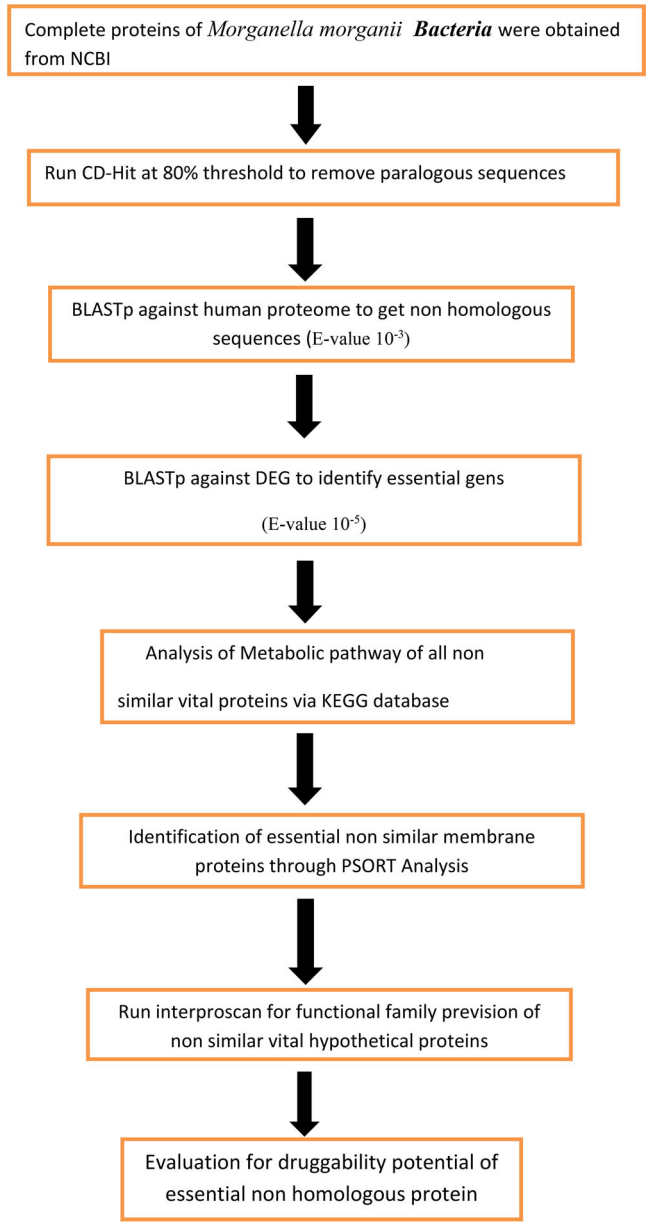

Figure 1. Overall workflow chart of the study showing steps from selection of bacterial strains to prediction of drug targets.

2.4. Determination of essential non-similar proteins in Morganella morganii

Sequences that weren't homologous were subjected to a BLASTp search using the DEG database (Yu et al., 2010; Zhang et al., 2004)and an e-value criterion of 10^{-5} was used. DEG is a database of essential genes which are used to identify the essential genes of an organism. The homologous proteins were screened out by DEG database search. The protein sequences identified these sequences as being crucial for the survival of the bacterial species (Zhang et al., 2004; Power et al., 2006).

2.5. Metabolic pathway analysis

The whole metabolic pathways of a living organism are contained in the Kyoto Encyclopedia of Genes and Genomes

(KEGG) (Moriya et al., 2007). The usage of the KAAS server field (Kanehisa et al., 2017; Sachs & Wilcox, 2006) allowed for the prediction of protein sequences participating in several metabolic pathways of *Morganella morganii*.

2.6. Subcellular localization prediction

The prediction of the subcellular localization of distinct proteins that are essential was done using PSORTb version 3.0 (Naga et al., 2021;Yang et al. 2020). The fundamental idea is to employ Sub Cellular Localization BLAST, which takes all essential non-homologous amino acid sequences and runs BLASTp vs. a repository of peptides with established subcellular locations. PSORTb offers prevision results for a variety of subcellular locations, such as the cytoskeleton, external cytoplasm, cell walls, and unknown.

2.7. Draggability potency of selected sequences

By using BLASTp to compare against the Drug Bank database (Kishida et al., 2006), which contains many target proteins about the IDs of drugs that are approved by the FDA, non-similar, all essential, and hypothetical sequences of protein were screened with an e value of 10^{-3} (Knox et al., 2011).

2.8. Human gut-metagenomes screening

It is well known that a variety of organisms live in the guts of living beings and are crucial to not only health but also disease (Savage, 1977) Instead of being purely commensally (a peaceful coexistence), the relationship between human gut flora is one of causality and symbiotic, reciprocal interaction. The gut microbiota helps the host by controlling the growth of pathogens, fermenting unused energy substrates, developing the immune system, and producing many miracle molecules, including hormones and vitamins. Moreover, through these, they also have been reported to mediate the cellular signaling in favor of the host. There could be negative implications if these species' proteins are accidentally inhibited (Savage, 1977; Herman et al., 2000) To investigate this potential and filter out these matching proteins, we used BLASTp with an E-value cutoff of 1 to evaluate the short-listed non-human homologous proteins *Morganella morganii* to the protein sequences of the intestinal bacteria at the human microbiome Project database server (Wu & Lewis, 2013; Wishart et al., 2018). This project's primary goal was to identify the effective targets for medication against *Morganella morganii*. The estimated drug interacting sites must adhere to the drug ability benchmark.

2.9. Docking, molecular dynamics and simulations

MGL tool Autodock 4.2 tools protocol was used to analyze the binding energies between selected drug compounds and target protein of *Morganella morganii* bacterial strain. The Lamarckian genetic algorithm (LGA) was used to carry out interactions. Auto Dock was used to calculate the ligand and receptor interaction/binding energy (DG). The docking and molecular simulation studies were conducted as described by Morris et al and other previously conducted studies (Kishida et al., 2006; Sajid Jamal et al., 2022). (Van Der Spoel et al., 2005).The Auto Dock tool uses the scoring function of the ligand and receptor molecules interaction according to the binding energy (ΔG) calculation based on the following formula:

$$\Delta G_{\text{binding}} = \Delta G_{\text{gauss}} + \Delta G_{\text{repulsion}} + \Delta G_{\text{hbond}} + \Delta G_{\text{hydrophobic}} + \Delta G_{\text{tors}},$$

where ΔG_{gauss}: attractive term for dispersion of two gaussian functions; $\Delta G_{\text{repulsion}}$: square of the distance if closer than a threshold value; ΔG_{hbond}: ramp function—also used for interactions with metal ions; $\Delta G_{\text{hydrophobic}}$: ramp function; ΔG_{tors}: proportional to the number of rotatable bonds (Van Der Spoel et al., 2005). 3D Crystal structure of penicillin binding protein 4 (dacB) from *Escherichia coli*, complexed with FLOMOX (FMX) (PDB ID: 2EXB) (Kishida et al., 2006) was obtained from Protein Data Bank (www.pdb./org) (Berman et al., 2000; Kufareva & Abagyan, 2012) Furthermore, Drugs control Flomoxef, DB00303, DB00417, DB00760, DB01328, DB01329, DB01331, and DB04570 from Drug Bank(https://go.drugbank.com/) (Wishart et al., 2018; Kuzmanic & Zagrovic, 2010) SMILES (Simplified Molecular Input Line Entry System) is a chemical notation used to generate3D structures of all selected drug compounds using NovaPro online tools (https://www.novoprolabs.com/tools/smiles2pdb).

2.10. Molecular dynamics simulation techniques (MDS)

DB01329_2exb and FMX_2exb complexes docking results need to be further evaluated through Molecular Dynamics Simulations (MDS) techniques. Therefore, MDS was executed for 100nanoseconds (ns) for both complexes, also 2exb simulation in water for results comparison using Groningen Machine for Chemical Simulations (GROMACS) tool 2018 version (Van Der Spoel et al., 2005; Miller et al., 2012) developed by University of Groningen, Netherlands.

The necessary 2exb topology file was created using the pdb2gmx package, and then an all-atom CHARMM27 force field was chosen. The Swiss Param server produced files with drug compound topologies after that (Zoete et al., 2011; Valdés-Tresanco et al., 2021).

To perform the solvation stage, a three-cell triclinic box was filled with water. To stabilize the system and reduce energy consumption, Na + and Cl-ions were added. The system needed to be set up in equilibrium before the two-step ensembles NVT (constant number of particles, temperature, and volume) and NPT (N;constant number of particles, temperature, and pressure) could be used. Both ensembles allow for temperature and pressure coupling management, which leads to system consistency and stability through thorough simulation (Gupta et al., 2020; Maisuradze et al., 2009). GORMACS, an integrated package, for MDS analysis, we used gmx rms for Root Mean Square Deviation (RMSD) (Kufareva & Abagyan, 2012; Tiana et al., 2004) gmxrmsf for Root Mean Square fluctuation (RMSF), gmx gyrate for the calculation of Radius of Gyration (Rg) (Kuzmanic & Zagrovic, 2010; He et al., 2022).

