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TYROSINE PROTEIN KINASE MODELING AND MOLECULAR CHARACTERIZATION OF SOME VIRULENCE FACTORS IN MULTIDRUG RESISTANT ACINETOBACTER BAUMANNII IN IRAQ

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ABSTRACT
Background: Hospital-acquired infections are often caused by multidrug-resistant Acinetobacter baumannii, an opportunistic bacterium. Resistance and multivirulence explain its clinical persistence.
Objective: A.baumannii clinical isolates in Iraq will be evaluated for antibiotic resistance, biofilm formation, efflux pump activity, elastase, and phospholipase at phenotypic and molecular levels.
Material and Methods: thirty A.baumannii isolates were characterized using standard biochemical assays and PCR primers specific to the species. The Vitek 2 compact system screened antibiotic susceptibility. The microtiter plate method was employed for biofilm growth and ethidium bromide for efflux pump activity screening. PCR was used to detect virulence and resistance genes ompA, adeB, LasB, plcN and PTK. Molecular modeling of the Tyrosine Protein Kinase (PTK) enzyme using homology modeling predicted its three-dimensional structure and assessed its therapeutic potential. **Results:** Thirty isolates of A.baumannii showed (100%) resistant to cefotaxime, 93%ciprofloxacin, (92%) ampicillin-sulbactam, (91%) amikacin, (89%) meropenem, (86%) cefepime, ceftazidime, imipenem and piperacillin-tazobactam, (83%) gentamicin, (72%) trimethoprim-sulfamethoxazole, and (3%) colistin. Biofilm analysis showed that 29 (96%) isolates produced biofilm to varying degrees (weak, moderate, strong), and qualitative and quantitative results were similar. Phenotypically, 22 (73.3%) isolates had efflux pump activity. PCR demonstrated significant incidence of virulence genes ompA 29 (96.6%), adeB 27 (90%), LasB 17 (56%), plcN 20 (66.6%) and PTK 30(100%). Molecular modeling showed that PTK has a membrane domain and a spherical extracellular domain with helices and folded sheets, similar to the Wzc protein in E. coli, highlighting its potential as a therapeutic target. **Conclusion:** Drug-resistant A. baumannii isolates with biofilm formation, efflux pumps and proteases are capable to survive and cause severe infection. PTK introduced as a well-defined hypothetical structure and a potential drug target for future therapeutic development**.**

KEYWORDS: Acinetobacter baumannii, Biofilm formation, efflux pump, PCR, resistance, PTK

INTRODUCTION

A.baumannii is a well-known opportunistic pathogen responsible for a wide range of nosocomial infections, including urinary tract infections ,respiratory tract infections, peritonitis , wound and soft tissue infections and meningitis, especially between patients with compromised immune systems [1]. The rapid increase in antimicrobial resistance rates in past years, scientific innovation efforts must concentrate on effective diagnostic and therapeutic strategies to address this challenge [2]. Carbapenem-resistant invasive A. baumannii (CRAB) form a major challenge to the efficacy of currently available antibiotics [3]. Recent studies indicate elevated resistance levels of A. baumannii to gentamicin, ciprofloxacin and amikacin [4]. Although carbapenems, such as imipenem and meropenem, were once considered highly effecacious therapeutic alternatives against A. baumannii, resistance rates approached 87%, leaving colistin as the sole available therapy option in most cases [5]. This disturbing trend reflects the increasing global incidence of β-lactam-resistant bacteria, which cause many hospital-acquired infections and have driven clinicians to use colistin as a last resort in the face of falling efficacy of traditional antibiotics [6]. The rising resistance of A. baumannii to antibiotics is a global health hazard, aggravated by its extensive dispersion [7]. The resistance pattern of this bacterium is linked to numerous endogenous and external mechanisms, including the synthesis of antibiotic-deactivating enzymes, mutation of drug binding sites, overexpression of efflux pump proteins and increased cell membrane permeability [8]. Numerous studies have addressed the relationship among virulence factors, antibiotic resistance patterns and phenotypic diversity in bacterial isolates, allowing a deeper understanding of the evolution of A. baumannii strains and their acquisition of characteristics that enhance their ability to spread and resist treatments

[9]. Efflux pumps contribute greatly to antibiotic resistance, either by directly expelling them outside the cell, or by indirectly increasing biofilm formation. The association between the substrate specificity of these pumps and their capacity to promote biofilm formation has emerged as a significant area of research [10]. Moreover, multidrug resistance (MDR)-associated efflux pumps play multiple roles in the transition from planktonic to biotic communities, expelling endogenous quorum-sensing signals, antibiotic agents and metabolic molecules, leading to modulation of both biofilm formation and the quorum sensing mechanism [11]. Certain pumps also modify the composition of the cell membrane, which is evident in the structural and functional characteristics of biofilms [12]. In addition, some bacterial infections can hemolysis host cells by generating phospholipases that target phospholipids in host cell membranes, leading to the creation of unstable chemicals that cause cell lysis and release of internal components [13]. The elastase enzyme LasB, a protease encoded by the lasB gene, is a virulence factor in A. baumannii. The presence of this gene in clinical isolates has been demonstrated to improve the cytotoxic capability and the ability of the bacteria to infiltrate human cells, contributing considerably to the severity of infection [14]. Polysaccharide capsules serve as critical virulence factors in A.baumannii, provide protection against environmental stressors and immunological responses, whereas alterations in their structure facilitate immune evasion and enhance pathogenicity [15]. Molecular modeling tools offer an accurate method for identifying protein structures linked to capsule formation and for selecting pharmacologically targetable locations [16]. The PTK gene, which governs the phosphorylation of proteins involved in capsule assembly and synthesis at the K locus, is a target; its inactivation results in impaired capsule formation, diminished virulence, and heightened immunological and antigenic susceptibility [17,18]. Its action is linked to improved biofilm stabilization, serving as a convergence of two principal virulence pathways in this bacterial species [19]. This study aimed to evaluate the phenotype of antibiotic resistance, biofilm formation, and efflux pump activity, as well as identifying some genes associated with virulence in clinical isolates of A.baumannii in Iraq, with the prediction of the three-dimensional structure of PTK as a potential drug target in this bacterium using computational biology techniques.

MATERIALS AND METHODS

Bacterial isolates

In the other study (data not shown) thirty clinical isolates of A. baumannii were obtained from patients at Imam Hussein Medical City in Karbala and other private clinics in Karbala and Najaf between August 2024 and October 2024. The isolates were identified using biochemical and molecular assays, using the Vitek 2 system and PCR amplification of the blaOXA-51 gene, respectively.

Antimicrobial Susceptibility Test (AST) using the VITEK 2 Compact System

Following the protocol laid out by BioMerieux, the VITEK device was employed to ascertain the susceptibility and resistance of A.baumannii isolates. The test was administered using a 64-hole antibiotic sensitivity test card, with multiple concentrations of each antibiotic being noted [20].

