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# COMPREHENSIVE *IN SILICO* RESISTOME PROFILING OF *STAPHYLOCOCCUS AUREUS* GENOMES: CHARACTERIZATION OF BROAD-SPECTRUM ANTIMICROBIAL RESISTANCE

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**Abstract:** Antimicrobial resistance (AMR) is increasingly turning in to global challenge, as pathogens are rapidly developing resistance to a wide range of antimicrobials at an alarming rate. The goal of present *in silico* study is to analyze and characterize AMR regions, their products and to evaluate the mechanisms of resistance in pathogenic *Staphylococcus aureus* strains isolated from clinical samples of Pakistani population. For this, 41 available WGS of *Staphylococcus aureus* were extracted from NCBI and subjected to Resfinder 4.6.0 for the detection of AMR genes among the sequences. Further, the molecular structure of protein products of most prevalent AMR genes was evaluated *in silico* using PDB and PDBsum. Total 18 AMR genes were found in 38 genomes; while 3 sequences showed no resistance genes. Most dominant resistant genes were found against beta lactams (n=37; 90 %) followed by genes against aminoglycosides (n=28; 68 %) > folate pathway antagonists (n=23; 56 %) > macrolides (n=19; 46 %) > tetracycline (n=18; 44 %) > lincosamide (n=12; 29 %) > fusidic acid (steroid antibacterial) (n=12; 29 %) > fosfomycin (n=7; 17 %) and pseudomonic acid (n=4; 9.7 %). In addition, *blaZ* (n=32; 78 %) and *mecA* (n=29; 71 %) were most predominant genes. The 3D structural analysis of β-lactamase and PBP2a reveals the resistance based on structure. This study plays crucial role for the development of effective therapeutic interventions that ultimately leads to enhance public health by reducing morbidity and mortality associated with resistant pathogens' infections.

**Key words**: Antimicrobial resistance; *Staphylococcus aureus;* blaZ, mecA, β-lactamase, PBP2a

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## INTRODUCTION

Antimicrobial resistance (AMR) is one of the major global concerns that threaten effective prevention and treatment of an increasing range of infections (Salam *et al*. 2023). AMR is one of the concerns particularly for under-developed and developing countries. It is reported that 70% of increase in Antibiotic Resistance is seen in Asian Region. Study from 2012-2022 demonstrated that Multi drug resistant(MDR) and Extensive Drug Resistant (XDR) Strains were reported which includes *Straphyloccus aureus*, *E Coli*, *Pseudomonas spp*, *Salmonella* spp, *Acinetobacter spp*, *Klebsiella spp*, *Proteus spp*, *Shigella spp* and *Entero-coccus spp* (Bilal *et al*. 2021). The WHO has published its priority pathogen list 2024 which grouped them as 3 groups of bacteria based on the urgency of need for new antibacterials(WHO bacterial priority pathogens list 2024. Scientific basis of AMR Key strategies to combat growing problem of antimicrobial resistance caused by microbes. (Holmes *et al.* 2016). The Gram-positive bacterium Staphylococcus aureus is a member of the Micrococcaceae family. It frequently colonises mucous membranes, skin, and skin glands. Staphylococcus aureus colonises about 25% of the human population. Staphylococcus aureus can cause serious conditions like osteomyelitis, infective endocarditis, skin and soft tissue infections, bloodstream infections, hospital-acquired pneumonia, and even death, while healthy people may only get minor skin infections. It is one of the most common pathogens in Pakistan, causing a variety of infections in both community and hospital settings. It also affects farm and domestic animals (Idrees *et al.*, 2023; Ullah *et al*., 2021; Peacock & Paterson, 2015). The majority of the virulence factors that this bacterium can produce, such as toxins and adhesins, are encoded by genes found on mobile genetic elements like integrons, transposons, and plasmids (Rao *et al*., 2022; Stapleton & Taylor, 2002).

Penicillin was the first antibiotic created for human use. More than 90% of people are now resistant to penicillin, despite the fact that it was once very effective in treating *S. aureus* infections. Multidrug-resistant (MDR) pathogens have emerged as a result of antibiotic overuse (Fetsch *et al.*, 2021). The presence of specific antimicrobial genes with in the genome of *S. aureus* make them resistant to different antimicrobial agents like tetracyclines, penicillins, aminoglycosides, cephalosporins and fluoroquinolones (Urban-Chmiel *et al*., 2022). In order to identify virulence factors, toxin genes and resistome (entire collection of antimicrobial genes with in microbial genome) in S. aureus, the strategies of molecular genomics are significant tools. These tools help in elucidating drug-resistance and toxicity of this pathogen. Moreover, advancements in bioinformatics tools has led to effective identification of AMR genes with in pathogenic genomes which could aid in the development of efficient therapeutic approaches.

