

Research Article

Comprehensive Characterization of Efflux Pump Genes, Biofilm Formation, and Virulence Determinants in *Staphylococcus aureus*

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A B S T R A C T

Background: *Staphylococcus aureus* is associated with high mortality rates in adults, particularly among immunocompromised individuals. However, only few predictive reports exist for this specific population. The emergence of multidrug-resistant *S. aureus* strains poses a significant clinical challenge. Activity of efflux pump and biofilm formation are considered to be playing crucial roles in driving resistance against antibiotics and limiting therapeutic efficacy.

Objective: The study aimed to investigate the prevalence of *S. aureus* among patients and to explain the mechanisms of resistance in isolates, focusing particularly on biofilm formation and efflux pump genes (*icaA*, *norB*, and *norC*). Additionally, molecular docking analysis was performed to explicate the molecular interactions underlying efflux- and biofilm-mediated resistance.

Methods: A total of 24 samples were collected from patients, of which ten were identified as *S. aureus* and confirmed via biochemical assays and PCR amplification of species-specific genes. Antimicrobial susceptibility testing was performed using Kirby–Bauer disk diffusion method. PCR was used to determine the presence of *icaA*, *norB*, and *norC* genes. Molecular docking technique was used to evaluate the binding interactions of ciprofloxacin, methicillin, carbonyl cyanide m-chlorophenyl hydrazone (CCCP), and reserpine.

Results: The *icaA*, *norB*, and *norC* genes were found in 100%, 50%, and 60% of the isolates, respectively. All the isolates were resistant to methicillin, while ciprofloxacin resistance was detected ABC (ATP-Binding Cassette) in four isolates. Reserpine exhibited the strongest binding affinity.

Conclusion: These findings underscore the importance of integrating molecular surveillance with in silico analyses while developing strategies for targeted therapeutic interventions.

Keywords: Antimicrobial Resistance, Biofilm, Efflux Pump, *icaA*, *norB*, *Staphylococcus aureus*

Introduction

S. aureus is considered harmful because it possesses three virulence factors - protein A, capsular polysaccharides, and ability to produce toxins such as Panton-Valentine Leucocidin, and toxic shock syndrome type 1 toxin (TSST-1). These pathogenic strains also form biofilms as an additional defence mechanism to protect against various medications and harmful chemicals, and to develop resistance to antibiotics as a means of survival.¹ An important mechanism that has evolved and led to the emergence of bacteria capable of pushing harmful substances out of the cell is known as efflux pumps. These pumps have been linked to development of multiple drug resistance (MDR).² Nearly all bacterial species contain efflux pumps, and their genes are present on chromosomes or plasmids. The efflux pumps belong to five families -ATP (ABC- binding cassette), RND, MFS, SMR and MATE).³ Additionally, methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a serious public health concern due to the global spread of several clones that carry the *Staphylococcal* Cassette Chromosome *mec* (SCCmec) gene.

S. aureus stands out due to its ability to develop resistance to multiple antibiotics. The challenge is further compounded by the emergence of multidrug-resistant (MDR) strains, which are often driven by genetic mechanisms such as efflux pump activity and biofilm formation.⁴ The efflux pump genes (*norA*, *norB* and *norC*) along with biofilm-related genes (*icaA*) play a crucial role in bacterial persistence against treatment agents. Therefore, identifying these genes is important for understanding the resistance mechanisms in clinical isolates of *S. aureus*, with an ultimate goal to refine treatment strategies and infection control measures.⁵

Methods

A total of 24 clinical samples were collected from patients at Baghdad Medical Hospital between December 2024 and January 2025. Ten patients had infection with *S. aureus*. Bacteria were cultured on specific media and their identity confirmed using standard biochemical methods and PCR amplification of resistance genes. Antibiotic susceptibility of the bacteria to Methicillin (M) and Ciprofloxacin (Cip) was tested using disk diffusion method according to CLSI (2020) guidelines. Genomic DNA was extracted from the isolates using a commercial DNA extraction kit. PCR assays were conducted to detect *norB*, *norC*, and *icaA* genes using gene-specific primers. The amplified gene products were

then analysed by gel electrophoresis on a 1.5% agarose gel stained with ethidium bromide.

Morphological examination

The basic diagnostic tests for morphological characteristics of isolates' growth involved morphological examination on media agar, which examined the colour, form, edges, and texture of the colony.⁶ For microscopic examination, a single bacterial colony was transferred to a microscopic slide, fixed, and then stained with Gram's stain. The configuration and form of the cells were then noted.⁷

Bacterial isolates

A total of ten *S. aureus* isolates were collected from the specimens at Baghdad medical hospital. The isolates were first identified using morphological traits, and then confirmed using genetic methods.

