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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 becoming a global challenge, as pathogens are rapidly developing resistance to a wide range of antimicrobials at an alarming rate. The goal of the 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 the Pakistani population. A total of forty-one available Whole Genome Sequences of *Staphylococcus aureus* were extracted from NCBI and subjected to ResFinder 4.6.0 for the detection of AMR genes among the sequences. Furthermore, the molecular structure of protein products encoded by the most prevalent AMR genes was evaluated *in silico* using PDB and PDBsum databases. 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 acid (steroid antibacterial) (n=12; 29 %) > fosfomycin (n=7; 17 %) and pseudomonic acid (n=4; 9.7 %). In addition; 56 %) > macrolides (n=19; 46 %) > tetracycline (n=18; 44 %) > lincosamide (n=12; 29 %) > fusidic, *blaZ* (n=32; 78 %) and *mecA* (n=29; 71 %) were the most predominant genes. The three-dimensional structural analysis of β-lactamase and PBP2a reveals the resistance based on structure. This study systematically characterizes the resistome of *Staphylococcus aureus* through *in silico* analysis, identifying broad-spectrum antimicrobial resistance determinants and providing a foundation for improved resistance monitoring and therapeutic decision-making.

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 threatens effective prevention and treatment of an increasing range of infections (Salam *et al*. 2023). It is one of the crucial problems particularly for underdeveloped and developing countries. Approximately 70% of the global increase in antibiotic resistance has been reported in the Asian region. Studies conducted between 2012 and 2022 have documented the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp., *Acinetobacter* spp., *Klebsiella* spp., *Proteus* spp., *Shigella* spp., and *Enterococcus* spp.(Bilal *et al*. 2021). The WHO has published its priority pathogen list 2024 which groups them as three categories of bacteria based on the urgency of need for new antibacterial (WHO bacterial priority pathogens list 2024, 2024 ; Holmes *et al.* 2016).

The Gram-positive bacterium *Staphylococcus aureus* is a member of the *Micrococcaceae* family. It frequently colonizes mucous membranes, skin, and skin glands. *Staphylococcus aureus* can cause serious conditions in immunocompromised patients including osteomyelitis, infective endocarditis, skin and soft tissue infections, bloodstream infections, hospital-acquired pneumonia, and even death. In contrast, healthy individuals may experience only 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 produced by this bacterium such as toxins and adhesins, are

encoded by genes located on mobile genetic elements including integrons, transposons, and plasmids (Rao *et a*l., 2022; Stapleton and Taylor, 2002). Penicillin was the first antibiotic developed for human use. More than 90% of people are now resistant to penicillin, even though 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 within the genome of *S. aureus* makes it resistant to various antimicrobial agents such as tetracyclines, penicillin, aminoglycosides, cephalosporins and fluoroquinolones (Urban-Chmiel *et al*., 2022). To identify virulence factors, toxin genes, and the resistome (the entire collection of antimicrobial genes in the microbial genome) in *S. aureus*, molecular genomics strategies are significant tools. These tools help to elucidate drug resistance and toxicity. Furthermore, advancements in bioinformatics tools have led to the effective identification of AMR genes within pathogenic genomes, which could aid in the development of efficient therapeutic approaches.

Recent studies have used whole genome sequencing (WGS) technology to identify bacteria and their antimicrobial resistance (AMR) gene profiles from a variety of sources (Ruppé *et al*., 2017; Tyson *et al*., 2015; Stoesser *et al*., 2013). To find AMR genes in bacterial genomes, specialized bioinformatics-based databases, such as ResFinder (Zankari *et al*., 2012), CARD (McArthur *et al*., 2013), and ARG-ANNOT (Gupta *et al*., 2014), are utilized. The Center for Genomic Epidemiology (www.genomicepidemiology.org) developed ResFinder, a well-known in silico tool for bacterial resistome analysis. This study represents the first comprehensive report from Pakistan involving the analysis of forty-one 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 Pakistani *S. aureus* strains.

MATERIALS AND METHODS

Data Retrieval and Sample Selection

The Biosample database of NCBI was used to extract relevant accession numbers (https://www.ncbi.nlm.nih.gov/biosample) for Staphylococcus aureus entries.

Next Generation Sequencing Data Acquisition

The Biosample accession numbers were then used to assess Whole Genome data in FASTA format via NCBI Genomes resource(https://www.ncbi.nlm.nih.gov/datasets/genome/).

Whole genome sequences (WGS) of Staphylococcus aureus were systematically retrieved from the NCBI database using predefined inclusion criteria: (i) isolates derived from clinical samples of the Pakistani population, and (ii) availability of complete or high-quality draft genome assemblies with adequate annotation and metadata. Genomes lacking relevant metadata or originating from non-clinical or non-Pakistani sources were excluded. At the time of data collection, forty-one genomes fulfilled these criteria and were included, representing all publicly available and eligible datasets for the target population.