Using whole genome sequencing (WGS) technology, recent studies have identified bacteria and their antimicrobial resistance (AMR) gene profiles from a range of sources (Ruppé *et al*., 2017; Tyson *et al*., 2015; Stoesser *et al*., 2013). To find AMR genes in bacterial genomes, specialised bioinformatics-based databases such as ResFinder (Zankari *et al*., 2012), CARD (McArthur *et al.*, 2013), and ARG-ANNOT (Gupta *et al*., 2014) are utilised. ResFinder, a well-known in silico tool for bacterial resistome analysis, was developed by the Center for Genomic Epidemiology (CGE) (www.genomicepidemiology.org). However, the National Center for Biotechnology Information (NCBI) database provided 41 complete whole genome sequences of *Staphylococcus aureus* that were isolated from different clinical samples of the Pakistani population for this study. The ResFinder tool was used to determine the resistance profiles and antimicrobial resistance (AMR) genes of each isolate. This study represents the first comprehensive report from Pakistan involving the analysis of 41 complete *Staphylococcus aureus* genomes. Utilizing advanced bioinformatics tools, this research assembled a substantial genomic dataset to perform an in-depth, large-scale investigation of antimicrobial resistance (AMR) gene profiles. This novel approach offers valuable insights into the regional resistome landscape and enhances the understanding of the genetic basis of drug resistance in *S. aureus* strains circulating in Pakistan.

## MATERIALS AND METHODS

### *Data Retrieval and Sample Selection*

To assess antimicrobial resistance in *Staphylococcus aureus* genomes, the term "*Staphylococcus aureus* Pakistan" was used to search the Biosample database of NCBI (https://www.ncbi.nlm.nih.gov/biosample). Relevant accession numbers were extracted for further analysis.

### *Next Generation Sequencing Data Acquisition*

The Biosample accession numbers were utilized to retrieve next-generation sequencing (NGS) data in FASTA format via the NCBI Genomes resource.
(https://www.ncbi.nlm.nih.gov/datasets/genome/).

A total of 41 NGS datasets (WGS) corresponding to different *S. aureus* (Pakistani patient isolates) isolates were available and selected for analysis

### *Antimicrobial Resistance Genes Analysis*

The FASTA nucleotide (FNA) files of the 41 isolates were downloaded and uploaded to ResFinder version 4.6.0 (http://genepi.food.dtu.dk/resfinder) using default settings (Zankari *et al*., 2012). This tool was used to identify antimicrobial resistance (AMR) genes present in the genomes and predict the corresponding resistance phenotypes. Further antibiotic resistance mechanisms of detected genes were studied using CARD (The Comprehensive Antibiotic Resistance Database) (McArthur *et al*., 2013).

### *Three-dimensional structure evaluation of proteins associated with AMR*

The study on 3D structures of protein product of most prevalent AMR genes was carried out using Protein Databank (PDB) (https://www.rcsb.org/) and PDBsum (https://www.ebi.ac.uk/thorntonsrv/databases/pdbsum/) (Burley *et al*., 2022).

## RESULTS AND DISCUSSION

The emphasis of current investigation is to detect antimicrobial resistance genes with in the whole genomes of *S. aureus* submitted to NCBI (patient isolates across Pakistani population). Many studies have acquired the potential of WGS to characterize AMR genes with in genomes of various pathogenic bacterial genomes (Wattimury *et al*., 2023; Irum *et al*., 2021; Nwaiwu and Onyeaka, 2021; Demirci *et al*., 2021; Salih and Shafeek, 2019).

### *Profiling of Antimicrobial Resistance Agents*

The antimicrobial resistance analysis of 41 *Staphylococcus aureus* strains reported in various clinical samples of Pakistani population using the ResFinder 4.6.0 tool revealed the presence of resistance genes associated with 10 different classes of antimicrobial agents (Figure 1). The AMR genes that show resistance to beta lactams were most prevalent (n=37; 90 %), followed by genes against aminoglycosides (n=28; 68 %), folate pathway antagonists (n=23; 56 %), macrolides (n=19; 46 %), tetracycline (n=18; 44 %), lincosamide (n=12; 29 %), fusidic acid (steroid antibacterial) (n=12; 29 %), fosfomycin (n=7; 17 %) and pseudomonic acid (n=4; 9.7 %). Several previous researches support the findings of current study. Rao *et al*. (2022) reported that the highest antimicrobial resistance among various *Staphylococcus aureus* strains isolated from human clinical samples was observed against penicillin (a β-lactam antibiotic) at a rate of 81.6%. This was followed by resistance rates of 44% to clindamycin and 43% to erythromycin. Similarly, according to Ibrahim *et al*. (2023), out of 172 *Staphylococcus aureus* isolates tested for antimicrobial resistance, 152 (88.37%) showed resistance to penicillin, 90 (52.32%) were resistant to trimethoprim-sulfamethoxazole (SXT), and 45 (26.16%) exhibited resistance to tetracycline.