Genotypic detection of isolates

In accordance with manufacturer's protocol, genomic DNA was extracted from the isolates using the Genomic DNA purification kit (Geneaid, Taiwan). The *16S rRNA* gene sequence of *S. aureus* was used to confirm the identity of isolates using PCR technique. The forward primer 5'- TCACCGTAGCATGCTGATCT -3' and reverse primer 5'- AGGTGGGGATGACGTCAAAT -3' were applied in this study to amplify a 195bp product.⁸

The amplification conditions were: Initiation denaturation 95°C (2 min) denaturation, annealing and elongation (94°C for 30 sec., 58°C for 30 sec. and 72°C for 30 sec., respectively), Final extension 72°C (for 1min), 35 cycling was applied in the program of PCR. Primers used for resistance genes are: *icaA* 188bp (F: ACACTTGCTGGCGCAGTCA, R: TCTGGAACCAACA TCCAACA)⁹, *norB* 236bp (F: TCGCCTTCAACACCAT CAAC,R: GGC GTAGGAGAT GATGGTCA)⁸, *norC* 441bp (F: GCG GGAGTGTGTTCTTCATC, R: CTGGAGGAAGGTGTTGAAGC).⁸

Super Mix for PCR with Gel electrophoresis

This method was performed on a final reaction volume of 25µl according to manufacturer's instructions. The master mix was allowed to thaw at room temperature (25°C). The PCR program was designed for this study as follows: 2×EasyTaq® PCR SuperMix12.5 ml), 1 Forward Primer (10 picom), Reverse Primer (10 picoml), (Template DNA3ml) with conditions including: 94 C for 30 sec. in denaturation, 62C for 30 sec. in annealing, 72 C for 1 min extension. Electrophoresis was performed at 100 volts for 30 min. The PCR products were then put in the ultraviolet trans illuminator for visualization.

In Silico ADMET Prediction

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the selected compounds -ciprofloxacin, methicillin, carbonyl cyanide m-chlorophenyl

hydrazone (CCCP), and reserpine - were evaluated using ICM-Pro software (MolSoft LLC, San Diego, CA, USA).

The chemical structures of all compounds were obtained from public chemical databases and imported into the ICM molecular modeling environment. Each structure was subjected to geometry optimization using the built-in ICM force field to obtain energetically favorable conformations prior to ADMET analysis.

Docking simulation

To study the binding mode and strength of interaction molecular docking is^{10,11} conducted. Molecular docking was performed using the MOE 2024 Dock module. The binding sites of NorB, NorC, and IcaA were identified based on predicted functional cavities using the Site Finder tool and literature-reported active-site residues. Dummy atoms were generated to define the docking pocket, and a rigid receptor–flexible ligand docking approach was applied.

Initial ligand placement was performed using the Triangle Matcher algorithm, followed by scoring with the London dG scoring function. The top-ranked poses were further refined using force field-based energy minimization, and final rescoring was conducted using the GBVI/WSA dG scoring function. For each ligand–protein complex, multiple docking poses were generated, and the best-scoring conformation was selected for interaction analysis.

Results

Phenotypic identification

Phenotypic identification of the isolates was done by culturing these on selective media (mannitol salt agar) beside culturing on blood agar. Then, the definitive presence of *S. aureus* was confirmed using biochemical tests.

Genotypic identification of bacterial isolates DNA extraction

DNA Extraction and interpretation the results via gel electrophoresis, (Agarose 1%, at 100 V for 60 min.) (Figure 1).

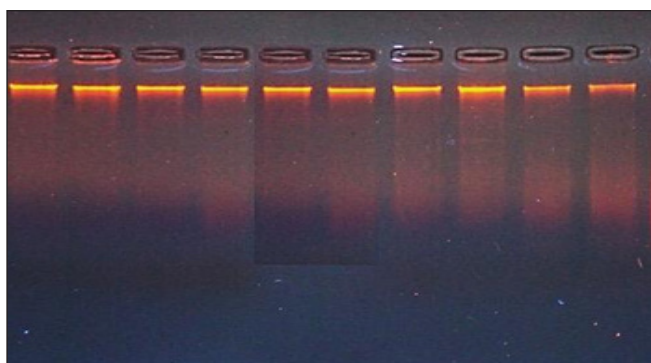


Figure 1. Gel electrophoresis for DNA Extraction

Antibiotic Sensitivity test

Antibiotic sensitivity was tested based on disc diffusion method as listed in CLSI 2022. The results indicated different susceptibility to M and Cip antibiotics. Four of the ten isolates were resistant to Cip (40%) while all the isolates (100%) were resistant to methicillin (Figure 2, Table 1). These results conform with another Iraqi study.

Table 1. Pattern of antibiotic resistance.

Antibiotic	No. of resistances	%
Cip	4	40
M	10	100

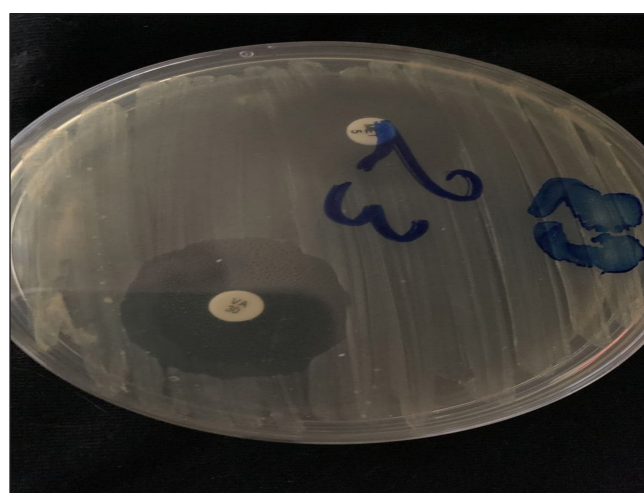


Figure 2. Sensitivity test

Genotypic identification using PCR technique was performed to detect *16s rRNA* and efflux genes in this study. The results, based on amplification of *16s RNA* (195 bp) gene, showed that 100% isolates were *S. aureus*. Figure 3 illustrated shine lines as a positive band when measure the volume with DNA ladder (100pb) *16s RNA* gene is considered one of the basic criteria in the bacterial classification because it contains highly conserved regions.