Isolates of A.baumannii were cultivated in nutrient broth medium and incubated at 37°C for 24 hours. Subsequent to incubation, bacterial colonies were transferred into a Khan tube holding 3 ml of normal saline with a conveyor loop to prepare a bacterial suspension. The suspension density was then assessed with the VITEK Densichek instrument, resulting in a turbidity range of 0.50–0.63 McFarland. Subsequently, 145 microliters of the bacterial suspension were introduced into the sensitivity test tube. The test strips were placed into the VITEK machine and the antimicrobial susceptibility findings were printed automatically**.**

Phenotypic Investigation of Some Virulence Factors of A.baumannii

Detection of Biofilm

Different phenotypic techniques, such as qualitative biofilm production assays (i.e., tube method) and quantitative biofilm production assays (i.e., microtiter plate assays), are used to detect biofilm formation A. baumannii [21][22].

Qualitative Tube Method for Biofilm Formation

The bacterial isolates were cultured in separate tubes with 10 ml of nutrent broth. The tubes incubated at 37 °C for 24 hours. After incubation, the bacterial culture was removed and the tubes were rinsed with physiological saline (NaCl 0.9%) and dry at 25°C. After drying, the tubes inner walls were stained with crystal violet (0.1%) for 5-10 minutes before being inverted and dried. The growth of a clear coloured layer on the tube inner walls was thought to indicate the formation of biofilms. The potential of A. baumannii isolates to generate biofilms has been classified into four categories: The density of the violet layer created inside the tubes and its base determines whether it is strong, medium, weak, or non-adhesive.

Quantitative Microtiter Plate Assays for Biofilm Formation

In this method bacterial isolates were inoculated into 5 mL of Brain Heart Infusion Broth and cultured at 37°C for 24 hours using this procedure. Following incubation, the density of bacterial cultures was compared to the McFarland 0.5 standard. Subsequently, 200 µL of the diluted culture were dispensed to each well of a 96 well plate, with four replicates per sample, whereas the negative control wells contained uncultured broth. Following a 24-hour incubation at 37°C, the contents were extracted from the wells and washed 3 times with physiological saline to eliminate single cells. The plates were allowed to dry at 60°C for 30 minutes, after which 200 µL of 0.5% crystal violet dye was introduced to each well and staining was conducted for 15 minutes at ambient temperature. Afterward, the excess dye was eliminated and the wells were rinsed with distilled water. To remove the dye, 200 µL of 33% glacial acetic acid was introduced to each well and the visual density was quantified at 630 nm using ELISA reader to evaluate biofilm-forming ability according to [18]. Biofilm production was classified based on the average optical density (OD) value and compared to the control optical density (ODc) using the following criteria: OD ≤ ODc: Absence of biofilm development (None). ODc < OD ≤ 2×ODc: weak biofilm production. 2×ODc < OD ≤ 4×ODc: Moderate biofilm production. OD > 4×ODc: strong biofilm formation.

Phenotypic detection of efflux pump

The identification of efflux pumps in 30 isolates of A.baumannii was conducted using the Ethidium Bromide agar cartwheel method (EtBr-CW), which employs agar medium infused with varying concentrations of ethidium bromide dye as a phenotypic indicator of efflux pump activity [23,24]. A.baumannii isolates were inoculated in 5 ml of brain heart infusion broth and cultured for 24 hours at 37°C, afterward diluted with physiological saline and compared to McFarland's standard solution at a concentration of 0.5%. Ethidium bromide dye was subsequently generated at varying concentrations (0, 0.5, 1, 1.5, 2, 2.5) mg/L by adding (0, 1, 2, 3, 4, 5) microliters of the dye to plates containing sterilized and partially cooled Muller-Hinton medium. The medium was meticulously combined, transferred into sterile plates, permitted to harden at ambient temperature and subsequently, the plates were radially sectioned on the outside. Sterile cotton swabs were utilized to transfer the diluted bacterial isolates, applying pressure against the inner wall of the dish to eliminate surplus. Then, each isolate was inoculated radially from the center of the dish towards its periphery for each concentration. The plates were incubated at 37°C for 24 hours and subsequently analyzed with a UV source to evaluate fluorescence intensity as a measure of efflux pump activity.

Molecular identification of genes encoding for virulence factors

The existence of ompA, adeB, LasB , plcN and PTK genes was determined using specific primers, as listed in Table 3. Genomic DNA was performed using a commercial extraction DNA kit (Addbio, Korea) and was subsequently subjected to PCR amplification using the primers detailed in Table 2. PCR reactions were applied in a thermal cycler (Edison, NJ, USA) using a commercially available PCR master mix (Microgen, South Korea).

Each PCR reaction carried out in a final volume of 25 µL, comprising 10 µL of Master Mix containing Taq DNA polymerase, 2 µL of forward primer and 2 µL of reverse primer (10 µmol concentration), 3 µL of DNA template and 8 µL of nuclease-free water. The reaction products were examined by electrophoresis on a 1.5% agarose gel at 50 V for 30 minutes. The bands that were generated were visualized under ultraviolet illumination using a DNA loading dye.

The PCR cycling conditions varied according to the target gene. The initial denaturation step was carried out at 94°C for 5 minutes for ompA, 94°C for 2 minutes for adeB, 95°C for 3 minutes for PTK and 95°C for 10 minutes for both LasB and plcN. This was followed by 33 amplification cycles consisting of denaturation at 94°C for 1 minute for ompA, 94°C for 30 seconds for adeB and 95°C for 30 seconds for PTK, LasB and plcN. Primer annealing was conducted at 58°C, 62°C and 55°C for 33 seconds, as specified in Table 1.

The extension step at 72°C was performed for 1 minute for ompA ,PTK genes , 2 minutes for adeB and 30 seconds for LasB and plcN genes. A final extension step was incorporated at 72°C for 10 minutes for ompA, LasB and plcN as well as 2 minutes for adeB and 5 minutes for PTK gene to ensure complete elongation of PCR products.