2. Results and discussion

D-alanyl-D-alanine carboxypeptidase (DD-CPase) is an enzyme that cleaves the terminal D-alanine residue from muramyl pentapeptide, a precursor of peptidoglycan. Peptidoglycan is the major component of the bacterial cell wall, and DD-CPase plays an important role in regulating its structure and function in several bacteria, including *Streptomyces coelicolor*, *Enterococcus faecium*, and *Bacillus subtilis*. In these studies, it has been shown that DD-CPase is involved in Spore maturation. DD-CPase is required for the maturation or -germination of bacterial spores. In the absence of DD-CPase, spores are less resistant to heat, acid, and other environmental stresses. Moreover, it plays a role in the development of resistance to antibiotics: DD-CPase is involved in the resistance of bacteria to certain antibiotics, such as vancomycin and teicoplanin. Moreover, DD-CPase has been shown to affect the permeability of the cell wall, the ability of the cell to grow and divide, and the response of the cell to environmental (Torraca & Mostowy, 2018; Rioseras et al., 2016). The initial phase in the development of drug is to identify binding targets for the repression of genes for therapeutic purposes. Because of recent improvements in whole genome sequencing, computational methods in biology and chemistry, and the assignment of both host genome and pathogen sequences, *in-silico* technology has been used a lot to find important vaccine and drug targets at the molecular level in a wide range of disease-causing microbes. This project's primary goal was to identify the therapeutic targets that were most effective against *Morganella morganii*. The planned therapeutic sites must meet the drug ability standard, which says they can't be the same as the human host, they must be essential to the bacteria, and they must play a key role in the main metabolic process of the bacteria. The observed results of this study scheme are presented in Table 1. The NCBI was used to get the 2098 protein sequences that make up the full proteome of the studied bacterial strain.

The CD-hit tool (Hou et al., 2020) is used to screen out the promising drug targets with an 80% (0.8) sequence similarity cutoff, which identified paralogs (duplicated) proteins out of a total of 2430 proteins. In this step, homologous proteins and short peptides having fewer amino acids than 100 were filtered out. Therefore, the 358 short peptides (less than 100 amino acids) and 69 identical sequences were filtered away, leaving the sequences. After that, BLASTp was run against the human genome on these 1671 non-paralogous sequences. According to the findings, 730 proteins (non-homologous) did not have any homologs in the genome of the human host (Homo sapiens). sequences that are identical to the host genome, did not consider them targets for drugs, may cause patients to have cytotoxic responses and other unfavorable side effects. The BLASTp program was then used to compare homologous studied pathogen proteins to DEG with an Evalue cutoff score of 10^{-5}, yielding a collection of 406 proteins. Pathogen survivability genes, together with the need that they be non-homologous to the genetic material of the host, have a tremendous potential for becoming species-specific. drug target. As documented in multiple recent research, the subtractive genome methodologies outlined here for the analysis of *Morganella morganii* bacterial strains are currently successfully used for harmful pathogens (Hassan et al., 2022).

3.1. Subcellular location of the non-similar and vital proteins

The localization of a protein is a crucial prerequisite for it to demonstrate activity. The protein needs to be in a specific position in order to perform its job at its best. A serine-type D-alanyl-D-alanine carboxypeptidase in *Ochrobactrum* sp. 11a has been described to survive under high salt concentration (Príncipe et al., 2009). There are techniques for predicting the subcellular localization of proteins that involve looking at the proteins. The PSORTb is most effective technique for predicting sub cellular distribution of functional protein The PSORTb was applied to the nonhomologous and essential proteins, and the results suggested that the majority of the functional proteins (60%) are related to the cytoplasm (Figure 2). The next-highest concentration of proteins was found in the cytoplasmic membrane while 7% protein were unknown and 2% protein were limited to the cell wall. The sequences present in the cytoplasmic membrane could be effective target for development of vaccine (Zou et al., 2011).

Table 1. The steps and relative number of retrieved proteins of subtractive genomics.

S. No.	Steps conducted and analyzed	*Morganella morganii*
1.	Total number of proteins	3565
2.	Abstracted duplicates (>80% identical) in CDHIT	2430
3.	No. of sequences non-homolog to *Human* applying BLASTp (E-value 10^{-3})	534
4.	Indispensable sequences in DEG (E-value 10^{-5})	497
5.	Indispensable sequences take part in metabolic pathways (KEGG)	315
6.	No. of indispensable proteins of membrane (PSORT)	123
7.	Vital proteins targets for drug (DBD)	1
8.	No. of non-similar hypothetical protein as essential proteins (Interproscan	0

(1) The complete protein sequence of *Morganella morganii* was taken from NCBI. (2) The CD-HIT was used at an 80% sequence identity threshold for the screening of duplicate protein sequences and also proteins with less than 100 amino acids, which were removed. (3) For proteins in *Morganella morganii* that are not similar to human proteins, BLASTp was used in conjunction with an e value of 10^{-3} threshold expectation rate, and proteins similar to human proteins were excluded from the study. (4) The sequences that weren't homologous were subjected to a BLASTp search using the DEG database, and an e-value criterion of 10^{-5} was used to find out the essential proteins only for pathogen Morganella. (5) The KEGG database was used to analyze the significance of pathogen essential proteins in metabolic pathways. (6) The PSORTb was used to screen the localization of proteins. D-alanyl-D-alanine carboxypeptidase vital protein targets by seven drugs were identified; a fast format of these target proteins was BLAST with the drug bank, and Cefoperazone was screened for predicted drug targets, D-alanyl-D-alanine carboxypeptidase. No one of the non-similar hypothetical proteins as essential proteins was analyzed.

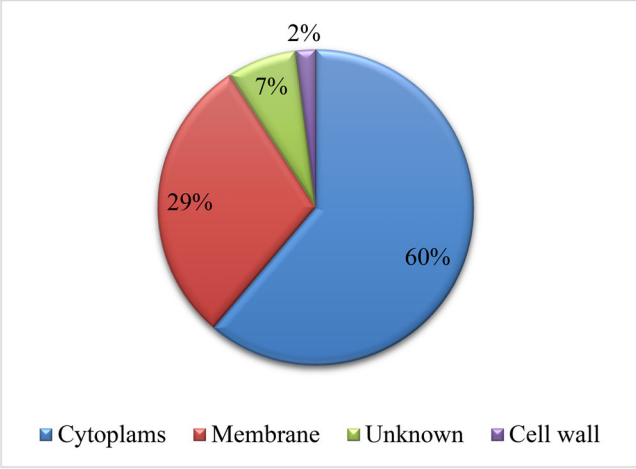

Figure 2. Subcellular location of non-similar essential proteins of *Morganella morganii* shown in percentage; cytoplasmic location 60%; cell membrane 29%; unknown 7%; and cell wall 2%, and these could be desirable location for drug binding to inhibit the life supporting process of studied pathogenic bacterium.

3.3. Analysis of metabolic pathways KEGG

- KASS, an internet server, was used to test the crucial non-similar sequences. The role of the reported proteins in various important metabolic pathways that are active in pathogens was ascertained using this service. 406 Proteins in all were uploaded to this site. The KAAS uses the BLAST algorithm to compare the prokaryotic proteome's metabolic pathways to those of humans (Hassan et al., 2022).The analyzed metabolic pathways and their involved proteins are; Glycolysis/ Gluconeogenesis (2 protein), Citrate cycle (TCA cycle) (1), Pentose phosphate pathway (2), Fructose and mannose metabolism (1), Starch and sucrose metabolism (3), Amino sugar and nucleotide sugar metabolism (8), Pyruvate metabolism (3), Glyoxylate and dicarboxylate metabolism (1), Propanoate metabolism (2), Butanoate metabolism (1), Oxidative phosphorylation (6), Photosynthesis (2), Carbon fixation in photosynthetic organisms (1), Carbon fixation pathways in prokaryotes (2), Methane metabolism (1), Nitrogen metabolism (2), Sulfur metabolism (4), Fatty acid biosynthesis (2), Glycerolipid metabolism (1), Glycerophospholipid metabolism (1), Purine metabolism (8), Pyrimidine metabolism (5), Alanine, aspartate and glutamate metabolism (3), Glycine, serine and threonine metabolism (3), Cysteine and methionine metabolism (7), Valine, leucine and isoleucine biosynthesis (1), Lysine biosynthesis (4), Arginine biosynthesis (3), Arginine and proline metabolism (1), Histidine metabolism (2), Tyrosine metabolism (1), Phenylalanine metabolism (1), Phenylalanine, tyrosine and tryptophan biosynthesis (2), beta-Alanine metabolism (1), Taurine and hypotaurine metabolism (2), Selenocompound metabolism (2), Cyanoamino acid metabolism (1), D-Amino acid metabolism (3), Glutathione metabolism (3), Lipopolysaccharide biosynthesis (12), Antigen nucleotide sugar biosynthesis (1), Peptidoglycan biosynthesis (12), Teichoic acid biosynthesis (2), Exopolysaccharide biosynthesis (1), Thiamine metabolism (2), Riboflavin metabolism (3), Vitamin B6 metabolism (2), Nicotinate and nicotinamide metabolism (2), Pantothenate and CoA biosynthesis (3), Biotin metabolism (2), Folate biosynthesis (8), Porphyrin metabolism (5), Ubiquinone and other terpenoid-quinone biosynthesis (3), Terpenoid backbone biosynthesis (6), Phenylpropanoid biosynthesis (1), Tropane, piperidine and pyridine alkaloid biosynthesis (1), Monobactam biosynthesis (1), Novobiocin biosynthesis (1), Aminobenzoate degradation (1), Nitrotoluene degradation (1), Atrazine degradation (2), Ribosome (6), Aminoacyl-tRNA biosynthesis (1), Protein export (3), Sulfur relay system (3), RNA degradation (4), NA replication (6), Base excision repair (1), Nucleotide excision repair (1), Mismatch repair (4), Homologous recombination (8), ABC transporters (15), Phosphotransferase system (PTS) (5), Bacterial secretion system (3), Two-component system (23), Cell cycle - *Caulobacter* (4), Quorum sensing (10), Biofilm formation – *Vibrio cholerae* (2), Biofilm formation - *Pseudomonas aeruginosa* (1), Biofilm formation – *Escherichia coli* (7), Bacterial chemotaxis (5), Flagellar assembly (3), PPAR signaling pathway (1), Pertussis (2), beta-Lactam resistance (5), Vancomycin resistance (3), and Cationic antimicrobial peptide (CAMP) resistance (7). The distinct metabolic pathways are shown in the Table 2. The pathways described above that are unique and required for survival or pathology of disease. Because there are no competing routes in humans, the sequences associated with those pathways are the most effective pharmacological targets.