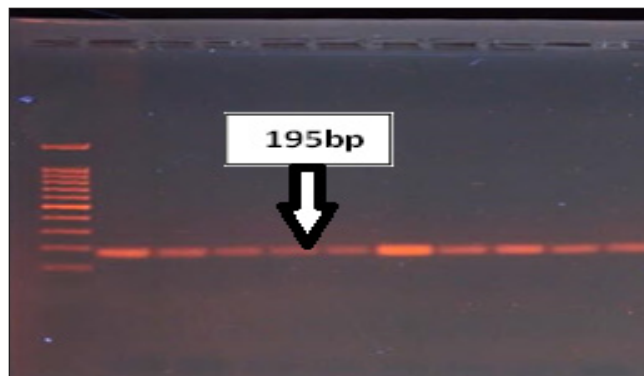


Figure 3. PCR product of *16S rRNA* primer (195bp) in gel electrophoresis

Molecular Identification of *S. aureus* with resistance genes

This investigation involved a comprehensive analysis of the ten clinical isolates. Polymerase Chain Reaction (PCR) was used to scrutinize the genetic landscape related with their epidemiological characteristics, with a particular emphasis on *icaA*, *norB*, and *norC* genes.

Molecular assessment was performed in all the isolates, which were resistant to methicillin, to amplify a specific segment of *icaA* gene (188bp) and products bands showed in Figure 4.

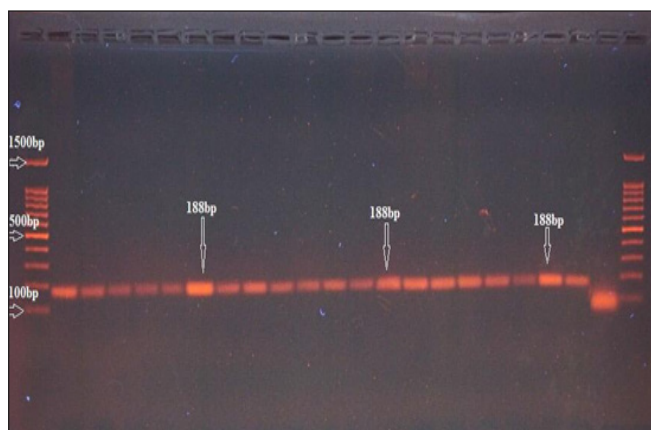


Figure 4. Gel electrophoresis for PCR product of *icaA* gene (188bp)

The study of prevalence of *nor* (B & C) genes in total resistance samples found *norB* in 5/10 (50%), and *norC* in 6/10 (60%)

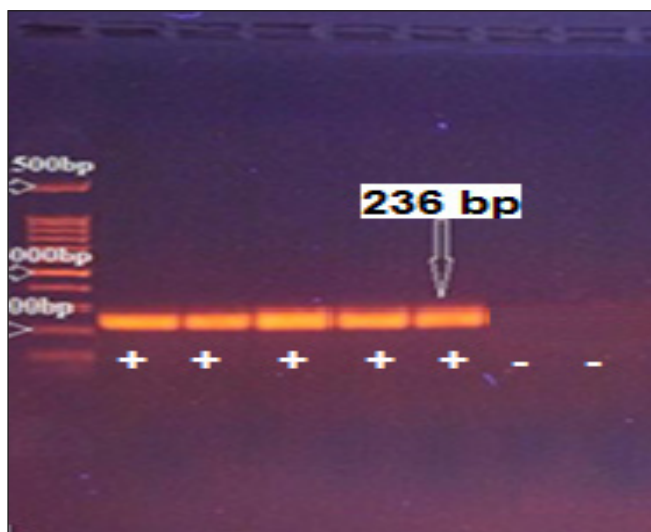


Figure 5. PCR product of *norB* primer (236bp) by gel electrophoresis. Lane L: DNA ladder (100-1500bp), Lanes (2-6) represented positive results, Lane (7,8) represented negative results