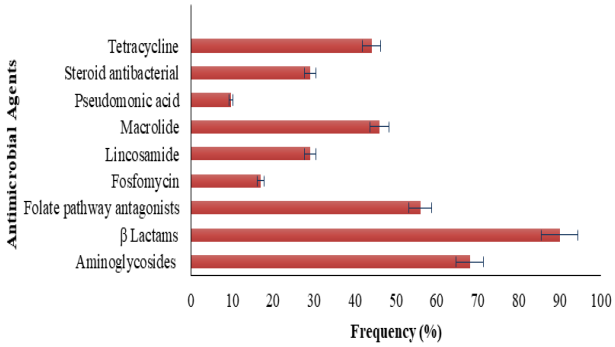


**Figure 1.** AMR genes were identified using ResFinder 4.6.0 from WGS data of 41 *S. aureus* strains retrieved from the NCBI database

### *Genomic Detection of AMR Determinants*

A total of eighteen antibiotic resistance genes were detected in 38 whole genomes sequences of *Staphylococcus aureus*. Most prevalent among the genes were those encoding beta lactam resistance including *blaZ* (n=32; 78 %) and *mecA* (n=29; 71 %). The only observed gene for fosfomycin resistance was *fosY* (n=8; 19.5 %). Aminoglycoside resistant was encoded by five genes namely *aac(6')-aph(2'')* (n=15; 36.5 %), *aph(3')-III* (n=13; 32 %), *aadD* (n=7; 17%), *ant(6)-Ia* (n=6; 15 %) and *ant(9)-Ia* (n=3; 7 %). For lincosamide resistance, the two detected gene were *erm(C)* (n=12; 29 %) and *lnu(A)* (n=7; 17%). Three genes *tet(M)* (n=11; 27 %), *tet(K)* (n=6; 15 %) and *tet(L)* (n=1;2 %) were observed for tetracycline resistance. As for macrolide resistance, two genes were recorded i.e. *msr(A)* (n=8; 19.5 %) and *mph(C)* (n=8; 19.5 %). While *fusC* gene (n=2; 5 %) was identified for fusidic acid resistance. One gene *dfrG* (n=23; 56 %) associated with trimethoprim (a folate pathway antagonist) resistance was identified. *mupA* gene (n=1; 2%) was identified for pseudomonic acid resistance and no gene was detected for quinolone resistance (Figure 2).

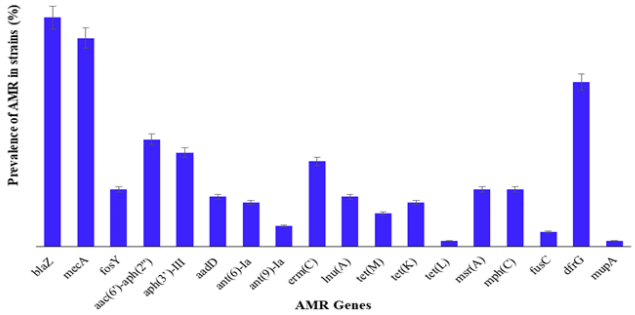


**Figure 2.** Prevalence of sixteen antimicrobial resistant genes in genomes of *S. aureus*

According to results by Resfinder 4.6.0, three sequences of *Staphylococcus aureus* does not harbor any gene. The highest number of resistant genes in a single strain was ten, exhibiting resistance to seven different groups of antimicrobial agents including Beta lactam. The data of occurrence of AMR gene in each of 41 strains is highlighted (Table 1)