Table 1. List of primers for detection of virulence genes used in this study

Virulance factor	Target gene	Primer sequence	product Size	Annealing Tem.	references
Biofilm	ompA	F:CGCTTCTGCTGGTGCTGAAT R:CGTGCAGTAGCGTTAGGGTA	531	58	[25]
Efflux pump	adeB	F: AAAGACTTCAAAGAGCGG R: TCACGCATTGCTTCACCC	623	55	[26]
Phospholipase	plcN	F:GTTATCGCAACCAGCCCTAC R:AGGTCGAACACCTGGAACAC	466	55	[27]
Protease	LasB	F:GGAATGAACGAAGCGTTCTC	300	55	[27]

		R:GGTCCAGTAGTAGCGGTTGG			
Capsule	PTK	F: GGCTGAGCATCCTGCAATGCGT R: ACTTCTGGAGAAGGGCCTGCAA	597	62	[28]

Molecular modeling tyrosine protein kinases

To construct the three-dimensional structure of PTK in A.baumannii AYE 17879 (ABAYE3818), the enzyme's amino acid sequence from the Genome and Protein Database (KEGG: Kyoto Encyclopedia of Genes and Genomes) was used. The enzyme comprises 727 amino acids, categorizing it as a medium-sized protein with intricate regulatory and functional characteristics. The sequence was subsequently input into Swiss-Model to predict the enzyme's structure via homology modeling techniques (https://swissmodel.expasy.org/). The program used the framework of a homologous enzyme from Escherichia coli (E. coli PTK) as a reference template, with its three-dimensional structure elucidated via X-ray diffraction and recorded in the Protein Data Bank (PDB) (PDB ID: 3XYZ). This template was selected due to its significant amino acid sequence homology with the examined enzyme and the quality of the crystallographic data. After the construction of the model by Swiss-Model, the resultant structure was assessed using MolProbity to evaluate the quality of the three-dimensional model, including the examination of angles, bonds, and spatial placements.

RESULTS AND DISCUSSIONS

Antimicrobial Resistance Pattern

Thirty clinical isolates of A. baumannii from another study (data not shown) were subjected to susceptibility testing with the Vitek 2 compact system against 12 antibiotics, as illustrated in Figure (1).

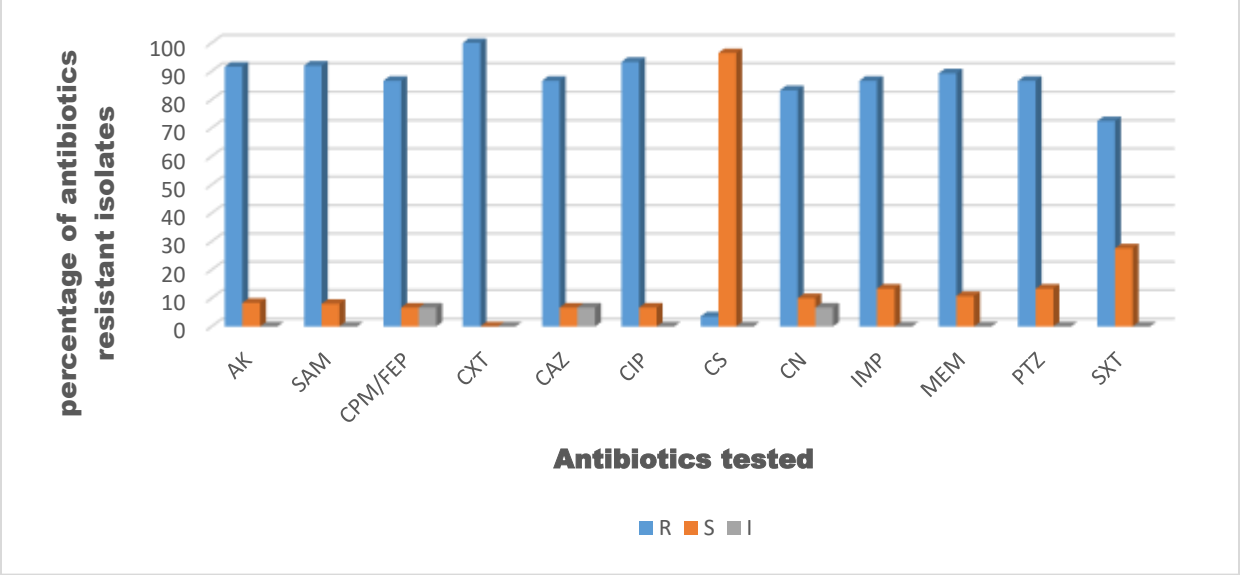

Figure (1). A. baumannii isolates resistance profile to several antibiotics,

indicating elevated resistance rates to the majority of drugs, with the exception of colistin (CS). Antibiotics evaluated include amikacin (AK), ampicillin/sulbactam (SAM), cefepime (FEP), cefotaxime (CTX), ceftazidime (CAZ), ciprofloxacin (CIP), colistin (CS), gentamicin (CN), imipenem (IMP), meropenem (MEM), piperacillin/tazobactam (PTZ) and trimethoprim/sulfamethoxazole (SXT).

Among 30 isolates of A.baumannii, It was shown that the highest resistance 30\30 (100%) toward cefotaxime, followed by 28\30 (93%) resistance toward ciprofloxacin and 23\25 (92%) resistance toward ampicillin-sulbactam, subsequently 22\24 (91%) toward amikacin and after that 25\28 (89%) toward meropenem. With respect to cefepime, ceftazidime, imipenem and piperacillin-tazobactam 26\30 (86%) were resistant, whereas 25\30 (83%) of gentamicin were resistant. Furthermore, A.baumannii isolates showed 21\29 (72%) resistance toward trimethoprim-sulfamethoxazole and 1/28 (3%) toward colistin.

Biofilm Production

Investigation of biofilm according to the microtiter plate method revealed that 1(3.3%) of A. baumannii isolated in this study were strong biofilm producers (SBF), but 4(13.3%) of the isolates were moderate producers (MBF), followed by 24(80%) weak biofilm producers (WBF) and one isolate non-biofilm producer as shown in figure (2).

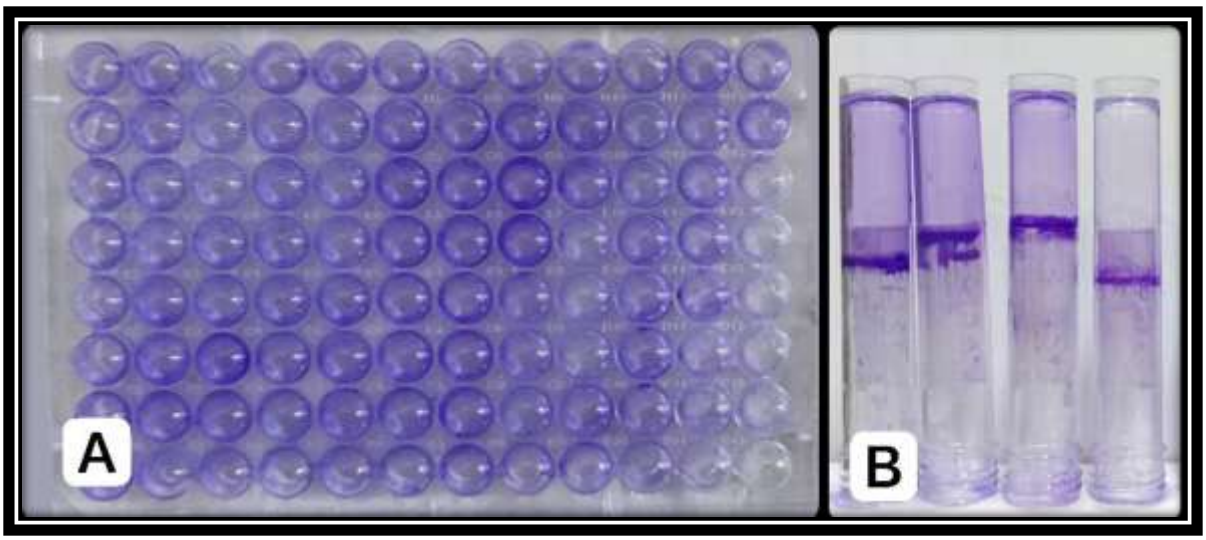

Figure (2): Comparative assessment of biofilm forming ability in A.baumannii using quantitative and qualitative methods. (A) Quantitative detection (microtiter plate assay) of biofilm production based on crystal violet staining intensity. (B) Tube method indicating qualitative evaluation of biofilm formation by visible adherence and staining on the inner wall of the tubes.