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 parameters including a minimum identity threshold of 80% and minimum coverage of 60% (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 databases (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 products of the most prevalent AMR genes was carried out using the Protein Databank (PDB) (https://www.rcsb.org/) and the PDBsum databases (https://www.ebi.ac.uk/thorntonsrv/databases/pdbsum/) (Burley *et al*., 2022).

Statistical Analysis

Descriptive statistical analysis was performed to evaluate antimicrobial resistance (AMR) gene profiles identified in the study genomes. Frequency and percentage were calculated for each AMR gene and for the distribution of resistance determinants across isolates. The data were further summarized according to antibiotic resistance classes based on the presence/absence of corresponding genes.

RESULTS AND DISCUSSION

The emphasis of the present investigation is to detect antimicrobial resistance genes within the whole genomes of *S.aureus* submitted to NCBI (patient isolates from the 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 forty-one *Staphylococcus aureus* strain sequences reported in various clinical samples of the Pakistani population using the ResFinder 4.6.0 tool revealed the presence of resistance genes associated with ten 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 studies support the findings of the 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.

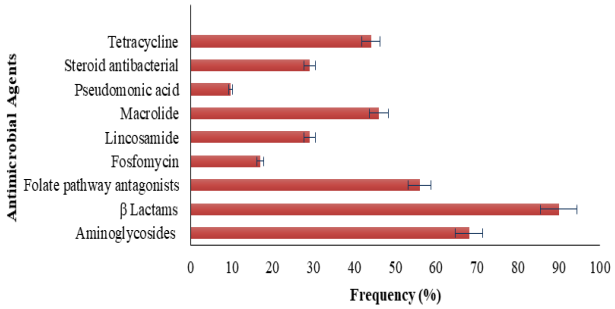

Figure 1. Identification of AMR genes using ResFinder 4.6.0 from WGS data of 41 *S. aureus* strains retrieved from the NCBI database

Genomic Detection of AMR Determinants

A total of eighteen antibiotic resistance genes were detected in thirty-eight whole genome 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 characterized for fusidic acid resistance. One gene dfrG (n=23; 56 %) associated with trimethoprim (a folate pathway antagonist) resistance was detected. mupA gene (n=1; 2 %) was observed for pseudomonic acid resistance and no gene was detected for quinolone resistance (Figure 2).

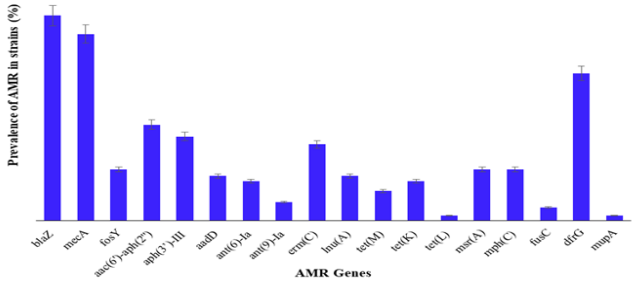

Figure 2. Prevalence of eighteen antimicrobial resistant genes in genomes of *S. aureus*

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	*aac(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 (Phosphoni c acid derivative) resistance	Cell wall synthesis inhibitor
mup A	1	2	Mupirocin (Pseudomoni c acid)	Protein Synthesis inhibitor

Among eighteen AMR genes identified in forty-one genomes of *S. aureus*, total thirteen 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 forty-one *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 thirty-two genomes (~78 %), followed by ***mecA*****,** found in twenty-nine genomes (~71 %). Akpaka *et al* (2016) 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, 31 % prevalence was 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).

An important 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). In current study, *BlaZ* and *mecA* genes are prevalent in various *Staphylococcus aureus* strains. High prevalence of *blaZ* and *mecA* is consistent with global datasets where these are the dominant β-lactam resistance determinants in both chromosomes and plasmids (Silva-de-Jesus *et al*., 2025; Snoussi *et al*., 2023; Namoune *et al*., 2023: Pennone *et al*., 2022) Frequent co-occurrence of genes to aminoglycosides, macrolides, tetracyclines, folate antagonists, fusidic acid and other mirrors multidrug profiles seen across clinical and food associated isolates (Alkuraythi *et al*., 2024; Ullah *et al*., 2021; Wang *et al*., 2021). These patterns likely result from prolonged antibiotic selection pressure and the dissemination of resistance genes through mobile genetic elements. From a clinical perspective, the dominance of β-lactam resistance genes, along with additional MDR determinants, suggests reduced efficacy of commonly used first-line antibiotics and highlights challenges in empirical treatment strategies. The presence of resistance genes against alternative and topical agents further complicates therapeutic and decolonization approaches (Ullah *et al*., 2022; Batool *et al*., 2021) Collectively, these findings underscore the importance of integrating whole-genome sequencing with routine antimicrobial susceptibility testing, strengthening antibiotic stewardship programs to minimize selective pressure, and maintaining continuous genomic surveillance to monitor emerging resistant lineages and inform effective infection control strategies (Shelenkov *et al*., 2025).