**Table 1. Frequency of AMR Genes present in each strain**

| S. No | Bio sample Accession | AMR Genes |
|---|---|---|
| 1 | SAMEA111505390 | *aph(3')-III, blaZ, tet(L), fusC* |
| 2 | SAMEA111505391 | *aph(3')-III, mecA, blaZ, msr(A), mph(C)* |
| 3 | SAMEA111505392 | *aac(6')-aph(2''), aph(3')-III, mecA, blaZ, dfrG, msr(A), mph(C), lnu(A)* |
| 4 | SAMEA111505387 | *aac(6')-aph(2''), aph(3')-III, mecA, blaZ, dfrG, msr(A), mph(C)* |
| 5 | SAMEA111505388 | *aadD, blaZ, dfrG, msr(A), mph(C), tet(K), fusC, lnu(A)* |
| 6 | SAMEA111505377 | *aph(3')-III, mecA, blaZ, dfrG, msr(A), mph(C)* |
| 7 | SAMEA111505378 | *mecA, blaZ, dfrG, lnu(A)* |
| 8 | SAMEA111505379 | *mecA, blaZ, dfrG, lnu(A)* |
| 9 | SAMEA111505380 | *aph(3')-III, mecA, blaZ, dfrG, fosY, lnu(A)* |
| 10 | SAMEA111505381 | *aadD, blaZ, lnu(A)* |
| 11 | SAMEA111505382 | *aadD, blaZ, lnu(A)* |
| 12 | SAMEA111505383 | *mecA, blaZ, dfrG* |
| 13 | SAMEA111505384 | *mecA, dfrG* |
| 14 | SAMEA111505385 | *blaZ* |
| 15 | SAMEA111505386 | *mecA, blaZ, dfrG* |
| 16 | SAMN29417126 | *aadD, blaZ, dfrG, msr(A), mph(C), tet(K), lnu(A)* |
| 17 | SAMN31113469 | *aac(6')-aph(2''), mecA, blaZ, dfrG, tet(M), fosY* |
| 18 | SAMN29417124 | - |
| 19 | SAMN29417123 | - |
| 20 | SAMN11096030 | *aac(6')-aph(2''), mecA, blaZ, erm(C)* |
| 21 | SAMN19679277 | *ıac(6')-aph(2''), mecA, blaZ, erm(C), tet(K)* |
| 22 | SAMN19679289 | *dfrG* |
| 23 | SAMN22108560 | *aadD, mecA, blaZ, tet(K)* |
| 24 | SAMN29417122 | - |
| 25 | SAMN19679286 | *aac(6')-aph(2''), mecA, blaZ, erm(C), tet(M), ant(9)-Ia* |
| 26 | SAMN19679287 | *mecA, blaZ, erm(C), tet(M)* |
| 27 | SAMN19679288 | *aadD, mecA, blaZ, dfrG, tet(K)* |
| 28 | SAMN19679285 | *ac(6')-aph(2''), mecA, dfrG, erm(C), tet(M), fosY, ant(6)-Ia, aph(3')-III* |
| 29 | SAMN19679284 | *aph(3')-III, mecA, blaZ, dfrG, tet(M), fosY, ant(6)-Ia* |

| | | |
|---|---|---|
| 30 | SAMN19679283 | *aac(6')-aph(2''), aph(3')-III, mecA, dfrG, erm(C), tet(M), fosY, ant(6)-Ia* |
| 31 | SAMN19679282 | *ac(6')-aph(2''), mecA, blaZ, erm(C), tet(M), ant(6)-Ia* |
| 32 | SAMN19679281 | *aac(6')-aph(2''), mecA, blaZ, tet(M)* |
| 33 | SAMN19679278 | *ac(6')-aph(2''), mecA, dfrG, erm(C), tet(M), fosY, ant(6)-Ia, aph(3')-III* |
| 34 | SAMN11096691 | *aadD, aac(6')-aph(2''), mecA, blaZ, dfrG, erm(C), bleO* |
| 35 | SAMN11080949 | *aph(3')-III, mecA, blaZ, dfrG, msr(A), mph(C)* |
| 36 | SAMN19679280 | *ac(6')-aph(2''), mecA, blaZ, erm(C), tet(M), ant(9)-Ia* |
| 37 | SAMEA111505389 | *blaZ, dfrG* |
| 38 | SAMN03112991 | *blaZ* |
| 39 | SAMN03160855 | *aph(3')-III, mecA, dfrG, msr(A), mph(C), erm(C)* |
| 40 | SAMN03161113 | *aac(6')-aph(2''), mecA, blaZ, tet(M), ant(9)-Ia* |
| 41 | SAMN11080858 | *aac(6')-aph(2''), aph(3')-III, mecA, blaZ, dfrG, erm(C), tet(K), mupA, fosY, ant(6)-Ia* |

**Table 2. AMR gene profiles detected in 41 WGS of clinical *Staphylococcus aureus* isolated from clinical samples in the Pakistani population**