Figure (3) of the study demonstrated that highly efficient biofilm-producing bacterial isolates (SBF) exhibited high resistance to the majority of the tested antibiotics, with resistance rates exceeded 90% of the tested antibiotics, including gentamicin (CN), imipenem (IMP), ceftazidime (CAZ) and amikacin (AK). The lowest resistance rate was observed against colistin (CS), indicating its sustained efficacy as a last-resort antibiotic against multiresistant isolates. Conversely, isolates exhibiting moderate (MPF) and weak (WBF) biofilm-forming capabilities demonstrated reduced levels of resistance.

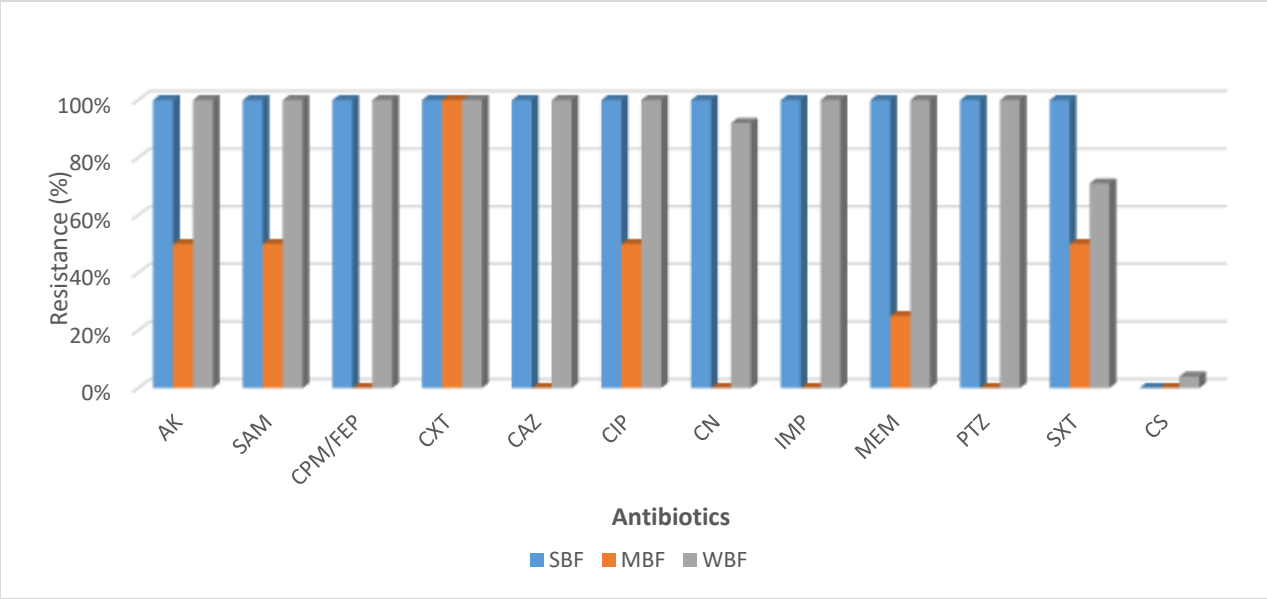

Figure (3): Distribution of A.baumannii isolates categorized by biofilm-forming ability — strong (SBF), moderate (MBF) and weak (WBF) — in relation to various antibiotic resistance profiles.

Efflux pump activity

The identification of efflux pumps in 30 bacterial isolates of A. baumannii was conducted using the EtBr-CW method for phenotypic detection, revealing that 22 (73.3%) of the isolates tested positive for this characteristic. This was determined by the minimal concentration at which the isolates exhibited no fluorescence under the UV source, as illustrated in Figure (4).

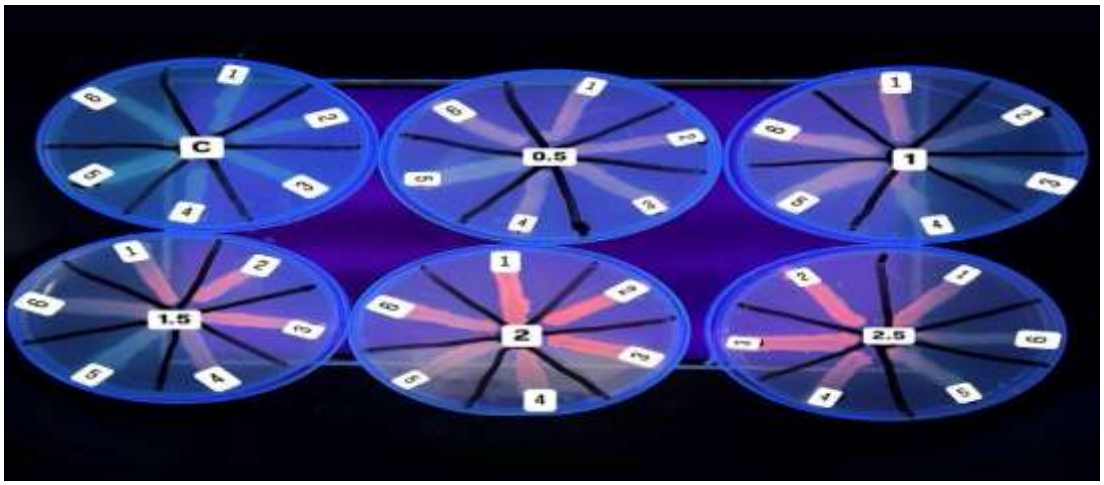

Figure (4): The evaluation of efflux pump activity in bacterial isolates is done using the Ethidium bromide (EtBr) cartwheel method. Different sectors of plates are infected with different bacterial strains, and the amounts of EtBr range from 0.5 to 2.5 µg/mL. Low efflux activity is shown by weak fluorescence under UV light, but bright fluorescence suggests active efflux pumps.