Cyclohexadienyl dehydrogenase involved in Novobiocin biosynthesis, Ammonia ligase, Aspartate–ammonia ligase are catalyzed Cyanoamino acid metabolism, Lipopolysaccharide biosynthesis protein RffC involved in O-Antigen nucleotide sugar biosynthesis, 4-alpha-L-fucosyltransferase Exopolysaccharide biosynthesis. The Peptidoglycan biosynthesis is catalyzed by many enzymetic proteins like -UDP-N-acetylglucosamine 1-carboxyvinyl-transferase, UDP-N-acetylmuramoylalanine–D-glutamate ligase, UDP-N-acetylmuramoylalanyl-D-glutamate–2,6-diaminopimelate ligase, D-alanine–D-alanine ligase, Undecaprenyl-diphosphatase, Putative permease, Phospho-N-acetylmuramoyl-pentapeptide-transferase, pyrophosphoryl-undecaprenol N-acetylglucosamine transferase, Penicillin-binding protein 2, D-alanyl-D-alanine carboxypeptidase. Teichoic acid biosynthesis is mediated by Undecaprenyl-diphosphatase and putative permease. Other specific proteins that were analyzed with bacterium but not for the human host are Aminobenzoate degradation protein-3-polyprenyl-4-hydroxybenzoate carboxy-lyase UbiX, Nitrotoluene degradation by Uptake hydrogenase small subunit precursor, Methane metabolism by Acetate kinase. Many two components proteins- Phosphate regulon transcriptional regulatory protein PhoB, Transcriptional regulatory protein PhoP, Osmolarity sensory histidine kinase EnvZ, Two-component system response regulator OmpR, Response regulator BaeR, UDP-4-amino-4-deoxy-L-arabinose–oxoglutarate aminotransferase, DNA-binding response regulator and Two-component sensor protein RcsC. The RNA-binding protein Hfq catalyzing the Quorum sensing and Methyl-accepting chemotaxis protein mediate the bacterial chemotaxis for the servial of the bacterial cells.

The identified drug target, D-alanyl-D-alanine carboxypeptidases (DD-CPases), is a class of penicillin-binding proteins (PBPs), inhibited by ß-lactam antibiotics, and implicated in the formation and remodeling of peptidoglycans (PGs). Glutathione (GSH), according to a number of reports, possesses antimicrobial properties. The way that GSH prevents microbial development, however, is unknown. The Glutathione (GSH), works as

Table 2. Unique metabolic pathways of *Morganella morganii* from KEGG.

S. No.	*Morganella morganii* metabolic pathway	*Morganella morganii* metabolic enzymes
1.	Novobiocin biosynthesis	Cyclohexadienyl dehydrogenase [KO:K14187] [EC:5.4.99.5 1.3.1.12]
2.	Cyanoamino acid metabolism	Ammonia ligase [KO:K01914] [EC:6.3.1.1]
3.	Cyanoamino acid metabolism	Aspartate–ammonia ligase
4.	O-Antigen nucleotide sugar biosynthesis	Lipopolysaccharide biosynthesis protein RffC
5.	Exopolysaccharide biosynthesis	4-alpha-L-fucosyltransferase [KO:K12582] [EC:2.4.1.325]
6.	Peptidoglycan biosynthesis	UDP-N-acetylglucosamine 1-carboxyvinyltransferase [KO:K00790] [EC:2.5.1.7]
		UDP-N-acetylmuramoylalanine–D-glutamate ligase [KO:K01925] [EC:6.3.2.9]
		UDP-N-acetylmuramoylalanyl-D-glutamate–2,6-diaminopimelate ligase [KO:K01928] [EC:6.3.2.13]
		D-alanine–D-alanine ligase [KO:K01921] [EC:6.3.2.4]
		Undecaprenyl-diphosphatase [KO:K06153] [EC:3.6.1.27]
		Putative permease [KO:K19302] [EC:3.6.1.27]
		Phospho-N-acetylmuramoyl-pentapeptide-transferase [KO:K01000] [EC:2.7.8.13]
		pyrophosphoryl-undecaprenol N-acetylglucosamine transferase [KO:K02563] [EC:2.4.1.227]
		Penicillin-binding protein 2 (PBP-2) [KO:K05515] [EC:3.4.16.4]
		D-alanyl-D-alanine carboxypeptidase [KO:K07258] [EC:3.4.16.4]
		D-alanyl-D-alanine carboxypeptidase [KO:K07259] [EC:3.4.16.4 3.4.21.-]
7.	Teichoic acid biosynthesis	Undecaprenyl-diphosphatase [KO:K06153] [EC:3.6.1.27]
		Putative permease [KO:K19302] [EC:3.6.1.27]
8.	Aminobenzoate degradation	3-polyprenyl-4-hydroxybenzoate carboxy-lyase UbiX [KO:K03186] [EC:2.5.1.129]
9.	Nitrotoluene degradation	Uptake hydrogenase small subunit precursor [KO:K06282] [EC:1.12.99.6]
10.	Methane metabolism	Acetate kinase [KO:K00925] [EC:2.7.2.1]
11.	two components	Phosphate regulon transcriptional regulatory protein PhoB (SphR) [KO:K07657]
		Transcriptional regulatory protein PhoP [KO:K07660]
		Osmolarity sensory histidine kinase EnvZ [KO:K07638] [EC:2.7.13.3]
		Two-component system response regulator OmpR [KO:K07659]
		Response regulator BaeR [KO:K07664]
		UDP-4-amino-4-deoxy-L-arabinose–oxoglutarate aminotransferase [KO:K07806] [EC:2.6.1.87]
		DNA-binding response regulator KdpE [KO:K07667]
		Two-component sensor protein RcsC [KO:K07677] [EC:2.7.13.3]
12.	Quorum sensing	RNA-binding protein Hfq [KO:K03666]
13.	Bacterial chemotaxis	Methyl-accepting chemotaxis protein [KO:K03406]

The pathways described in this table are observed to be unique, and they could be important drug targets to inhibit the survival or pathology of disease mediated by Morganella morganii. In these, D-alanyl-D-alanine carboxypeptidase was observed to be the primary choice for drug development.

Table 3. Non-homologous essential proteins of *Morganella morganii* similar to FDA sanctioned drugs against drug Bank database applying BLAST.

Protein ID	Protein name as drug target	Drug bank ID	Drug bank organism
P24228	D-alanyl-D-alanine carboxypeptidase DacB	DB00303; DB00417; DB00760; DB01328; DB01329; DB01331; DB04570)	*Escherichia coli* (strain K12)

Cefonicid (DB01328), Cefoperazone (DB01329),Cefoxitin (DB01331), Ertapenem (DB00303), Latamoxef (DB04570), Meropenem (DB00760), Phenoxymethylpenicillin (DB00417).

antibiotic Penicillium precursors that inhibit the D-alanyl-D-alanine-carboxypeptidase (DacC), and stop formation of peptidoglycan from glycopeptides (D-alanyl-alanine) (Mustikaningtyas et al., 2021). Recently, it has been reported that *Streptomyces coelicolor* uses a D-alanyl-D-alanine carboxypeptidase for resistance development, spore cell wall formation, and spore germination (Berman et al., 2000).

3.4. Draggability potential of the shortlisted

As a way to improve the current investigation, the drug ability of each of the nominated sequences of critical proteins was also checked. It led to the discovery of x proteins that were similar to the Drug Bank database's accessible targets of FDA-approved medications. Table 3 displays specifics of chosen proteins and target IDs. The protein has a homolog in the Drug Bank with at least 25% sequence similarity, all of the proteins can be thought of as potent drug targets. The majoronly therapeutic targets connected was observed to be D-alanyl-D-alanine carboxypeptidase DacB (P24228-Protein id)) that resemble homologs in the Drug database Ertapenem (DB00303) is a carbapenem antibiotic used for the treatment of moderate to severe bacterial infections caused by specific sensitive organisms (Otarigho & Falade, 2023); Phenoxymethylpenicillin (DB00417), Phenoxymethylpenicillin, Penicillin K, and Penicillin V are other names for the narrow-spectrum antibiotic. It is Phenoxymethylpenicillin, a naturally occurring oral penicillin, is used to treat minor to moderate infections of the respiratory tract, skin, and soft tissues that are brought on by microorganisms that are penicillin-sensitive (Miller, 2002).

Meropenem (DB00760) is a broad spectrum carbapenem antibiotic used to treat different kinds of bacterial infections; Cefonicid (DB01328) a second-generation cephalosporin used to control lower respiratory tract infections, soft tissue, urinary tract infections, and bone infections.Cefoperazone (DB01329); Cefoxitin (DB01331) is a semi-synthetic, broad-spectrum cephalosporin antibiotic; Latamoxef (DB04570) is a broad-spectrum beta-lactam antibiotic with a structure that is similar to that of cephalosporins, with the exception that it has an oxaazabicyclo moiety in place of the thiaazabicyclo moiety found in some cephalosporins. Because it crosses the

blood-brain barrier and is effective against anaerobic infections, it has been suggested particularly for meningitides.