| AMR Genes | Prevalence (n) | Percentage (%) | Predicted Phenotype | Classification of antibiotic |
|---|---|---|---|---|
| *aadD* | 7 | 17 | Aminoglyc | Protein Synthesis |
| *aac(6')-* | 15 | 36.5 | oside | inhibitor |
| *aph(2'')* | 13 | 32 | resistance | |
| *aph(3')-III* | 6 | 15 | | |
| *ant(6) -Ia* | 3 | 7 | | |
| *ant (9)-Ia* | | | | |
| *mecA* | 29 | 71 | Beta lactam | Cell wall synthesis |
| *blaZ* | 32 | 78 | resistance | inhibitor |
| *dfrG* | 23 | 56 | Trimethopri m resistance | Metabolic pathway inhibitor |
| *msr(A)* | 8 | 19.5 | Macrolide | Protein Synthesis |
| *mph(C)* | 8 | 19.5 | resistance | inhibitor |
| *erm(C)* | 12 | 29 | Lincosamid | Protein Synthesis |
| *lnu (A)* | 7 | 17 | e resistance | inhibitor |
| *tet(L)* | 1 | 2 | Tetracyclin | Protein Synthesis |
| *tet(M)* | 11 | 27 | e resistance | inhibitor |
| *tet(K)* | 6 | 15 | | |
| *fusC* | 2 | 5 | Fusidic acid (Steroid antibacteria l) resistance | Cell wall synthesis inhibitor |
| *fosY* | 8 | 19.5 | Foscomyci n | Cell wall synthesis inhibitor |
| *mup A* | 1 | 2 | (Phosphoni c acid derivative) resistance Mupirocin (Pseudomoni c acid) | Protein Synthesis inhibitor |

Among eighteen AMR genes identified in 41 genomes of *S. aureus*, total 13 genes were linked with resistance to protein synthesis inhibitors. This was followed by four genes involved in resistance to cell wall synthesis inhibitors and only one gene is associated with resistance against metabolic pathway inhibitors. Despite a higher number of AMR genes associated with protein synthesis inhibitors, their distribution across the 41 *Staphylococcus aureus* genomes was relatively limited. In contrast, resistance genes targeting cell wall synthesis inhibitors demonstrated higher prevalence (Table 2). The ***blaZ*** gene was the most frequently detected, present in 32 genomes (~78%), followed by ***mecA,*** found in 29 genomes (~71%). Akpaka *et al* (2017) also reported that the *blaZ* gene, encoding penicillinase and representing the most common β-lactam resistance mechanism in *Staphylococcus aureus*, was identified in 88.7% of methicillin-susceptible *S. aureus* (MSSA) isolates, whereas methicillin resistance associated with the *mecA* gene was found in 13.6% of the strains. According to Alkuraythi *et al* (2024), clinical isolates of *Staphylococcus aureus* harbored *mecA* gene (96.3 %) and *blaZ* (62%).

For mecA gene, prevalence was 31% identified in *Staphylococcus aureus* strains from clinical samples of human origin (Rao et al., 2022), while the most common genes detected were ermC followed by ermB and blaZ with prevalence of 87.29%, 83.05%, 63.98% respectively, followed by aacA-aphD, with a low frequency (Gan *et al*., 2021). So based on findings of present study and previous studies reported worldwide, the blaZ and mecA can be reported as one of the most common antimicrobial resistance genes detected in *Staphylococcus aureus* strains.

A significant class of antimicrobial agents known as β-lactam antibiotics is distinguished by the presence of a β-lactam ring in their molecular structure. This class is further classified in to several major groups including penicillin, cephalosporins, carbapenems and monobactams. These are most frequently used antibiotics that target the synthesis of pathogenic bacterial cell wall thus ultimately results in cell lysis and death of pathogen. Due to extensive utilization of these antibiotics, major clinical pathogens have developed resistance towards them (Zango *et al*., 2019). The resistance mechanisms employed by *Staphylococcus aureus* against β-lactam antibiotics are multifaceted and complex. In the genera of staphylococci, two biological strategies mediate penicillin resistance. One mechanism suggests the production of β-lactamase enzyme that hydrolyzes β-lactam ring present in the antibiotics of penicillin group (i.e. penicillin G, penicillin V, amoxicillin and ampicillin) leading to the inactivation of antibiotics. *BlaZ* gene is responsible for the production of β-lactamase or penicillinase enzyme. Another

strategy confers resistance specifically to methicillin due to the presence of *mecA* gene that encodes penicillin-binding protein (PBP2a) in *Staphylococcus aureus* strains (Hnini *et al*., 2024; Olsen *et al*., 2006). So, in current study *BlaZ* and *mecA* genes are prevalent in various *Staphylococcus aureus* strains. The resistance mechanisms associated with the identified AMR genes in various *Staphylococcus aureus* strains, as analyzed using the CARD database, are presented in Table 3.

***In silico Protein Structural characterization of Dominant AMR Determinants***

In this study, *blaZ* and *mecA* emerged as the most predominant genes, and their corresponding protein products were selected for three-dimensional structural analysis. Ptotein databank and PDBsum were utilized for 3D structure evaluation.