Identification of Virulence associated Genes using molecular techniques

In this study, PCR was employed to detect virulence-related genes in A. baumannii isolates. The ompA gene (531 bp) was identified in 29 (96.6%) isolates, indicating biofilm-forming ability. The adeB gene (623 bp), associated with efflux-mediated resistance, was detected in 27 (90%) isolates. The LasB gene (300 bp), encoding a zinc-dependent elastase, was present in 17 (56.6%) isolates, whereas the plcN gene (466 bp), linked to phospholipase activity, was amplified in 20 (66.6%), and the PTK gene (597bp) which responsible for capsular polysaccharide, was detected in 30(100%) isolates, as illustrated in Figures (5–9).

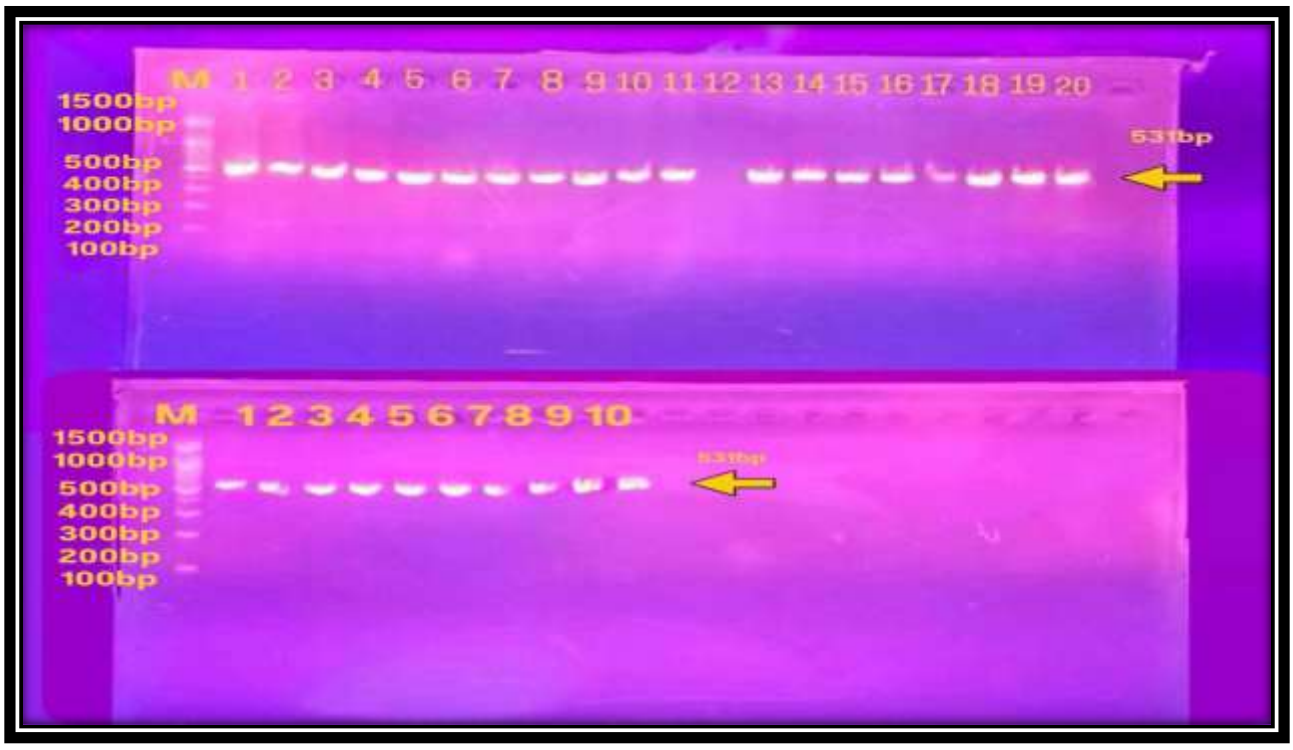

Figure (5): PCR amplification of the ompA gene (531 bp) in A.baumannii. PCR amplicons were electrophoresed on a 1.5% agarose gel at 70 V for1 hour. Lane M: DNA ladder (100–1500 base pairs).

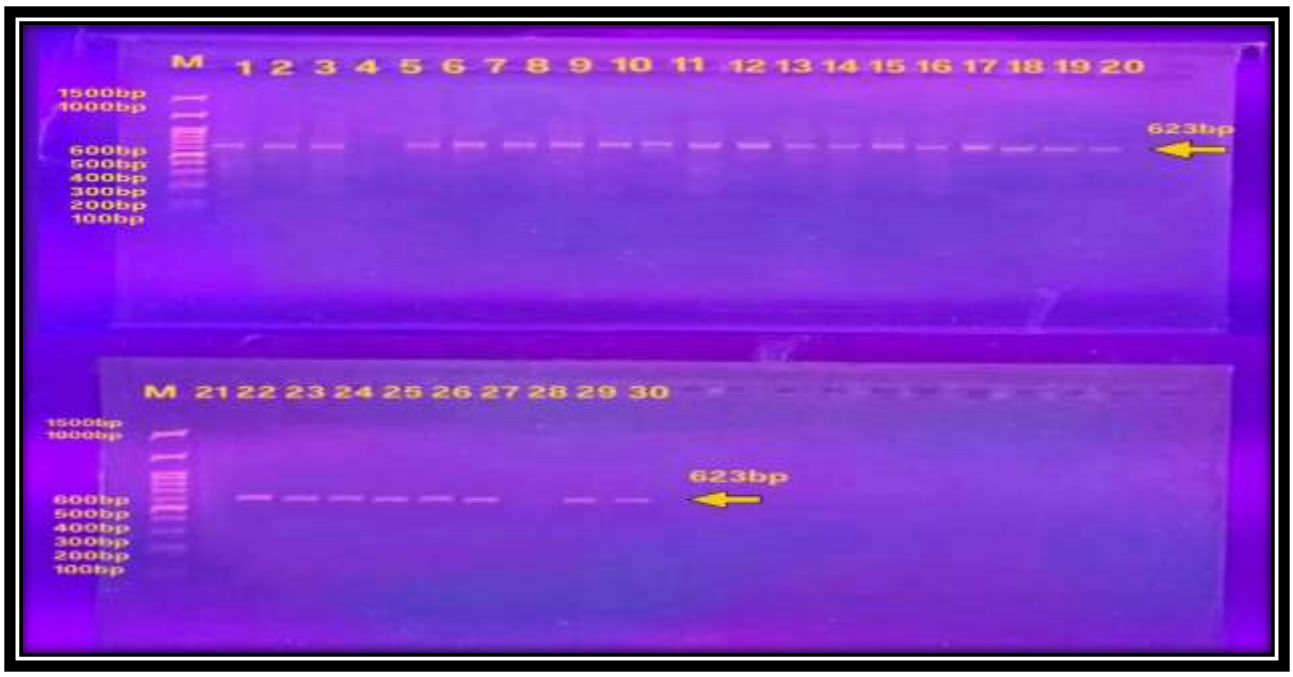

Figure (6): PCR amplification of the adeB gene (623 bp) in A.baumannii. PCR amplicons were electrophoresed on a 1.5% agarose gel at 70 V for1 hour. Lane M: DNA ladder (100–1500 base pairs).