The resistance mechanisms associated with the identified AMR genes in various *Staphylococcus aureus* strains, as analyzed by 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 so their corresponding protein products were selected for three-dimensional structural analysis. The Protein 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.
	tet(K)	membrane-bound efflux pump protein	Tetracycline efflux transporter activity	Tetracycline	Antibiotic efflux by bacteria to lower intracellular drug levels.
	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 zinc binding 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
	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).

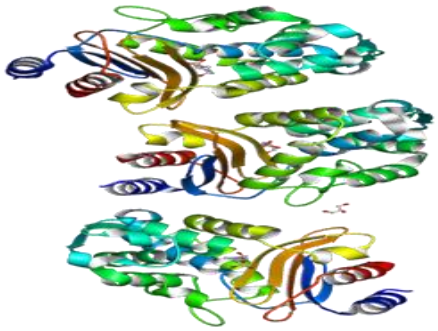

Figure 3. Three-dimensional structure of β-lactamase of Staphylococcus aureus (PBD ID: 6WGR)

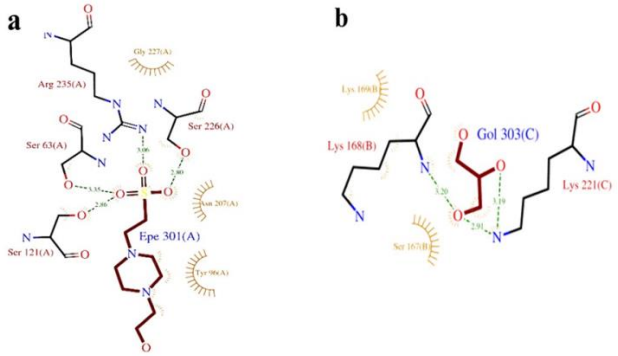

Figure 4. A LIGPLOT (a) Interactions of ligand EPE with amino acid residues (Arg, Ser, Gly, Asn and Tyr) of β-lactamase; (b) Interactions of ligand glycerol with amino acid residues (Lys and Ser) of β-lactamase

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).The three-dimensional structural features of β-lactamase and PBP2a are consistent with structural studies showing altered active sites and target protein that prevent β-lactam binding providing a mechanistic basis for the high-level resistance observed *in silico* (Alexander *et al*., 2022 Alkuraythi, *et al*., 2005).

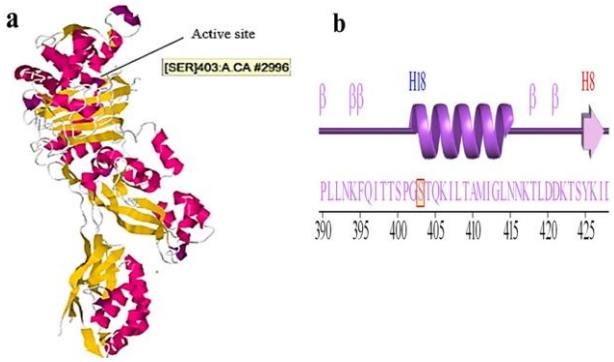

Figure 5. (a) Crystalline structure of PBP2a (b) Active site highlighted

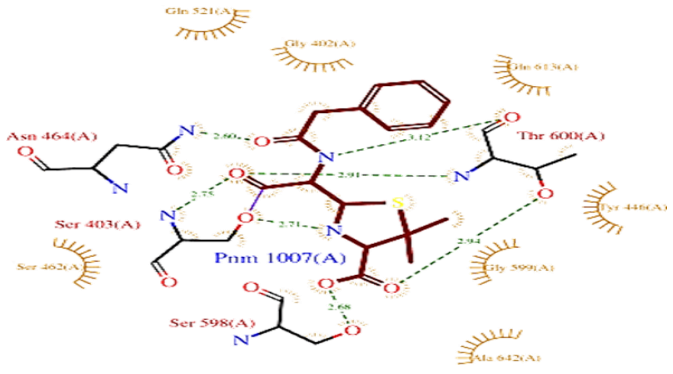

Figure 6. Interactions of ligand PNM with amino acid residues of PBP2a (A LIGPLOT)

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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