***Three-dimensional structure of β-lactamase***

The enzyme crystallizes as a monomer (~29 kDa) in an asymmetric unit, with an overall molecular mass of ~88.5 kDa across all three modeled chains (A, B, C) (Figure 3). It belongs to the class A β-lactamase/transpeptidase superfamily, featuring the typical α/β-fold core seen in serine hydrolases. The high-resolution crystal structure was determined using X-ray diffraction at 1.88 Å, with excellent refinement metrics (R-work ≈0.17, R-free ≈0.21), indicating strong model reliability. The active site houses the signature serine nucleophile, which is essential for catalyzing the hydrolysis of β-lactam rings. Additionally, several ligands such EPE - 4-(2-Hydroxyethyl)-1-Piperazine ethanesulfonic acid [HEPES] (3 molecules) , glycerol, and water molecules (434) are captured near the active-site region, offering insight into potential stabilizing interactions. The LIGPLOT analysis revealed the binding interactions of the ligand EPE (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), commonly used as a buffering agent during crystallization, within the active site region of *Staphylococcus aureus* β-lactamase (PDB ID: 6WGR). The diagram highlighted multiple hydrogen bonds between EPE and key amino acid residues such as., Arg 235, Ser 410, Gly 227, Asn 207 and Tyr 96, suggesting that the binding pocket accommodates polar interactions. Additionally, hydrophobic contacts were observed with surrounding non-polar residues, indicating a well-defined binding environment (Figure 4a). On the other hand, for glycerol interactions it is revealed that multiple hydrogen bonds and hydrophobic contacts between the glycerol molecule (GOL) and key residues within the enzyme's binding pocket are present (Figure 4b).

**Table 3. Protein products of detected AMR genes along with their mechanisms**

| Antibiotic Class | Gene | Protein | Molecular Function | Target antimicrobial agents | Resistance mechanism |
|---|---|---|---|---|---|
| Aminoglycoside | *aph(3')-III* | aminoglycoside 3'-phosphotransferase | Phosphorylation | Kanamycin Neomycin Ribostamycin Paromomycin | Antibiotic inactivation by blocking ribosomal binding |
| | *aac(6')-aph(2'')* | Aminoglycoside 6′-N-acetyltransferase and 2″-O-phosphotransferase | Transferase activity | Gentamicin Tobramycin Amikacin Netilmicin | Antibiotic inactivation by preventing the binding to the 30S ribosomal subunit |
| | *ant(6) -Ia* | aminoglycoside nucleotidyltransferase 6-Ia) | adenylation | Streptomycin | Antibiotic inactivation by preventing inhibition of bacterial protein synthesis. |
| | *ant (9)-Ia* | aminoglycoside nucleotidyltransferase 9-Ia | adenylation | Spectinomycin | Antibiotic inactivation by preventing the binding to the 30S ribosomal subunit. |
| | *aadD* | aminoglycoside adenyltransferase D | adenylation | Kanamycin Neomycin Paromomycin Ribostamycin | Antibiotic inactivation by preventing the binding to the 30S ribosomal subunit. |
| Beta-lactam | *mecA* | PBP2a (Penicillin-Binding Protein 2a) | transpeptidation in peptidoglycan cross-linking, maintaining cell wall synthesis even when other PBPs are inhibited | Methicillin Oxacillin Penicillins Cephalosporins | Low affinity for β-lactam antibiotics, as a result cell wall synthesis continues leading to resistance. |
| | *blaZ* | Beta-lactamase | Beta-lactamase activity | Penicillin Amoxicillin Ampicillin | Enzymatic inactivation of β-lactam antibiotics. |

| | | | | | |
|---|---|---|---|---|---|
| Macrolide streptogramin | *msr(A)* | Methionine Sulfoxide Reductase (ATP binding efflux protein) | ATP binding efflux protein that actively transfer antibiotics out of bacterial cell | Macrolides (e.g.,erythromycin) Streptogramin B | Antibiotic target protection and active efflux. |
| Macrolide | *mph(C)* | Macrolide 2'-phosphotransferase | Transferase activity | Spiramycin | Antibiotic inactivation via phosphorylation. |
| Tetracycline | *tet(M)* | Ribosomal protection protein (RPP) | GTP dependent binding | Tetracycline Oxytetracycline Doxycycline Minocycline | Ribosomal protection by binding with 30s ribosomal submit in the presence of GTP. |
| Tetracycline | *tet(K)* | membrane-bound efflux pump protein | Tetracycline efflux transporter activity | Tetracycline | Antibiotic efflux by bacteria to lower intracellular drug levels. |
| Tetracycline | *tet(L)* | membrane-associated efflux protein | Tetracycline transmembrane efflux transporter activity | Tetracycline | Antibiotic efflux by bacteria to lower intracellular drug levels |
| Fusidane | *fusC* | 2-domain zincbinding protein | Fusidic acid resistance via target protection of elongation factor G (EF-G) | Fusidic acid | Antibiotic target protection |
| Diaminopyrimidine | *dfrG* | Dihydrofolate reductase | Dihydrofolate reductase activity with reduced affinity for trimethoprim | Trimethoprim | Antibiotic target replacement |
| Streptogramin/ lincosamide/ macrolide | *erm(C)* | rRNA adenine N-6-methyltransferase | 23S rRNA (adenine-N6)-methyltransferase activity | Macrolides, Lincosamides, Streptogramin B | Antibiotic target alteration |
| Streptogramin/ lincosamide/ macrolide | *lnu (A)* | Lincosamide nucleotidyltransferase A | Nucleotidylation of lincosamides | Clindamycin lincomycin | Antibiotic inactivation by adenylation. |
| Phosphonic acid | *fosY* | glutathione S-transferase | Conjugation of glutathione with fosfomycin, that reduces the effectiveness of drug | foscomycin | fosfomycin resistance via drug inactivation by glutathione transferase |
| Pseudomonic acid | *mup A* | mupirocin-resistant isoleucyl-tRNA synthetase | mediates the binding of the amino acid isoleucine to its corresponding tRNA during protein synthesis. | mupirocin | Target modification |