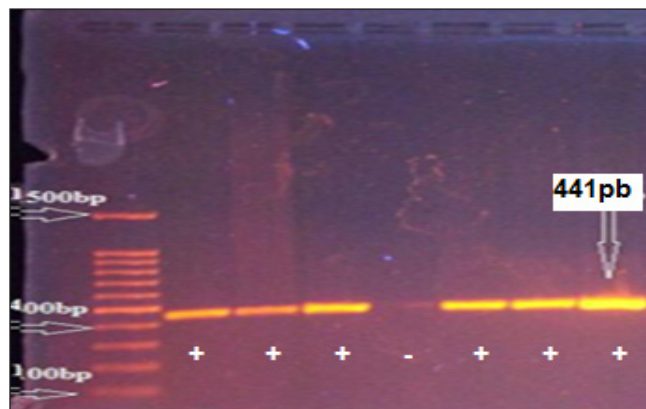


Figure 6. PCR product of *norC* primer (441bp) by gel electrophoresis. Lane 1: DNA ladder (100-1500bp), lanes (2,3,4,6,7,8) represented positive results, Lane (5) represented negative results

Binding site identification

In this work, potential binding pockets on *norB* gene surface were identified using ICM Pocket Finder. The results revealed varying geometric and physicochemical properties (Table 2).

Pocket 1 showed highest value in the volume of binding site ($625.3 \text{ \AA}^3$) and surface area ($615.9 \text{ \AA}^2$), which enables bulky ligands to be accommodated in the binding cavity (Figure 7). The observations also revealed that there is a moderate hydrophobicity (0.565) and a relatively high buriedness score (0.719) to indicate that the predicted binding site provides partially enclosed environment favorable for stable ligand binding. Its high nonsphericity (1.74) and radius ($5.30 \text{ \AA}$) further illustrate a complex and unique geometry.

Notably, Pockets 2 and 4 had volumes of $347.2 \text{ \AA}^3$ and $237.9 \text{ \AA}^3$, respectively. Pocket 4 showed high hydrophobicity (0.740) and buriedness (0.823), resulting in deeply embedded hydrophobic binding site. Pocket 3, on the other hand, had a lower buriedness (0.402) and a relatively smaller volume ($183.3 \text{ \AA}^3$). Despite its moderate size ($163.2 \text{ \AA}^3$), pocket 9 had the highest hydrophobicity (0.744) and buriedness (0.972) values. Smaller pockets (Pockets 5–10) exhibited reduced volumes and surface areas, with variable hydrophobicity and lower aromatic residue contributions. All identified pockets showed extremely low conservation and relative conservation values, indicating that these cavities are located in regions with low sequence conservation.

Binding pocket detection for the IcaA protein was also carried out using the ICM Pocket Finder module in MolSoft ICM Pro. The MolSoft ICM Pro was successful as it was able to identify one predominant binding pocket with defined geometric and physicochemical characteristics (Table 3). The detected pocket exhibited a volume of $305.9 \text{ \AA}^3$ and a surface area of $297.7 \text{ \AA}^2$. Of interest here is the cavity

size which is capable of accommodating small to medium-sized ligands. The more surprising feature from the table is hydrophobicity value (0.508) and buriedness score (0.723). It seems possible that these results are due to balanced hydrophobic–hydrophilic environment and that the pocket is partially enclosed within the protein structure.

The DLID score (–0.47) indicates moderate drug ability, suggesting that optimization may be necessary to improve ligand affinity. The binding site may be located in a non-conserved area of the lcaA protein because no evolutionary conservation was found for this pocket.

The protein databank's crystal structure of the NorC gene (Figure 9) provided us with the binding site.

Admet Profiling

In silico ADMET (absorption, distribution, metabolism, excretion, and toxicity)¹² was employed to evaluate the pharmacokinetic characteristics of ciprofloxacin, methicillin, carbonyl cyanide m-chlorophenyl hydrazone (CCCP), and reserpine¹² (Table 4). Except, reserpine, which exhibited a greater molecular weight of 608.27 Da, all compounds showed molecular weights within an acceptable range for small-molecule drugs. The range of lipophilicity values (molLogP) was 1.29 to 3.25 indicating moderate level of hydrophobicity that can facilitate membrane permeability.

Table 2. The predicted pockets for NorB gene by ICM pocket finder

I	Volume	Area	Hydrophobicity	Buriedness	Aromatic	DLID	Radius	Nonsphericity	Conservation	RelCons
1	625.3	615.915	0.565178	0.719234	0.05515	0.170086	5.30477	1.74172	7.40442e-316	7.40442e-313
2	347.162	330.336	0.518166	0.724048	0	-0.35211	4.35995	1.38288	7.40401e-316	7.40401e-313
4	237.886	228.455	0.739899	0.823232	0.011364	0.262932	3.84379	1.23047	7.40386e-316	7.40386e-313
3	183.348	271.655	0.397566	0.401623	0.040568	-2.36756	3.52421	1.74054	7.40394e-316	7.40394e-313
9	163.205	154.897	0.744444	0.972222	0.040741	0.582092	3.39012	1.07251	7.40376e-316	7.40376e-313
5	158.566	194.499	0.439655	0.617816	0	-1.52743	3.35769	1.37286	7.40382e-316	7.40382e-313
7	150.966	176.153	0.602649	0.789735	0.009934	-0.51634	3.30316	1.28476	7.40379e-316	7.40379e-313
10	129.365	147.236	0.451923	0.615385	0	-1.65943	3.13744	1.19029	7.40375e-316	7.40375e-313
6	123.65	169.569	0.420455	0.663961	0.001623	-1.57283	3.09054	1.41275	7.40379e-316	7.40379e-313