Figure (7): PCR amplification of the LasB gene (300 bp) in A.baumannii. PCR amplicons were electrophoresed on a 1.5% agarose gel at 70 V for1 hour. Lane M: DNA ladder (100–1500 base pairs).

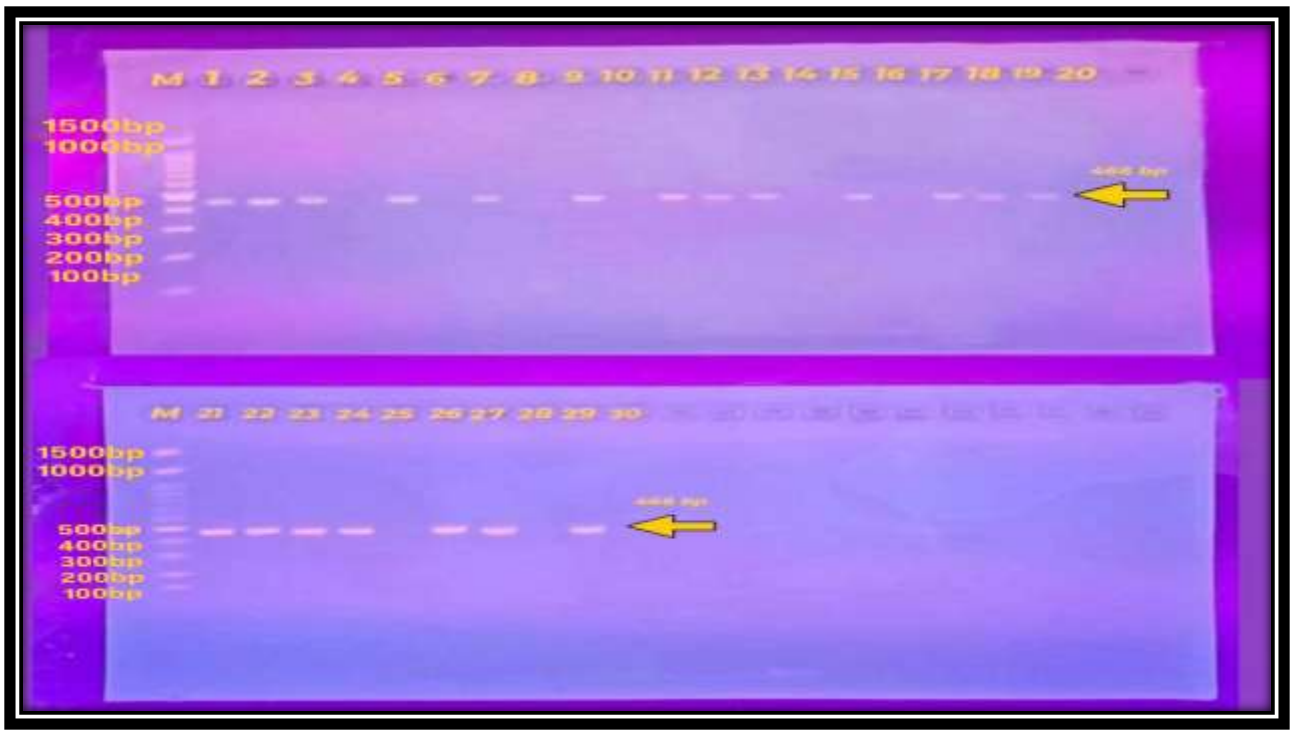

Figure (8): PCR amplification of the plcN gene (466 bp) in A.baumannii. PCR amplicons were electrophoresed on a 1.5% agarose gel at 70 V for1 hour. Lane M: DNA ladder (100–1500 base pairs).

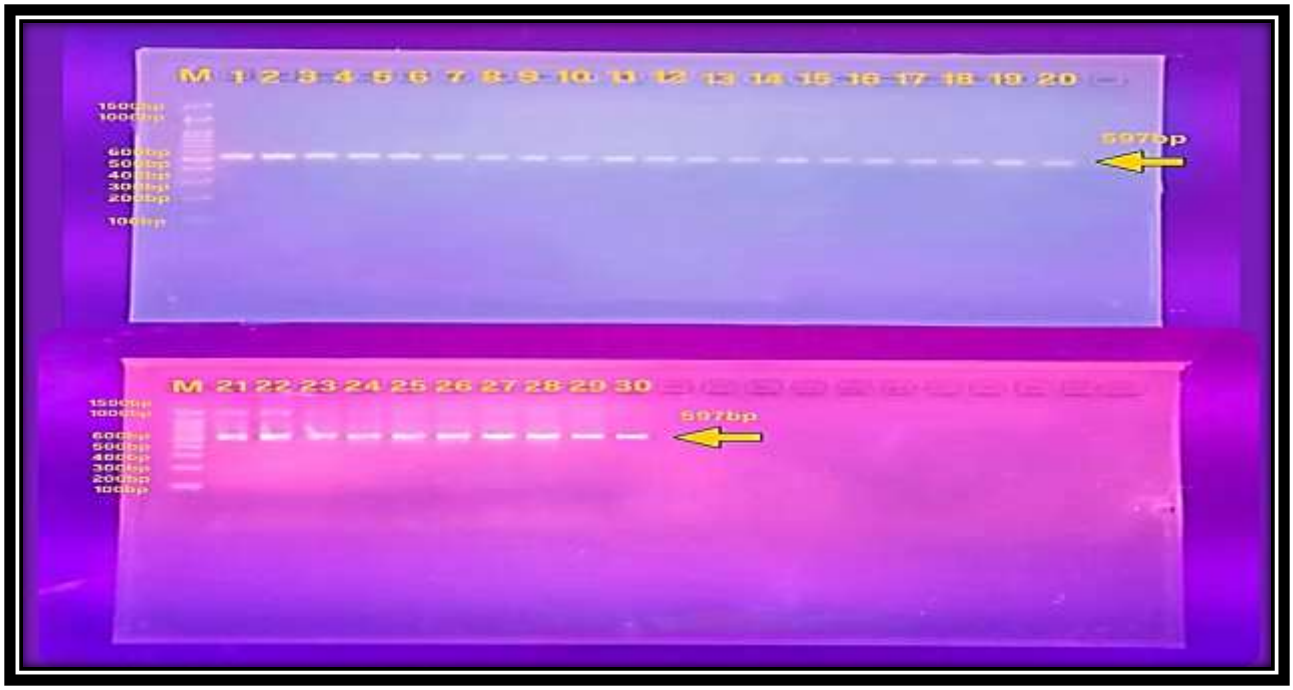

Figure (9): PCR amplification of the PTK gene (597 bp) in A. baumannii. PCR products were electrophoresed on a 1.5% agarose gel at 70 V for 60 minutes.