3.5. Human gut-metagenomes screening

As the majority of antibiotics target both infections and beneficial bacteria in the human microbiota, this has a long-lasting effect on the normal gut flora. Thus, eliminating the pathogen's proteins that have a strong similarity to the proteins of the gut flora as a treatment target may eventually lower the chance of medication-widening adverse effects. UsingB LASTp at the Human Microbiome Database, the entire genome of *Morganella morganii* strain was utilized as an input query against gastrointestinal flora as the reference genome for the investigation of evolutionary relationships with the gut flora. Sequence homology analysis results showed that in the input sequences, no one was found to be shared characteristics with the gut flora, leaving the remaining sequences as non-homologous to the gut flora. There no sequences were found to be essential as well as virulent proteins (Table 4).

It was found that observed drugs were the best ligands for targeting the *Morganella morganii* D-alanyl-D-alanine carboxypeptidase DacB and could be used to inhibit this target from working. The enzyme protein is human non-homologous, which makes it a target for future development of therapeutic drugs. But we suggested conducting *in vitro*/*in vivo* experiments to make sure that our findings are true in the real world. In the current study, analysis of genomes data in order to uncover potential treatment targets in *Morganella morganii* is highlighted. The pathogen's critical survival or vital proteins that are non-homologous to the host were taken into consideration rather than the entire genome.

3.6. Docking and molecular dynamics

The interaction of identified receptors and drugs was analyzed through docking and simulation studies. The drug that interacted optimally was observed to be Cefoperazone with the estimated free energy of binding −8.9 Kcal/mol and the least interacting drug was DB00417: ZINC3831282 (Bacillin V2-6.5 Kcal/mol. The interaction of other studied drugs with the identified protein DB00303, ZINC3874302; Flomoxef- Estimated Free Energy of Binding −7.1 Kcal/mol, DB00303: ZINC3918453-Ertapenem −8.4 Kcal/mol, DB00760: ZINC3808779 (Meropenem –7.0), DB01328: ZINC3830428 (Cefonicid –8.0 Kcal/mol), DB01329:
ZINC3830432 (Cefoperazone- −8.9 Kcal/mol) were also found to be significant (Table 4).FMX-2exb complex formed 11 hydrogen bonds and amino acid residues PRO152, SER398, THR418, GLY419, A SN308, ALA61, GLY358, LYS65, and LEU359 formed Van der Waals contacts. While Halogen bond was formed by ASP155 and Pi-cation contact built by LYS417 (Table 4; Figure 3(A,E)).DB01328 interaction with 2exb formed 7 hydrogen bonds and amino acid residues ASN308, LYS417, GLY419, SER306, THR418, GLY423, GLN422, VAL424, ARG459, ARG402 created Van der Waals contacts. Also, it was observed that PHE160 was forming Pi-Pi stacking (Table 4; Figure 3 (B,F)).DB01329 formed total 10 hydrogen bonds. The amino acid residues involved in Van der Waals interaction were GLN422, LYS65, GLY419, SER62, SER306, SER398, TYR425, PHE160, LEU59, GLY398, and ARG361. ARG459 was forming Pi-cation, ALA61, LEU359 LEU421 forming Pi-Alkyl bond (Table 4 and Figures 3(C,G)). DB00303 interacted with 2exb and formed 8 hydrogen bonds. The amino acid residues LEU421, ALA61, GLY358, LYS65, SER306, ALA162, ASP307 and LYS65 were involved in Van der Waals interaction. While LEU359 was forming Pi-Sigma , PHE160 formed Pi-Pi stacking and CYS73 was creating Pi-Alkyl bond (Table 4; Figures 3(D,H)).

Cefoperazone is belong to β-lactam class of antibiotics and bactericidal in nature, and its minimal inhibitory concentrations are influenced only by high inoculum concentrations of β-lactamase-producing strains. The β-lactamase-producing organisms are the sole factors that affect its minimal inhibitory concentrations. Similar to cefotaxime, cefoperazone appears capable of penetrating bacterial cell membranes. Studies show the improved killing of *Enterobacteriaceae,* and *P. aeruginosa* when cefoperazone is combined with -lactamase inhibitors or aminoglycosides (Jones & Barry, 1983; Kuang et al., 2022). The protein binding affinity with bacterial penicillin-binding proteins 3, 1a, 2, and 1bs of these drugs have been reported that support the result of this study.

3.7. Molecular mechanic/Poisson–Boltzmann surface area (MM-PBSA) methods

gmx_MMPBSA Version = v1.5.6 based on MMPBSA.py v.16.0 script utilized performed Poisson Boltzmann calculations using an internal PBSA solver in a sander using temperature = 298.15 K) for the estimation of binding free energies, All units are reported in kcal/mol. Calculations were performed using 51 complex frames.

After completion of 100 ns molecular dynamics simulation, analyses were extracted from trajectory files containing RMSD, RMSF, Rg, and the formation of numbers of hydrogen bond data—the deviation of both complexes and 2exb in water during the 100 ns MDS. The average RMSD values observed were between 0.15 and 0.35 nm for complexes and 2exb simulation in water (Figure 4(A)) Significantly was observed that DB01328-2exb and DB01329-2exbcomplexesshowed similar values as the control FMX-2exb complex near 0.2 nm with better stability, which is less than 2exb simulation in water, i.e. approximately 0.25 nm while selected third complex DB00303-2exb shown similar value 0.2 nm during 100 ns simulation but slight increase near to the value 0.3 nm until end of the simulation. RMSF calculation per residue shows all complexes' overall values between 0.1 and 0.55 nm (Figure 4(B)). The observed average value was 0.1 nm for all simulated molecules. Selected complexes, including 2exb simulation in water showed almost similar fluctuation patterns except for some major fluctuations observed at the 200–230 and 390–400 amino acid regions. The radius of gyration analysis is very important for assessing the compactness and stability of protein structures during the simulation period due to the presence of ligand molecules. The observed average value of Rg ranged between

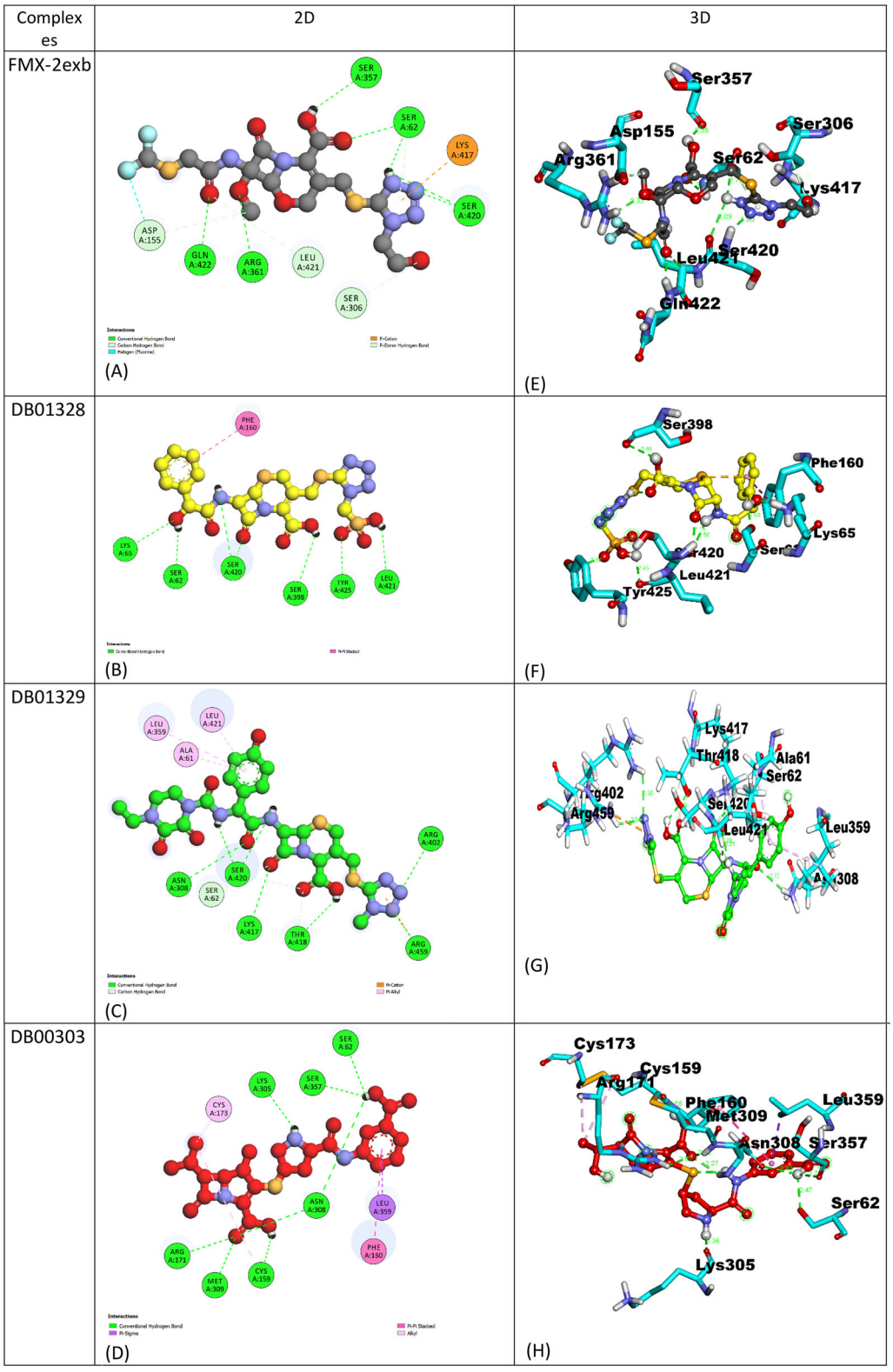

Figure 3. Representing the A, B, C, and D, 2D visualization of drug-receptor interaction where hydrogen bonds are shown by dotted green lines and Pi-Alkyl interaction by dotted Pink lines. Spheres show all interacting amino acid residues. E, F, G, and H showing 3D visualization of active site pocket and close view of drug molecules FMX (black), DB01328 (yellow), DB01329 (green), and DB00303(red) interaction with the penicillin-binding protein 4 (dacB) from *Escherichia coli* (PDB:2exb), interacting amino acid residues are shown by stick pattern in cyan color surrounding.Green dotted lines are showing the formation of hydrogen bonds.