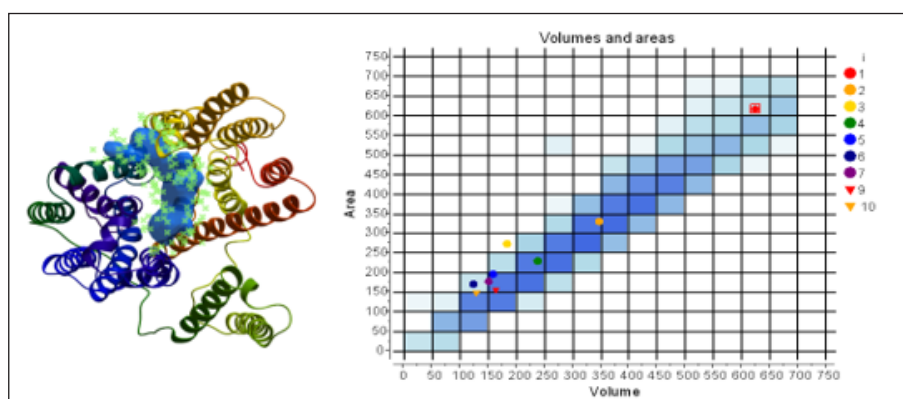


Figure 7. The geometry of top predicted pocket for NorB protein

Table 3.The predicted pockets for IcaA gene by ICM pocket finder

I	Volume	Area	Hydrophobicity	Buriedness	Aromatic	DLID	Radius	NonspHERicity	Conservation	RelCons
1	305.895	297.668	0.507692	0.723077	0.075	-0.47314	4.17985	1.35582	0	0

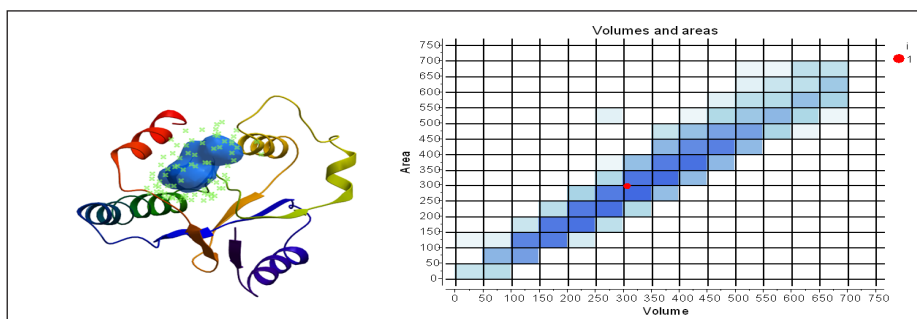


Figure 8.The geometry of top predicted pocket for IcaA protein

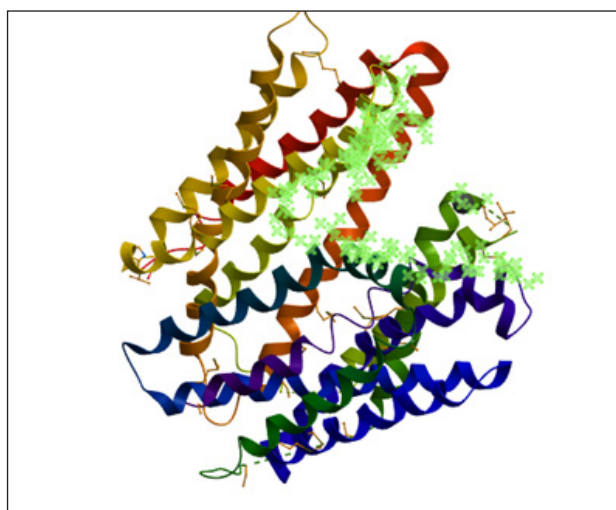


Figure 9.The binding site of NorC gene (green selection)

Table 4.ADMET features of the antibiotics

Name	Ciprofloxacin	Methicillin	reserpine	ccc
molWeight	331.133	380.104	608.273	204.02
molLogP	1.28891	2.41184	3.16113	3.25114
molLogS	-2.96006	-3.0679	-5.50929	-3.9446
molPSA	58.8011	85.2876	91.8555	55.4399
molArea	330.117	393.417	649.833	212.19
molHf	-89.9075	-189.544	-297.999	120.114
drugLikeness	0.84339	0.289588	0.999729	-2.02755
badGroups	Fluoroquinolone: allergies			Hydrazones
RotB	3	6	10	4
HBA	6	10	12	3
HBD	2	2	1	1

Caco2	-4.72154	-5.38389	-5.22744	-4.67118
HalfLife	3.32161	1.26027	4.71114	5.29214
HERG	0.468601	0.106808	0.64853	0.182113
LD50	597.463	435.703	245.72	547.715
PAINS	0.112684	0.0388055	0.490531	0.096554
PAMPA	-4.62842	-4.99967	-4.85461	-4.987
PGPinhib	0.00539333	0.164246	0.330263	0
PGPSubst	0.0183609	0.258018	0.356734	0
Tox_Names	Anil_di_alk_E;Carboxylic acids	Carboxylic acids;beta-lactam	Indole	Cyano_imine_B;N-N;hydrazone
Tox_Score	0.704124	1.5	0.505635	1.30747

Absorption and Permeability Properties

To assess absorption in the intestine, the Caco-2 and PAMPA parameters were used. All compounds displayed moderate-to-good absorption. Longer half-lives and higher permeability values displayed by CCCP and reserpine result in prolonged biological activity. Methicillin and ciprofloxacin have relatively low permeability. All the compounds were found to be within acceptable oral bioavailability parameters, with ciprofloxacin showing the lowest PSA, which may favor its absorption.