PTK gene modling

The three-dimensional structure of the studied protein was predicted using computational modeling techniques. The results indicated that the protein comprises of a periplasmic globular domain, multiple transmembrane helices and a cytoplasmic kinase domain including a tail abundant in tyrosine residues essential for autophosphorylation, as in Figure (10), signifying its classification within the membrane tyrosine kinase protein family, which is crucial for regulating intracellular phosphorylation reactions.

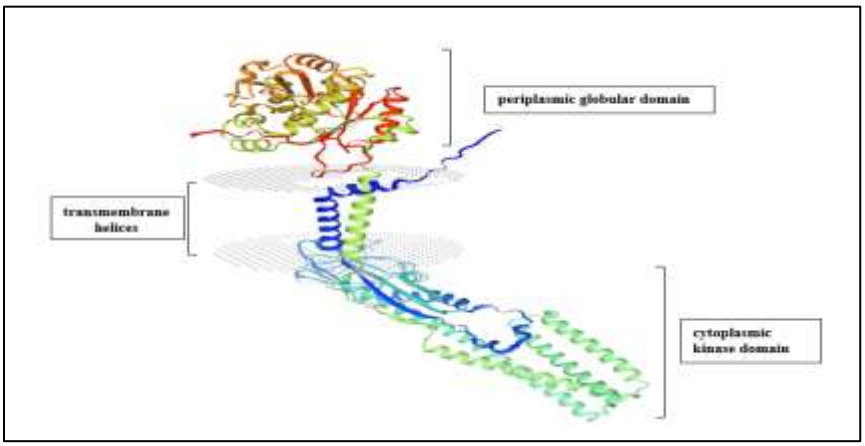

Figure (9): Three-dimensional structure of a model of a tyrosine protein kinase in A.baumannii AYE 17879 (ABAYE3818).

The structural model's quality was evaluated using MolProbity and the Ramachandran plot (Figure 10) , revealing that 96% of the amino acids residues situated within the allowed regions, whereas small percentage 0.55% in the disallowed regions. The model achieved a MolProbity Score of 1.02 and a Clash Score of 0.26, indicating the model's high accuracy. To ascertain the evolutionary and functional relationship, a BDP protein BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis was conducted, revealing that the examined protein exhibits the greatest similarity to the Wzc transmembrane tyrosine kinase protein in Escherichia coli, with 36% identity and an E-value of ≤ 1e-143. Furthermore, it demonstrated additional similarities to protein kinases from other pathogenic bacteria, including Staphylococcus aureus, Vibrio cholerae, and Burkholderia cepacia (Figure 11).

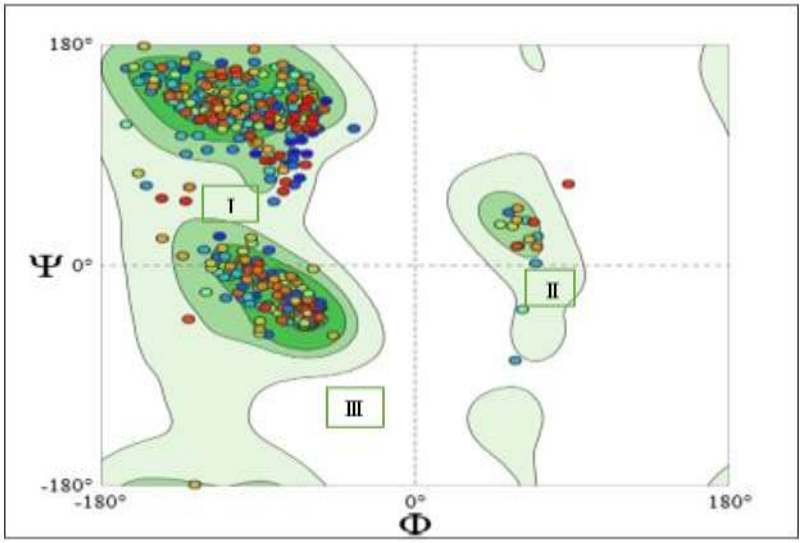

Figure (10): Rama-Khandran diagram of the tyrosine protein kinase autophosphorylates model in A.baumannii, obtained from the Swiss model program, all amino acids are located in the preferred or allowed regions (regions I and II), whereas region III (non-preferred or non-allowed) is devoid of amino acids.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Chain A, Putative transmembrane protein Wzc [Escherichia coli]	Escherichia coli	440	440	99%	5e-144	35.89%	727	7NHR_A
Chain A, Putative transmembrane protein Wzc [Escherichia coli]	Escherichia coli	437	437	99%	5e-143	35.98%	727	9EXO_A
Chain A, Putative transmembrane protein Wzc [Escherichia coli]	Escherichia coli	436	436	99%	9e-143	35.93%	727	9I2Q_A
Chain A, Tyrosine-protein kinase [Escherichia coli]	Escherichia coli	436	436	98%	2e-142	36.01%	727	7NI2_A
Chain A, Putative transmembrane protein Wzc [Escherichia coli]	Escherichia coli	426	426	96%	2e-138	35.85%	727	9EXQ_A
Chain A, Tyrosine-protein kinase wzc [Escherichia coli K-12]	Escherichia coli K-12	249	249	36%	2e-76	46.79%	286	3LA6_A
Chain A, Tyrosine-protein kinase etk [unidentified]	unidentified	232	232	34%	8e-70	44.40%	299	3CIO_A
Chain A, BceF [Burkholderia cepacia]	Burkholderia cepacia	228	228	37%	1e-68	43.12%	271	6Z0P_A
Chain A, Crystal structure of the chimerical protein CapA1B1 in complex with ADP-Mn [Staphylococcus aureus]	Staphylococcus aureus	137	137	35%	2e-35	33.07%	269	4JLV_A
Chain A, Membrane protein CapA1, Protein tyrosine kinase Protein tyrosine kinase [Staphylococcus aureus]	Staphylococcus aureus	135	135	35%	4e-35	33.07%	271	3BFV_A
Chain A, MEMBRANE PROTEIN CAPA1, PROTEIN TYROSINE KINASE [Staphylococcus aureus]	Staphylococcus aureus	133	133	35%	3e-34	32.68%	271	2VED_A
Chain A, C-terminal fragment of CapA, Protein tyrosine kinase [Staphylococcus aureus]	Staphylococcus aureus	129	129	36%	7e-33	31.54%	269	4JMF_A
Chain A, VpsD [Vibrio cholerae]	Vibrio cholerae	122	122	29%	1e-30	32.08%	235	6U1Q_A
Chain A, SpoOJ regulator (Soj) [Helicobacter pylori 26695]	Helicobacter pylori 26695	50.4	50.4	17%	5e-06	29.41%	276	6IUB_A
Chain A, SpoOJ regulator (Soj) [Helicobacter pylori 26695]	Helicobacter pylori 26695	49.7	49.7	17%	9e-06	28.99%	264	6JMJ_A
Chain A, ParA family protein [Mycococcus xanthus DK 1622]	Mycococcus xanthus D	46.6	46.6	16%	8e-05	29.01%	255	6RAY_A
Chain A, SITE-DETERMINING PROTEIN [Aquifex aeolicus]	Aquifex aeolicus	39.7	39.7	16%	0.014	26.56%	257	4V03_A

Figure 11: BLAST protein results illustrating the amino acid sequence similarity of the PTK in A.baumannii to Wzc tyrosine kinases in E. coli and related kinases from other bacterial pathogens.