2.46 and 2.58 nm for all complexes. DB01329-2exb showed an average value of 2.5 nm, and DB00303-2exb and 2exb simulation in water showed an approximately similar value ranging from 2.46 to 2.54 nm while DB01328-2exb showed a value ranged between 2.48 and 2.58 nm and with average 2.52 nm (Figure 4(C)). Total of 1–6 hydrogen bonds formed during selected drugs and 2exb receptor interaction (Figure 4(D)). FMX-2exb formed 4hydrogen bonds, and DB01328 and DB01329-2exb formed 6 hydrogen bonds, while DB00303-2exb formed 3 hydrogen bonds for the whole simulation period.

Table 4. Showing molecular docking data obtained from interaction analysis between selected drugs and penicillin-binding protein 4 (dacB) from *Escherichia coli* (PDB:2EXB).

Drug molecules	Estimated free energy of binding (Kcal/mol)	Hydrogen bond details	Hydrogen bond length (Angstrom)	Van der Waals interaction	Other type interaction
Control ZINC3874302 (Flomoxef)	−7.1	A:SER62:HN – :UNL1:O4 A:ARG361:HH22 – :UNL1:O1 A:SER420:HN – :UNL1:N4 A:GLN422:HN – :UNL1:O2 :UNL1:H3 – A:SER62:OG :UNL1:H3 – A:SER420:O :UNL1:H8 – A:SER357:O A:SER306:CB – :UNL1:O6 A:LEU421:CA – :UNL1:O2 :UNL1:C1 – A:ASP155:OD2 A:SER62:OG – :UNL1	3.05537 2.31939 1.90298 2.2639 2.72835 3.09183 2.37631 3.42455 3.54 3.30528 3.64201	PRO152,SER398, THR418,GLY419,ASN308, ALA61,GLY358,LYS65, LEU359	Halogen = ASP155 Pi-cation = LYS417
DB00303ZINC3918453 (Ertapenem)	−8.4	A:ARG171:HH22 – :UNL1:O3 A:ASN308:HN – :UNL1:O3 A:MET309:HN – :UNL1:O3 :UNL1:H11 – A:LYS305:O :UNL1:H7 – A:CYS159:O :UNL1:H17 – A:SER62:OG :UNL1:H17 – A:ASN308:OD1 :UNL1:H17 – A:SER357:O	2.52143 2.2745 2.22483 2.35627 2.58052 2.46861 2.82606 2.70927	LEU421,ALA61,GLY358, LYS65,SER306,ALA162, ASP307,LYS65	PI-SIGMA = LEU359 PI-PI STACKED = PHE160 Pi-Alkyl = CYS73
DB00417ZINC3831282 (Bicillin V2)	−6.5	A:SER398:HG – :UNL1:O4 A:SER420:HN – :UNL1:O3 :UNL1:H18 – A:SER398:O	2.27955 2.13917 2.80678	THR418,GLY419, LYS417,SER62,ARG361,GLY358, SER306, ASN308, LYS65	Pi-Alkyl = LEU421, LEU359,ALA61
DB00760ZINC3808779 (Meropenem)	−7.0	A:SER62:OG – :UNL1:S1 A:ARG361:HH12 – :UNL1:O1 :UNL1:H11 – A:SER306:O :UNL1:H7 – A:SER62:OG :UNL1:H7 – A:ASN308:OD1 :UNL1:H7 – A:SER357:O A:SER306:CB – :UNL1:O5	3.40825 2.02688 2.44475 2.37765 2.41163 3.06175 3.71448	ASP155, PHE160, SER420, LEU421, LEU359, ALA61, GLY358, LYS65,LYS305	na
DB01328ZINC3830428 (Cefonicid)	−8.0	A:SER62:OG – :UNL1:S1 A:ARG361:HH12 – :UNL1:O1 :UNL1:H11 – A:SER306:O :UNL1:H7 – A:SER62:OG :UNL1:H7 – A:ASN308:OD1 :UNL1:H7 – A:SER357:O A:SER306:CB – :UNL1:O5	3.40825 2.02688 2.44475 2.37765 2.41163 3.06175 3.71448	ASN308,LYS417,GLY419, SER306,THR418,GLY423,GLN422, VAL424,ARG459,ARG402	Pi-Pi stacked = PHE160
DB01329ZINC3830432 (Cefoperazone)	−8.9	A:ASN308:HD22 – :UNL1:O2 A:ARG402:HH22 – :UNL1:N7 A:LYS417:HZ1 – :UNL1:O3 A:ARG459:HH21 – :UNL1:N8 :UNL1:H12 – A:SER420:O :UNL1:H10 – A:SER420:O :UNL1:H14 – A:THR418:OG1 A:SER62:HB1 – :UNL1:O3 A:THR418:HB – :UNL1:O4 A:SER420:HB1 – :UNL1:O4	2.77248 2.90323 3.08205 2.53752 2.99247 2.20568 2.48437 2.98935 2.9024 2.5564	GLN422,LYS65,GLY419,SER62, SER306,SER398,TYR425, PHE160,LEU59,GLY398,ARG361	Pi-cation = ARG459 Pi-Alkyl = ALA61, LEU359,LEU421
DB01331ZINC3830449 (Cefoxitin)	−6.7	A:SER62:OG – :UNL1:O3 A:SER62:OG – :UNL1:O5 A:ASN308:HD22 – :UNL1:O2 A:LYS417:HZ1 – :UNL1:O4 :UNL1:H10 – A:SER420:O :UNL1:H13 – A:SER398:O	2.66603 3.19955 1.92241 2.76304 2.67913 2.37873	ALA402,LEU399,ARG361, PHE160, ALA61 ,SER306, LYS65,GLY419	Pi-sigma = LEU359, Pi-Alkyl = LEU421
DB04570ZINC3831157 (Moxalactam)	−7.6	A:ASN308:HD22 – :UNL1:O3 A:ARG402:HH21 – :UNL1:N4 A:SER420:HN – :UNL1:O6 A:ARG459:HH22 – :UNL1:N4 A:SER398:CB – :UNL1:O7	1.96967 3.08388 2.23561 2.72012 3.13882	ARG361, LEU59, GLY358, SER62,SER306, GLY419, THR418, TYR425PHE160	Pi-Sigma = LEU359, Pi-Alkyl = ALA61, LEU421

(Maisuradze et al., 2009) have reported that principal component analysis (PCA) shows how a protein expands generally during the course of simulations. A protein's considerable mobility under various circumstances can be assessed by calculating the total of the eigenvalues, which measure the motility of the whole system (Tiana et al., 2004). The patterns of the DB01329-2exb, DB01328-2exb, and FMX-2exb complexes coincide when trajectories are projected in 2D on