Drug-Likeness and Structural Alerts

Drug-likeness analysis revealed that reserpine (0.99) and ciprofloxacin (0.84) had the highest scores. Methicillin and CCCP showed lower and unfavorable drug-likeness values, particularly CCCP, which displayed a negative score. Structural alert analysis identified known toxicophoric groups, including hydrazone and cyano-imine groups in CCCP, beta-lactam moieties in methicillin, and fluoroquinolone-associated allergy alerts in ciprofloxacin.

Toxicity Assessment

Toxicity analysis of the tested compounds revealed clear differences in their toxicity scores (Table 4). Reserpine showed the lowest toxicity score (0.51) among the tested compounds suggesting a favorable safety profile. Ciprofloxacin showed moderate toxicity (0.70) while CCCP (1.31) and methicillin (1.50) demonstrated higher toxicity risks. No significant PAINS alerts were observed for ciprofloxacin or methicillin, whereas CCCP and reserpine showed limited alerts related to their functional groups. A combination of pharmacokinetic and toxicity parameters was used in to evaluate the overall ADMET suitability of the compounds which was ranked as follows: Reserpine > Ciprofloxacin > CCCP > Methicillin. The findings of the present study suggest that reserpine possesses the most favorable ADMET properties among the studied compounds.

Molecular docking

"Molecular docking was used to evaluate the molecular basis of resistance to methicillin, reserpine, carbonyl cyanide m-chlorophenyl hydrazone (CCCP), and ciprofloxacin, by targeting NorB, NorC, and IcaA." [13]. The tested compounds had varying binding affinities when docked against the NorB efflux pump. Reserpine showed the strongest interaction as shown in Figure 10D (-9.41 kcal per mole). This results in highly stable binding and a potent inhibitory potential towards NorB. Figure 10 shows schematically the interaction of Ciprofloxacin (-6.49 kcal/mol) presented in figure 10A and methicillin (-6.87 kcal/mol) presented in figure 10B showed moderate binding affinities. In contrast, CCCP displayed weak binding (-5.18 kcal/mol) (Figure 10C), indicating limited direct interaction with NorB and supporting its role as an indirect efflux inhibitor through proton motive force disruption rather than direct binding.

Docking analysis with the NorC efflux pump demonstrated overall moderate to weak interactions. Reserpine again showed the strongest binding affinity (-6.33 kcal/mol), suggesting relatively stable binding within the NorC cavity as displayed (11D). Methicillin (-5.21 kcal/mol) (Figure 11B) and ciprofloxacin (-5.00 kcal/mol) (Figure 11A) showed weaker interactions, consistent with limited inhibitory capacity and possible efflux susceptibility. CCCP presented minimal binding to NorC (-4.23 kcal/mol), further supporting its indirect mechanism of efflux inhibition (Figure 11D).

Docking against the IcaA biofilm-associated protein revealed differential ligand interactions. Reserpine demonstrated the strongest binding affinity (-7.64 kcal/mol), indicating a stable interaction within the active site and suggesting potential interference with polysaccharide intercellular adhesin synthesis (Figure 12D). Methicillin (-6.50 kcal/mol) (Figure 12B) and ciprofloxacin (-6.19 kcal/mol) (Figure 12A) showed moderate binding, while CCCP exhibited weak interaction (-4.65 kcal/mol), indicating minimal direct involvement with IcaA (Figure 12D).