As expected for class A β-lactamases, the structure exhibits a compact arrangement of α-helices and β-strands forming the canonical serine β-lactamase architecture. This includes the key structural features like 3 anti-parallel beta sheets, 4 beta hairpins, 2 beta bulges, 9 beta strands, 14 alpha helices, 16 helix helix interactions, 18 beta turns and 1 gamma turn. These all facilitates substrate binding and hydrolytic activity (PBD ID: 6WGR). Similar outcomes were also reported by Denesyuk *et al* (2025) and Majiduddin *et al* (2002).

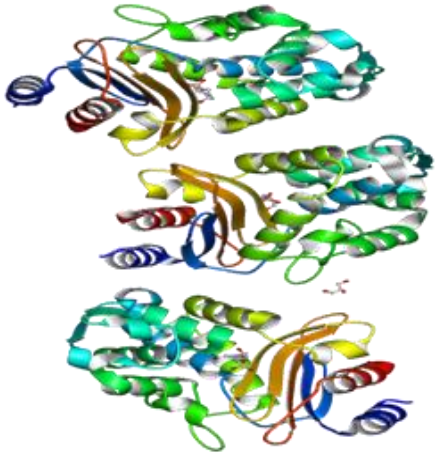

**Figure 3.** Three-dimensional structure of β-lactamase of Staphylococcus aureus (PBD ID: 6WGR)

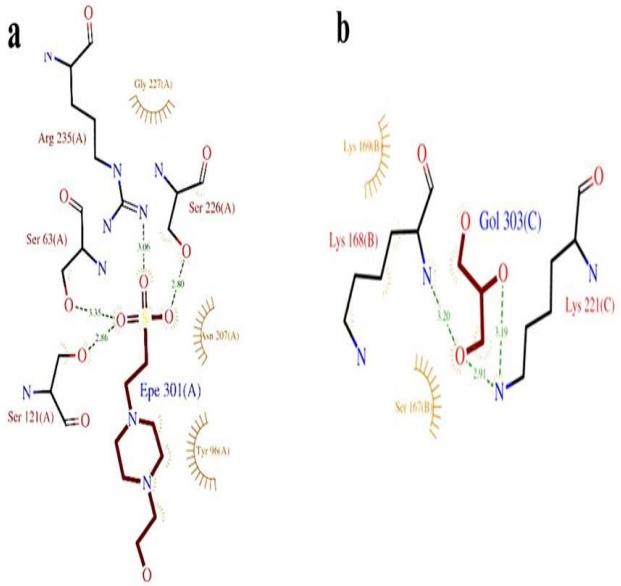


**Figure 4.** A LIGPLOT (a) Interactions of ligand EPE with amino acid residues (Arg, Ser, Gly, Asn and Tyr) of β-lactamase; (b) Interactions of ligand glycerol with amino acid residues (Lys and Ser) of β-lactamase

***Three-dimensional structure of PBP2a***

The three-dimensional structure of Penicillin-Binding Protein 2a (PBP2a) from *Methicillin-resistant Staphylococcus aureus* (MRSA), resolved at 2.45 Å resolution (PDB ID: 1MWT), provides critical insights into the molecular basis of β-lactam resistance and facilitates the development of novel therapeutic agents against MRSA. This crystal structure captures the enzyme covalently acylated by Penicillin G and methicillin, represented by ligand PNM, forming an acyl-enzyme intermediate at the active-site serine residue (Ser403) (Figure 5). The refinement statistics of the model include an R-work value of 0.225 and an R-free value of 0.285, indicating a well-resolved structure suitable for functional interpretation.