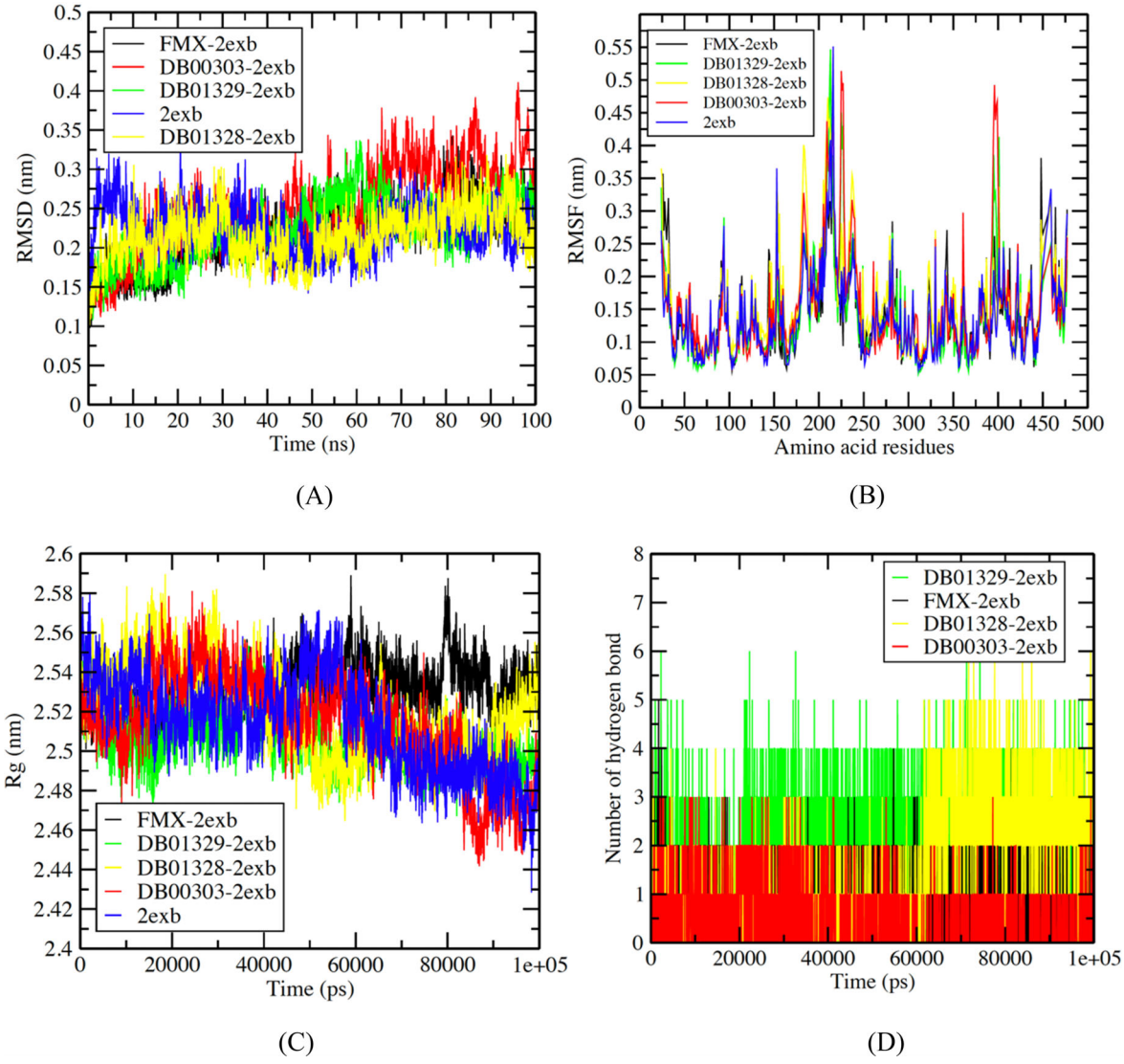

Figure 4. 2D Graphs showing (A) RMSD plot of FMX-2exb (black), DB01329-2exb (green), DB01328-2exb (yellow), DB00303-2exb (red) and 2exb in water (blue)deviation during 100 ns period, (B) RMSF plot with fluctuation per residues, (C) Hydrogen bond plot showing formation hydrogen bond FMX-2exb (black), DB01329-2exb (green), DB01328-2exb (yellow), and DB00303-2exb (red), (D) Radius of gyration (Rg) plot showing compactness of protease molecule during 100 ns simulation. Where nm: nanometer; ps: picosecond.

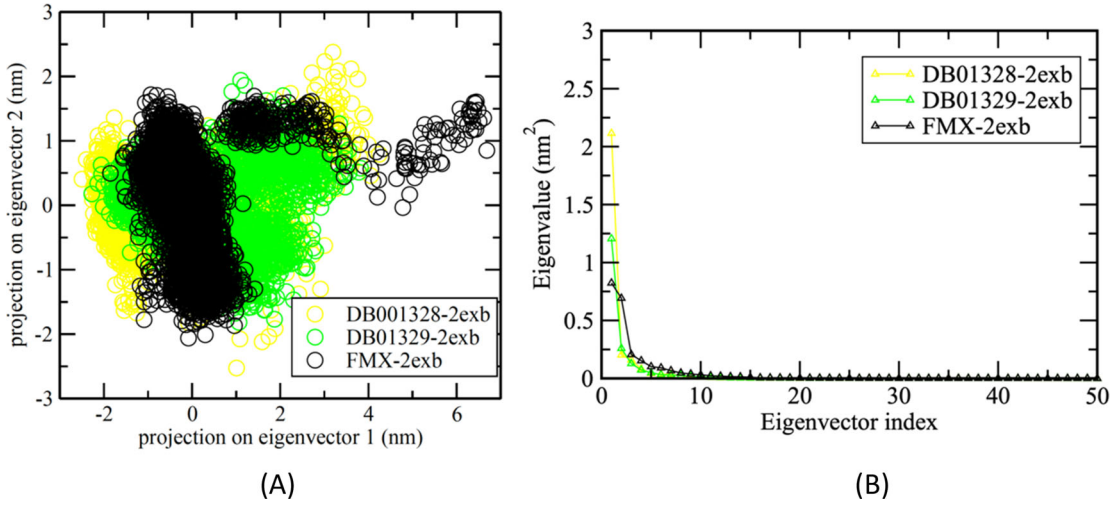

Figure 5. (A) PCA analysis for protein-ligand complex structure in Gromacs representing the 2D graph eigenvector1 Vs eigenvector2 (B) Eigenvector index graph representing the eigenvalues of DB01328-2exb (yellow), DB01329-2exb (green) and FMX-2exb (black).

Table 5. Representing the summarized data of Poisson Boltzmann complex energy components calculation with ± SEM (standard deviation error of the mean) of complexes FMX-2exb, DB01329-2exb, DB01328-2exb, and DB00303-2exb.

Complex	ΔVdwaals	ΔEEL	ΔEPB	ΔENPOLAR	ΔEDISPER	ΔGGas	ΔGSolv	ΔGTotal
Complex free energy calculation components								
FMX-2exb	−3538.22	−27014.61	−4277.93	101.21	0.00	−22826.42	−4176.73	−27003.15
	(±3.86)	(±19.87)	(±16.00)	(±0.16)	(±0.0)	(±19.74)	(±15.92)	(±9.71)
DB01329-2exb	−3547.83	−26966.31	−4278.05	102.42	0.00	−22376.42	−4175.63	−26552.04
	(±4.03)	(±16.84)	(±13.23)	(±0.8)	(±0.0)	(±19.93)	(±13.13)	(±11.27)
DB01328-2exb	−3542.74	−27039.78	−4266.47	101.81	0.00	−22421.38	−4164.67	−26586.05
	(±3.64)	(±16.25)	(±13.71)	(±0.16)	(±0.0)	(±17.57)	(±13.67)	(±9.61)
DB00303-2exb	−3522.38	−27072.56	−4220.42	101.81	0.00	−22839.90	−4118.61	−26958.51
	(±4.16)	(±17.26)	(±0.0)	(±0.16)	(±0.0)	(±17.62)	(±13.87)	(±9.68)

Where ΔVdwaals: Van der Waals energy; ΔEEL: electrostatic molecular energy; ΔEPB: polar contribution to the solvation energy; ΔENPOLAR: nonpolar contribution of repulsive solute-solvent interactions to the solvation energy; ΔEDISPER: nonpolar contribution of attractive solute-solvent interactions to the solvation energy; ΔG gas: total gas phase molecular energy; ΔG solv: total solvation energy; ΔG Total: total binding energy.

Table 6. Representing the summarized data of MMPBSA-based free energy calculation components with ± SEM (standard deviation error of the mean) of ligand-receptor.

Complex	ΔVdwaals	ΔEEL	ΔEPB	ΔENPOLAR	ΔEDISPER	ΔGGas	ΔGSolv	ΔGTotal
Ligand-receptor free energy calculation components								
FMX-2exb	−34.66	−4.63	19.71	−3.20	0.00	−39.29	16.52	−22.78
	(±0.43)	(±0.42)	(±0.66)	(±0.03)	(±0.00)	(±0.59)	(±0.65)	(±0.55)
DB01329-2exb	−49.10	−11.30	33.09	−4.18	0.00	−60.41	28.91	−31.50
	(±0.79)	(±0.10)	(±0.06)	(±0.04)	(±0.00)	(±1.23)	(±0.70)	(±0.93)
DB01328-2exb	−39.51	−25.80	52.96	−3.80	0.00	−65.30	49.17	−16.13
	(±0.86)	(±0.98)	(±1.30)	(±0.07)	(±0.0)	(±01.52)	(±1.27)	(±0.91)
DB00303-2exb	−32.17	−13.82	29.21	−3.52	0.00	−45.99	25.69	−20.30
	(±0.69)	(±0.75)	(±0.87)	(±0.05)	(±0.0)	(±0.94)	(±0.86)	(±0.55)

Where ΔVdwaals: Van der Waals energy; ΔEEL: electrostatic molecular energy; ΔEPB: polar contribution to the solvation energy; ENPOLAR: nonpolar contribution of repulsive solute-solvent interactions to the solvation energy: ΔEDISPER: nonpolar contribution of attractive solute-solvent interactions to the solvation energy; ΔG gas: total gas phase molecular energy; ΔG solv: total solvation energy; ΔG total: total binding energy.

eigenvectors. Additionally, the results demonstrated that binding of the complexes to 2exb results in a change in atom position (Figures 5(A,B)).

MDS trajectory files were further subjected to MMPBSA analysis. The obtained data of Poisson Boltzmann complex energy and ligand-receptor energy components calculations were presented in Tables 5 and 6. Selected complexes were shown near ΔVdwaals energies ranging from 3522.38 (±4.16) to 3547.83 (±4.03) kcal/mol for DB00303-2exb and DB01329-2exb complex respectively while observed ΔEEL was almost similar for all compounds and control (FMX-2exb). ΔGTotal was comparatively very near to the control(FMX-2exb)energy −27003.15(±9.71) kcal/mol (Table 5).

Another MMPBSA-based free energy components calculation revealed a significant ΔGTotal as compared to control where it was observed that DB01329 interaction with 2exb showed ΔGTotal −31.50 (±0.93) kcal/mol better than control drug FMX which showed ΔGTotal −22.78 (±0.55) kcal/mol. Also, Drugs DB01329 and DB01328 interaction with 2exb showed better ΔVdwaals i.e. −49.10 (±0.79) and −39.51(±0.86) kcal/mol, respectively, whereas control drug FMX showed −34.66 (±0.43) kcal/mol (Table 6).