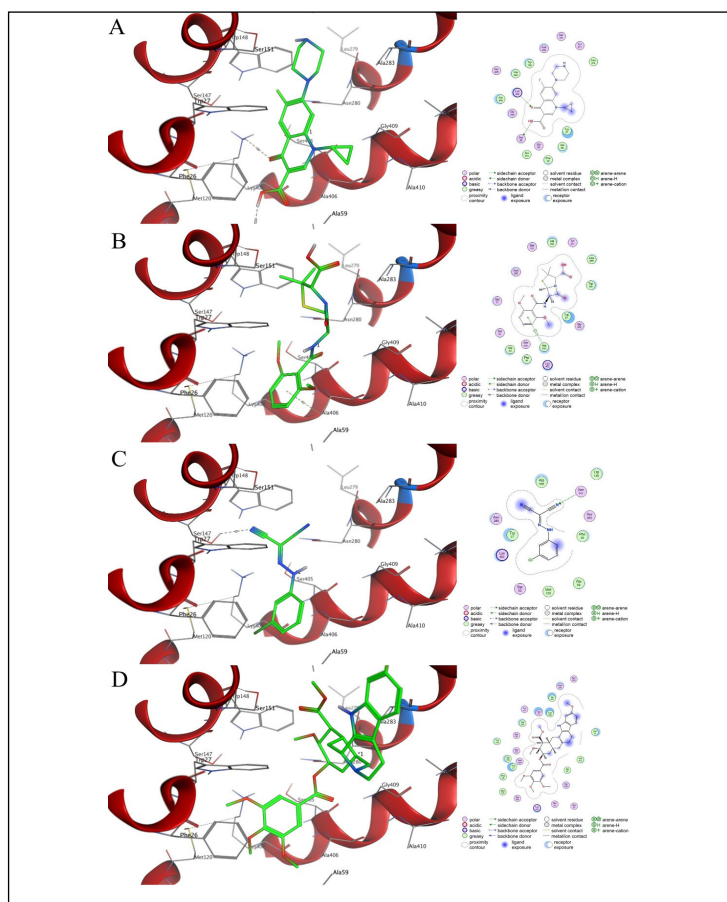


Figure 10. Molecular interaction profiles of the NorB with selected ligands. (A) Ciprofloxacin, (B) methicillin, (C) carbonyl cyanide m-chlorophenyl hydrazone (CCCP), and (D) reserpine. The docked complexes illustrate key binding interactions within the NorB active site

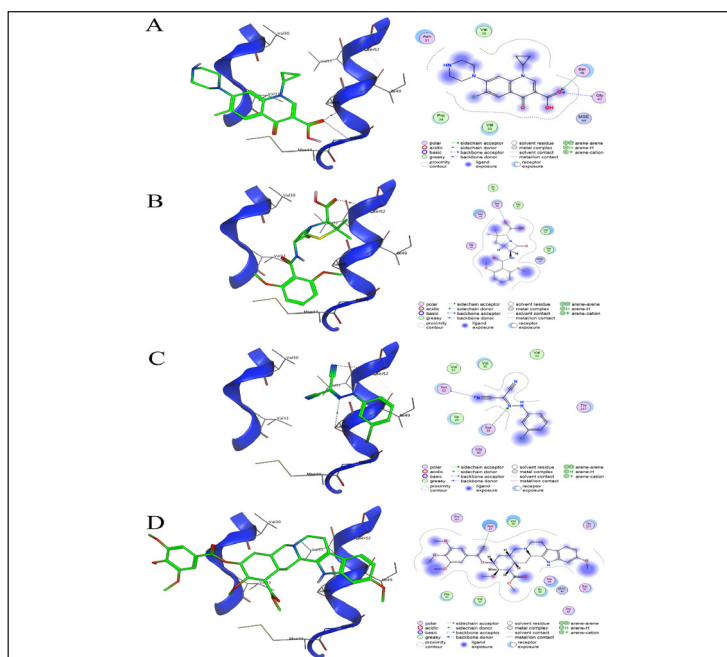


Figure 11. Molecular interaction profiles of the NorC with selected ligands. (A) Ciprofloxacin, (B) methicillin, (C) carbonyl cyanide m-chlorophenyl hydrazone (CCCP), and (D) reserpine. The docked complexes illustrate key binding interactions within the NorC active site

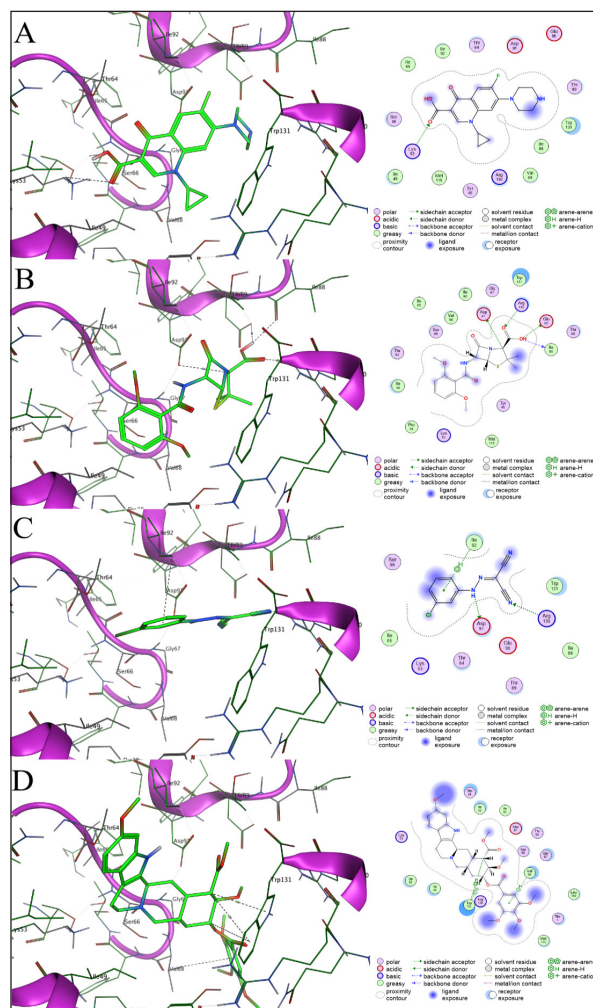


Figure 12. Molecular interaction profiles of the IcaA with selected ligands. (A) Ciprofloxacin, (B) methicillin, (C) carbonyl cyanide m-chlorophenyl hydrazone (CCCP), and (D) reserpine. The docked complexes illustrate key binding interactions within the NorB active site