DISCUSSION

A. baumannii is an important selective pathogen that increasingly endangers global public health. The infection has demonstrated resistance to most existing antibiotics and has been designated by the World Health Organisation (WHO), as a member of the most dangerous ESKAPE organisms “Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, and Enterobacter cloacae” a highly hazardous pathogen associated with persistent infections in healthcare settings [29, 30].

Our results showed A.baumannii isolates were resistant to the majority of the tested antibiotics. The resistance to beta-lactam antibiotics, particularly carbapenems, has emerged as a growing concern in the clinical setting, due to several acquired and overlapping defense mechanisms [31]. These results agree with a study in which the researchers indicated that this pattern of resistance is due to overuse of antibiotics [32]. This broad resistance is associated with several mechanisms, most notably: Production of beta-lactamase enzymes, inactivation of antibiotics, modification of their receptors, decreased permeability of the outer membrane, as well as hyperactivity of efflux pumps. Together, these mechanisms enhance the ability of bacteria to resist drug therapies and impede clinical response [33]

Our results revealed that the majority of A.baumannii isolates we examined were able to form biofilm phenotypically, which agree with previous studies indicating that biofilm formation in A. baumannii isolates is an important factor associated with antibiotic resistance and treatment [34, 35]. Other studies demonstrated that isolates exhibiting biofilm production displayed increased resistance to several antibiotics, indicating an association between the severity of biofilm production and resistance levels. This association can result from various variables, including the existence of resistance-associated genes, their regulation, enzyme synthesis, efflux pumps and biofilm development [36, 37].

Efflux pumps in A. baumannii are one of the major mechanisms of antibiotic resistance, especially in Gram negative organism. These systems contribute to the Extrusion of antibiotics by regulating the internal environment of the bacterial cell [38]. Studies revealed that the gene expression of these pumps is highly regulated, reflecting their importance in different resistance patterns, as antibiotic resistance via efflux pumps is distinguished from other mechanisms by its ability to expel a wide range of antibiotics and from several groups, making it a current challenge in the therapeutic strategies [39].

In this study, PCR was used to identify certain virulence- associated genes in A.baumannii isolates. we identified the OmpA gene in A. baumannii, which is one of the most prominent and common outer membrane proteins. OmpA plays a vital role in drug adhesion, control of immune response and biofilm development, and is associated with increased mortality in nosocomial pneumonia and bacteremia [40, 41]

The adeB gene is the main component of the adeABC efflux pumps system in A. baumannii. Several studies indicated a high prevalence of this gene in many clinical isolates [42, 26, 43, 44]. Efflux pumps are critically involved in many cellular processes such as nutrient regulation, internal pressure relief, secretion, pathogenicity and are important in their ability to reduce the effectiveness of antibiotics by expelling them out of the cell before they reach their biological targets [45]

The molecular investigation detected the LasB gene, which encodes a zinc-dependent elastase enzyme associated with the ability of A.baumannii to hydrolyze elastin in host tissues, thus contributing to tissue damage, immune evasion and promoting biofilm formation [46]. The existence of this gene may indicate Genetic material is transferred by genetic transformation, with mobile genetic elements like plasmids and transposons enhancing the efficiency and simplicity of this transfer [47]. Moreover, the LasB gene has been previously found in Pseudomonas aeruginosa and multiple investigations have shown that the elastase produced by this gene directly influences the pathogenicity of infections induced by these bacteria [48].

A plcN gene encoding a non-hemolytic phospholipase enzyme that contributes to the interaction of bacteria with host cell membranes by targeting phospholipids has been examined, reflecting its potential role as an important virulence factor in A. baumannii [49, 50]. This gene is particularly important in strains isolated from the respiratory tract, as some studies have shown the ability of the bacteria to metabolize phosphatidylcholine as the sole source of carbon and troponin, supporting the hypothesis of an association between this gene and the specific anatomical sites of clinical isolation [51]. In addition, the gene has been identified in A. baumannii isolates from wounds and burns, serving as a virulence factor linked to the degradation of cell membranes and subsequent tissue destruction [52].

PTK is a crucial enzyme in A.baumannii, largely contributing to capsule formation, an important virulence factor that protects the bacteria and enhances its survival capabilities inside the host environment. Research indicates that the disruption of the PTK gene results in the loss of the capsule and increased reactivity of the bacterium to human serum, hence diminishing its pathogenicity [17]. Recent genomic investigations have uncovered considerable variability in capsule production sites (KL locus) across several strains, highlighting the crucial role of this enzyme in influencing virulence and isolation diversity [53]. In addition, PTK lacks a homolog in human cells, providing it an attractive target for the development of antibacterial medicines that do not induce toxicity in humans [17]. Moreover, immunoinformatics research demonstrated that antigenic peptides may be derived from the PTK protein, presenting it as a viable target for future vaccine development [54].

Structural modeling indicated that the protein in question is part of the Bacterial Tyrosine Kinases (BY-kinases) family, comparative structural investigations revealed that this protein has a multi-unit membrane domain and a spherical extracellular domain, the same structural arrangement described in the Wzc protein of E.coli, this protein is crucial for regulating bacterial capsule formation; mutations in the Wzc gene result in capsule loss, diminishing the bacteria's resistance to human immune responses and impairing their infectious capability [16]. The Ramachandran plot reveals that 96% of residues are located within the allowed regions, and the low MolProbity score supports the model's high reliability. Models with a MolProbity score under 2 are dependable and appropriate for biological analysis [55].

CONCLUSION

PTK is a crucial pharmacological target for the advancement of future treatments against A.baumannii, because of its essential function in biofilm formation and the development of multidrug resistance. These bacteria acquire enhanced pathogenicity via their capsules, efflux pumps and proteases that facilitate biofilm development, in addition to phospholipases that assist in tissue damage. This enzyme represents a viable therapeutic target as a result of its necessity for bacterial critical processes.

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