Conclusions

The prediction of therapeutic targets has benefited from the exploration of the proteome and genome of many bacterial infections. In the present study, non-homologous essential druggable proteins against *Morganella morganii* engaging in the distinct metabolic pathway were predicted using a subtractive genomic-based metabolic pathway analysis technique. However, all important non-homologous proteins could also be effective therapeutic targets observed as unique through this study. Eliminating the pathogen from the relevant host may result from novel therapeutic candidates targeting the actions of these proteins, including D-alanyl-D-alanine carboxypeptidase. Carboxypeptidase from D-alanine An enzyme called DacB (DacB) is necessary for the formation of the bacterial cell wall. The components of the cell wall, called peptidoglycan pentapeptides, are stripped of their C-terminal D-alanine residues by DacB. For the cell wall to remain sturdy and intact, this procedure is required. DacB is a protein that binds penicillin, hence penicillin and other beta-lactam antibiotics inhibit it. Consequently, DacB becomes a viable therapeutic target for the creation of fresh antibiotics. Targeting DacB as a therapeutic target has a lot of benefits. First, because DacB is necessary for the creation of bacterial cell walls, targeting it should be extremely harmful to bacteria. Moreover, there are a variety of potential inhibitors for DacB because it is an enzyme that has been pretty well studied. Additionally, since DacB is not present in human cells, targeting it should provide little threat of harm. The creation of medications that target DacB is fraught with difficulties. First off, because DacB is a tiny enzyme, it can be challenging to develop medications that target it directly, and it is difficult to target with drugs that are introduced into the cell since it is found in the periplasm of the bacterial cell. DacB continues to be an attractive pharmacological target for the creation of novel antibiotics despite these difficulties. It is conceivable that new therapeutic molecules that target this enzyme will be created as our knowledge of DacB biology expands. The study's analysis and findings

included all crucial, potent drugs Cefoperazone, Phenoxymethylpenicillin, Meropenem, Latamoxef, Cefoxitin, Cefonicid, Ertapenem, against D-alanyl-D-alanine carboxypeptidase of *Morganella morganii*, which may make it easier for future researchers to create medication compounds or vaccines that could be effective against *Morganella morganii*.

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References

Al-Muhanna, A. S., Muhanna, S. Al., & Alzuhairi, M. A. (2016). Molecular investigation of extended-spectrum beta-lactamase genes and potential drug resistance in clinical isolates of *Morganella morganii*. *Annals of Saudi Medicine*, *36*(3), 223–228. https://doi.org/10.5144/0256-4947.2016.223

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., & Bourne, P. E. (2000). The protein data bank. *Nucleic Acids Research*, *28*(1), 235–242. https://doi.org/10.1093/nar/28.1.235

Gupta, S., Tiwari, N., Verma, J., Waseem, M., Subbarao, N., & Munde, M. (2020). Estimation of a stronger heparin binding locus in fibronectin domain III(14) using thermodynamics and molecular dynamics. *RSC Advances*, *10*(34), 20288–20301. https://doi.org/10.1039/d0ra01773f

Haag, N. L., Velk, K. K., & Wu, C. (2012). *In silico* identification of drug targets in methicillin/multidrug-resistant *Staphylococcus aureus*. *International Journal of Advanced Life Science*, *4*(1–2), 21–32.

Hassan, J., Mann, D., Li, S., Deng, X., & Kassem, I. I. (2022). First report of the mobile colistin resistance gene mcr-9.1 in *Morganella morganii* isolated from sewage in Georgia, USA. *Journal of Global Antimicrobial Resistance*, *29*, 540–541. https://doi.org/10.1016/j.jgar.2021.11.013

He, S., Almalki, A. A., Rafeeq, M. M., Sain, Z. M., Alqosaibi, A. I., Alnamshan, M. M., … & Jamal, Q. M. S. (2022). Targeting cytotoxin-associated antigen A, a virulent factor of Helicobacter pylori-associated gastric cancer: Structure-based *in silico* screening of natural compounds. *Molecules*, *27*(3), 732. https://doi.org/10.3390/molecules27030732

Herman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., … & Bourne, P. E. (2000). The protein data bank. *Nucleic Acids Research*, *28*, 235–242.

Hou, W., Wang, Z.-Y., Lin, J., & Chen, W.-M. (2020). Induction of differentiation of the acute myeloid leukemia cell line (HL-60) by a securinine dimer. *Cell Death Discovery*, *6*(1), 123. https://doi.org/10.1038/s41420-020-00354-3

Jamal, A., Jahan, S., Choudhry, H., Rather, I. A., & Khan, M. I. (2022). A subtraction genomics-based approach to identify and characterize new drug targets in *Bordetella pertussis:* Whooping cough. *Vaccines*, *10*(11), 1915–1925. https://doi.org/10.3390/vaccines10111915

Ji, X.-W., Zhu, X., Li, Y., Xue, F., Kuan, I. H. S., He, Q.-F., Meng, X.-R., Xiang, X.-Q., Cui, Y.-M., & Zheng, B. (2022). Model-informed drug development of new cefoperazone sodium and sulbactam sodium combination (3: 1): Pharmacokinetic/pharmacodynamic analysis and antibacterial efficacy against Enterobacteriaceae. *Frontiers in Pharmacology*, *13*, 856792. https://doi.org/10.3389/fphar.2022.856792

Jones, R. N., & Barry, A. L. (1983). Cefoperazone: A review of its antimicrobial spectrum, β-lactamase stability, enzyme inhibition, and other *in vitro* characteristics. *Reviews of Infectious Diseases*, *5* (Supplement_1), S108–S126. https://doi.org/10.1093/clinids/5.supplement_1.s108

Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., & Morishima, K. (2017). KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic acids research*, *45*(D1), D353–D361.

Kishida, H., Unzai, S., Roper, D. I., Lloyd, A., Park, S.-Y., & Tame, J. R. H. (2006). Crystal structure of penicillin binding protein 4 (dacB) from *Escherichia coli*, both in the native form and covalently linked to various antibiotics. *Biochemistry*, *45*(3), 783–792. https://doi.org/10.1021/bi051533t

Knox, C., Law, V., Jewison, T., Liu, P., Ly, S., Frolkis, A., Pon, A., Banco, K., Mak, C., Neveu, V., Djoumbou, Y., Eisner, R., Guo, A. C., & Wishart, D. S. (2011). DrugBank 3.0: A comprehensive resource for 'omics' research on drugs. *Nucleic Acids Research*, *39*(Database issue), D1035–41. https://doi.org/10.1093/nar/gkq1126

Köbel, M., Weidensdorfer, D., Reinke, C., Lederer, M., Schmitt, W. D., Zeng, K., Thomssen, C., Hauptmann, S., & Hüttelmaier, S. (2007). Expression of the RNA-binding protein IMP1 correlates with poor prognosis in ovarian carcinoma. *Oncogene*, *26*(54), 7584–7589. https://doi.org/10.1038/sj.onc.1210563

Kuang, S. F., Li, X., Feng, D. Y., Wu, W. B., Li, H., Peng, B., Peng, X. X., Chen, Z. G., & Zhang, T. T. (2022). Nitrite promotes ROS production to potentiate cefoperazone-sulbactam-mediated elimination to lab-evolved and clinical-evolved *Pseudomonas aeruginosa*. *Microbiology Spectrum*, *10*(4), e02327-21. https://doi.org/10.1128/spectrum.02327-21

Kufareva, I., & Abagyan, R. (2012). Methods of protein structure comparison. *Methods in Molecular Biology*, *857*, 231–257. https://doi.org/10.1007/978-1-61779-588-6_10

Kuki, Á., Nagy, L., Zsuga, M., & Kéki, S. (2011). Fast identification of phthalic acid esters in poly (vinyl chloride) samples by direct analysis in real time (DART) tandem mass spectrometry. *International Journal of Mass Spectrometry*, *303*(2–3), 225–228. https://doi.org/10.1016/j.ijms.2011.02.011

Kuzmanic, A., & Zagrovic, B. (2010). Determination of ensemble-average pairwise root mean-square deviation from experimental B-factors. *Biophysical Journal*, *98*(5), 861–871. https://doi.org/10.1016/j.bpj.2009.11.011

Laupland, K. B., Paterson, D. L., Edwards, F., Stewart, A. G., & Harris, P. N. A. (2022). *Morganella morganii,* an emerging cause of bloodstream infections. *Microbiology Spectrum*, *10*(3), e0056922. https://doi.org/10.1128/spectrum.00569-22

Li, W., & Godzik, A. (2006). Cd-hit: A fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics*, *22*(13), 1658–1659. https://doi.org/10.1093/bioinformatics/btl158

Liu, H., Zhu, J., Hu, Q., & Rao, X. (2016). *Morganella morganii*, a non-negligent opportunistic pathogen. *International Journal of Infectious Diseases*, *50*, 10–17. https://doi.org/10.1016/j.ijid.2016.07.006

Luo, X.-W., Liu, P.-Y., Miao, Q.-Q., Han, R.-J., Wu, H., Liu, J.-H., He, D.-D., & Hu, G.-Z. (2022). Multidrug resistance genes carried by a novel Transposon Tn7376 and a genomic island named MMGI-4 in a pathogenic *Morganella morganii* isolate. *Microbiology Spectrum*, *10*(3), e0026522. https://doi.org/10.1128/spectrum.00265-22