Collectively, these molecular docking results support the experimental findings by demonstrating that antibiotics commonly used in clinical practice exhibit moderate to weak binding to efflux pumps and biofilm-associated proteins, which may contribute to reduced therapeutic efficacy. In contrast, the strong and consistent binding of reserpine to NorB, NorC, and IcaA highlights the central role of efflux pump activity and biofilm formation in the persistence and multidrug resistance of *S. aureus* isolates.

Discussion The findings highlight the significant role of efflux pump genes and biofilm-associated genes in the antimicrobial resistance of *S. aureus* isolated from patients. The co-existence of *norB*, *norC*, and *icaA* may enhance resistance and complicate treatment. These genetic factors contribute to both intrinsic resistance and adaptation to the hospital environment. The correlation between age, gender and MRSA infection was not statistically significant in the current study which is in agreement with findings reported in other studies (8,14). A meaningful correlation

reported by other occasional studies may be influenced by anatomical factors, hygiene practices, environmental experiences, stress and exposure to risk. these factors seeking the medical attention especially in the presence of underlying diseases of cancer patients, or after radiation the hospitalization duration and adherence to hygiene and personal care principles in contact with patients because that all immune system of them in shutdown conditions.^{15, 16}

The study focused on the isolation of *S. aureus* from patients at Baghdad Medical Hospital with the highest infection rates in the intensive care unit, neonatal intensive care unit, and burn units. These areas show higher prevalence in comparison to other departments, which may be due to longer hospitalization duration, occurrence of multiple illnesses concurrently, or excessive use of antibiotics.¹⁷ Given the unique conditions of the intensive care units, the study emphasizes on the need for adherence to basic health care principles and strategies. In addition, those receiving radiation or chemotherapy treatment in hospitals are also

at higher risk due to their depleted immunity.¹⁸ Compared to similar studies, this study notes that the risk factors for colonization by these bacteria have increased leading to the emergence of resistant bacteria. Strengthening and implementing infection control measures is essential in these department, especially after the alarming increase in *S. aureus* strains with reduced susceptibility to Vancomycin which has led to acquired resistance to other types of antibiotics and glycopeptides. This conforms with our study as the results of antimicrobial testing for multidrug resistance isolates, showed all *S. aureus* isolates were methicillin resistant, with some also being ciprofloxacin-resistant and having probability to develop further resistance.

A study carried out in Imam Ahvaz Hospital by Shamakhteh et al.¹⁹ using the agar method and PCR identified 76 (63.3%) of the 120 clinical isolates as MRSA (methicillin resistant *Staphylococcus aureus*) with presence of the *mecA* gene. This result does not align with our study. However, the finding of 34 isolates (44.7%) being resistant to Cip is in agreement with our study. On the other hand, Sahle & Merid (20) found five (8.8%) out of 57 *S. aureus* isolates as MRSA and three (5.3%) were resistant to Cip.

Multi mechanism of antibiotic resistance in the *S. aureus* and the most effective one as efflux pumps especially in the past two decade, against fluoroquinolones (ciprofloxacin, norfloxacin and sparflloxacin) has been described in clinical isolates such as *nor* of the efflux pump family which have very different in their expression between different strains.²¹ Current study found that some isolates harboured efflux pump genes *norB* (23.30%), and *norC* (90.27%) simultaneously, while others carried all three efflux genes or only a pair of them. These results unconfirmed with those by Raksha et al²² where only two (50%) of the four isolates of *S. aureus* carried this gene. Moreover, *norB* and *norC* genes were detected in 30% and 27% of ciprofloxacin resistant isolates respectively.²³ From other hands Shamkhi et al.,⁸ study had identify that *norB* gene was present in 54 (56.25%) of isolates, and *norC* gene was founding in 17 (17.84%) of whole isolates, the variation of the percentage between searches' results possibility that different from the variation of collection samples from patients.^{24,25}

Conclusions

The high prevalence of efflux pump and biofilm genes in *S. aureus* isolates from patients suggests the need for definitive molecular diagnostic tools and targeted antimicrobial therapies. Continuous surveillance and infection control practices are essential to manage and prevent the spread of resistant *S. aureus* strains in vulnerable populations. Our findings provide valuable insights into the prevalence and potential clinical impact of these genes in *S. aureus* isolates. Due to the clinical significance of these infections, it is imperative for healthcare professionals to be aware

and work towards improving identification and control of these infections in healthcare systems.

Ethical clearance

The study was conducted following the receipt of participant consent and ethical approval from the ethics committee of the environmental health sciences department, college of Energy and Environmental Sciences at the university of L-Karkh of sciences.

Acknowledgment

This work was done in environmental health sciences department, college of Energy and Environmental Sciences at the university of AL-Karkh of sciences.

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