Structurally, PBP2a adopts a compact globular architecture composed of 8 beta sheet, 12 beta hairpins, 12 beta bulges, 25 beta strands, 29 alpha helices, 25 helix-helix interactions, 50 beta turns and 6 gamma turn as revealed by PDBsum secondary structure analysis. Functionally, the protein is divided into four major sections: (a) N-terminal transmembrane anchor (residues 1–23) – potentially involved in membrane association or structural stabilization; (b) Non-penicillin binding domain nBP (residues 27-326); (c) N-terminal extension subdomain (residues 27–138) and (d) C-terminal transpeptidase (catalytic) domain (residues 327–668), which contains the active site responsible for peptidoglycan cross-linking (Fishovitz *et al*., 2014; Lim and Strynadka, 2002).

The ligand PNM (Penicillin G) forms a covalent ester linkage with Ser403, mimicking the acylation step in peptidoglycan transpeptidation. Interaction diagrams (LIGPLOT) from PDBsum illustrate a stabilizing network of hydrogen bonds and hydrophobic contacts involving key residues such as Ser, Gly, Thr, Asn and Ala positioning the ligand effectively within the catalytic cleft (Figure 6).

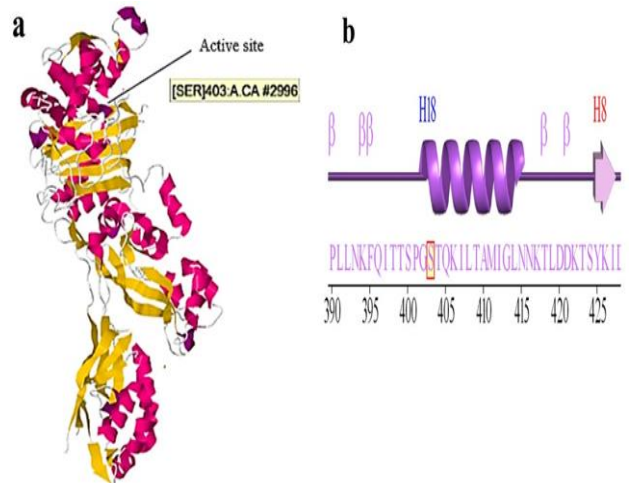


**Figure 5. (a)** Crystalline structure of PBP2a (b) Active site highlighted

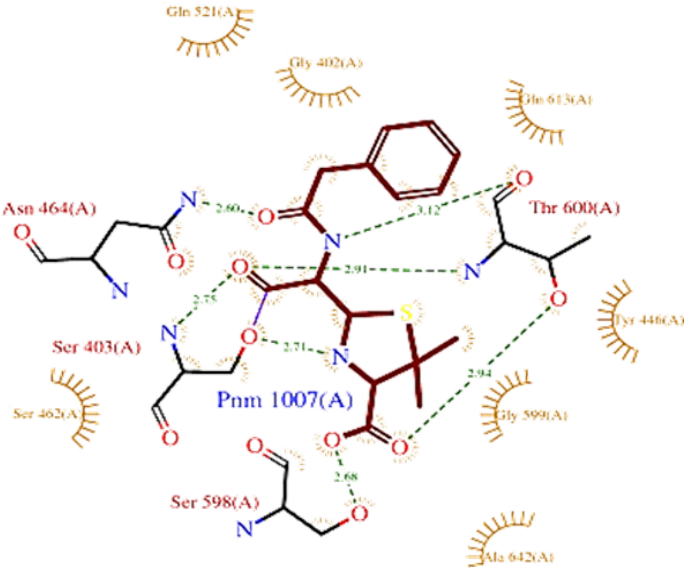


**Figure 6.** Interactions of ligand PNM with amino acid residues of PBP2a (A LIGPLOT)

## CONCLUSION

The current *in silico* research has revealed that ResFinder tool can be very effective for accurate detection AMR genes in multi drug resistant S. aureus and other pathogens. This study reports sixteen resistance genes in 38 complete WGS of *S.aureus* (Pakistani patient isolates) present in NCBI. Most frequently occurring genes associated with resistance were against beta-lactam and aminoglycoside antimicrobial agents. Correspondingly, *blaZ* and *mecA* genes were found to be most prominent in almost in all sequences of *S.aureus* that work against antibiotics of penicillin class such as penicillin V, amoxicillin, methicillin etc. Three-dimensional structural assessment of β-lactamase and PBP2a highlights distinct active site architectures, with hydrogen bonding and hydrophobic interactions guiding ligand accommodation. Together, these structures provide a foundation for designing more effective targeted inhibitors against resistant *Staphylococcus aureus* strains.

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