Maisuradze, G. G., Liwo, A., & Scheraga, H. A. (2009). Principal component analysis for protein folding dynamics. *Journal of Molecular Biology*, *385*(1), 312–329. https://doi.org/10.1016/j.jmb.2008.10.018

Miller, B. R., McGee, T. D., Swails, J. M., Homeyer, N., Gohlke, H., & Roitberg, A. E. (2012). MMPBSA. py: An efficient program for end-state free energy calculations. *Journal of Chemical Theory and Computation*, *8*(9), 3314–3321. https://doi.org/10.1021/ct300418h

Miller, E. L. (2002). The penicillins: A review and update. *Journal of Midwifery & Women's Health*, *47*(6), 426–434. https://doi.org/10.1016/s1526-9523(02)00330-6

Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A. C., & Kanehisa, M. (2007). KAAS: An automatic genome annotation and pathway reconstruction server. *Nucleic Acids Research*, *35*(Web Server issue), W182–5. https://doi.org/10.1093/nar/gkm321

Mustikaningtyas, D., Widyarti, S., Rifa'i, M., & Widodo, N. (2021). Proposed mechanism of antibacterial activity of glutathione by inhibition of the D-alanyl-D-alanine carboxypeptidase enzyme. *International Journal of Peptide Research and Therapeutics*, *27*(2), 843–849. https://doi.org/10.1007/s10989-020-10124-5

Naga, N. G., El-Badan, D. E., Rateb, H. S., Ghanem, K. M., & Shaaban, M. I. (2021). Quorum sensing inhibiting activity of cefoperazone and its metallic derivatives on *Pseudomonas aeruginosa*. *Frontiers in Cellular and Infection Microbiology*, *11*, 716789. https://doi.org/10.3389/fcimb.2021.716789

Neely, M. N., Pfeifer, J. D., & Caparon, M. (2002). *Streptococcus*-zebrafish model of bacterial pathogenesis. *Infection and Immunity*, *70*(7), 3904–3914. https://doi.org/10.1128/IAI.70.7.3904-3914.2002

Otarigho, B., & Falade, M. O. (2023). Computational screening of approved drugs for inhibition of the antibiotic resistance gene mecA in methicillin-resistant *Staphylococcus aureus* (MRSA) strains. *BioTech*, *12*(2), 25. https://doi.org/10.3390/biotech12020025

Park, S. Y., Lee, K., Cho, Y., Lim, S. R., Kwon, H., Han, J. E., & Kim, J. H. (2020). Emergence of third-generation cephalosporin-resistant *Morganella morganii in* a captive breeding dolphin in South Korea. *Animals*, *10*(11), 2052–2058. https://doi.org/10.3390/ani10112052

Power, P., Galleni, M., Ayala, J. A., & Gutkind, G. (2006). Biochemical and molecular characterization of three new variants of AmpC beta-lactamases from *Morganella morganii*. *Antimicrobial Agents and Chemotherapy*, *50*(3), 962–967. https://doi.org/10.1128/AAC.50.3.962-967.2006

Príncipe, A., Jofré, E., Alvarez, F., & Mori, G. (2009). Role of a serine-type D-alanyl-D-alanine carboxypeptidase on the survival of *Ochrobactrum* sp. 11a under ionic and hyperosmotic stress. *FEMS Microbiology Letters*, *295*(2), 261–273. https://doi.org/10.1111/j.1574-6968.2009.01604.x

Rioseras, B., Yagüe, P., López-García, M. T., Gonzalez-Quiñonez, N., Binda, E., Marinelli, F., & Manteca, A. (2016). Characterization of SCO4439, a D-alanyl-D-alanine carboxypeptidase involved in spore cell wall maturation, resistance and germination in *Streptomyces coelicolor*. *Scientific Reports*, *6*(1), 21659. https://doi.org/10.1038/srep21659

Sachs, J. L., & Wilcox, T. P. (2006). A shift to parasitism in the jellyfish symbiont *Symbiodinium microadriaticum*. *Proceedings-Biological Sciences*, *273*(1585), 425–429.

Sajid Jamal, Q. M., Alharbi, A. H., & Ahmad, V. (2022). Identification of doxorubicin as a potential therapeutic against SARS-CoV-2 (COVID-19) protease: A molecular docking and dynamics simulation studies. *Journal of Biomolecular Structure & Dynamics*, *40*(17), 7960–7974. https://doi.org/10.1080/07391102.2021.1905551

Savage, D. C. (1977). Microbial ecology of the gastrointestinal tract. *Annual Review of Microbiology*, *31*, 107–133. https://doi.org/10.1146/annurev.mi.31.100177.000543

Stock, I., & Wiedemann, B. (1998). Identification and natural antibiotic susceptibility of *Morganella morganii*. *Diagnostic Microbiology and Infectious Disease*, *30*(3), 153–165. https://doi.org/10.1016/s0732-8893(97)00243-5

Tiana, G., Simona, F., De Mori, G. M. S., Broglia, R. A., & Colombo, G. (2004). Understanding the determinants of stability and folding of small globular proteins from their energetics. *Protein Science*, *13*(1), 113–124. https://doi.org/10.1110/ps.03223804

Torraca, V., & Mostowy, S. (2018). Zebrafish INFECTION: From pathogenesis to cell biology. *Trends in Cell Biology*, *28*(2), 143–156. https://doi.org/10.1016/j.tcb.2017.10.002

Valdés-Tresanco, M. S., Valdés-Tresanco, M. E., Valiente, P. A., & Moreno, E. (2021). gmx_MMPBSA: A new tool to perform end-state free energy calculations with GROMACS. *Journal of Chemical Theory and Computation*, *17*(10), 6281–6291. https://doi.org/10.1021/acs.jctc.1c00645

Van Der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A. E., & Berendsen, H. J. C. (2005). GROMACS: Fast, flexible, and free. *Journal of Computational Chemistry*, *26*(16), 1701–1718. https://doi.org/10.1002/jcc.20291

Wadood, A., Jamal, A., Riaz, M., Khan, A., Uddin, R., Jelani, M., & Azam, S. S. (2018). Subtractive genome analysis for *in silico* identification and characterization of novel drug targets in *Streptococcus pneumonia* strain JJA. *Microbial Pathogenesis*, *115*, 194–198. https://doi.org/10.1016/j.micpath.2017.12.063

Wishart, D. S., Feunang, Y. D., Guo, A. C., Lo, E. J., Marcu, A., Grant, J. R., Sajed, T., Johnson, D., Li, C., Sayeeda, Z., Assempour, N., Iynkkaran, I., Liu, Y., Maciejewski, A., Gale, N., Wilson, A., Chin, L., Cummings, R., Le, D., … Wilson, M. (2018). DrugBank 5.0: A major update to the DrugBank database for 2018. *Nucleic Acids Research*, *46*(D1), D1074–D1082. https://doi.org/10.1093/nar/gkx1037

Wu, G. D., & Lewis, J. D. (2013). Analysis of the human gut microbiome and association with disease. *Clinical Gastroenterology and Hepatology*, *11*(7), 774–777. https://doi.org/10.1016/j.cgh.2013.03.038

Xiang, G., Lan, K., Cai, Y., Liao, K., Zhao, M., Tao, J., Ma, Y., Zeng, J., Zhang, W., Wu, Z., Yu, X., Liu, Y., Lu, Y., Xu, C., Chen, L., Tang, Y.-W., Chen, C., Jia, W., & Huang, B. (2021). Clinical molecular and genomic epidemiology of *Morganella morganii* in China. *Frontiers in Microbiology*, *12*, 744291. https://doi.org/10.3389/fmicb.2021.744291

Yang, J. H., Sheng, W. H., & Hsueh, P. R, SMART Program. (2020). Antimicrobial susceptibility and distribution of extended-spectrum β-lactamases, AmpC β-lactamases and carbapenemases among *Proteus, Providencia* and *Morganella* isolated from global hospitalised patients with intra-abdominal and urinary tract infections: Results of the Study for Monitoring Antimicrobial Resistance Trends (SMART), 2008–2011. *Journal of Global Antimicrobial Resistance*, *22*, 398–407. https://doi.org/10.1016/j.jgar.2020.04.011

Yang, Y., Yao, K., Ma, X., Shi, W., Yuan, L., & Yang, Y. (2015). Variation in *Bordetella pertussis* susceptibility to erythromycin and virulence-related genotype changes in China (1970–2014). *PLOS One*, *10*(9), e0138941. https://doi.org/10.1371/journal.pone.0138941

Yu, N. Y., Wagner, J. R., Laird, M. R., Melli, G., Rey, S., Lo, R., Dao, P., Sahinalp, S. C., Ester, M., Foster, L. J., & Brinkman, F. S. L. (2010). PSORTb 3.0: Improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. *Bioinformatics*, *26*(13), 1608–1615. https://doi.org/10.1093/bioinformatics/btq249

Zhang, R., Ou, H. Y., & Zhang, C. T. (2004). DEG: A database of essential genes. *Nucleic Acids Research*, *32*(Database issue), D271–2.

Zoete, V., Cuendet, M. A., Grosdidier, A., & Michielin, O. (2011). SwissParam: A fast force field generation tool for small organic molecules. *Journal of Computational Chemistry*, *32*(11), 2359–2368. https://doi.org/10.1002/jcc.21816

Zou, L., Wang, J., Huang, B., Xie, M., & Li, A. (2011). MtsB, a hydrophobic membrane protein of *Streptococcus iniae*, is an effective subunit vaccine candidate. *Vaccine*, *29*(3), 391–394. https://doi.org/10.1016/j.vaccine.2010.10.062