Original Article

Molecular Detection of *tox A* Gene in Multidrug Resistance *Pseudomonas aeruginosa* Isolated from Burn Infections

Zahraa Hamza Merza[1,2], Rabab Omran[1]

[1]Department of Biology, College of Science, University of Babylon, [2]Department of Medical Laboratory Techniques, Al-Mustaqbal University, Hillah, Babylon, Iraq

Abstract

Background: Exotoxin A (ExoA) is the most widespread and toxic virulence agent among pathogenic *Pseudomonas aeruginosa* species that acquire adenosine diphosphate-ribosyltransferase activity belonging to the class of exotoxins secreted by pathogenic bacteria that cause human diseases. **Objectives:** The aim of this study is to investigate ExoA in multidrug resistance *P. aeruginosa* isolated from burn infections. **Materials and Methods:** About 89 *P. aeruginosa* were isolated from burned infections. The Kirby–Bauer disc diffusion method on Mueller–Hinton agar was used to test different antibiotic susceptibilities. In addition, the ExoA encoding gene was analyzed using the polymerase chain reaction (PCR)-DNA-sequencing method. **Results:** The antibacterial susceptibility test of 89 *P. aeruginosa* showed a higher percentage of antibiotics resistant against amikacin for 14 isolates at (58.42%), then intermediate resistance against piperacillin was 21 isolates at (23.60%), while the higher sensitivity of antibiotics was against meropenem at 84 isolates (94.38 %). The presence of the *tox A* gene was not associated with antibiotic resistance ($P = 0.45$), but the multidrug-resistant (MDR) isolates became more virulent when they produced the ExoA. PCR-DNA sequencing results appeared the presence of several mutations in *tox A* gene within two of the studied isolates that leads to change the amino acids, which may be the effect on exotoxin functions in the *P. aeruginosa* isolate 18-GF and slightly effects in the *P. aeruginosa isolate* 61-NR1 by effecting on protein conformation of domain III that participate in forming exotoxin complexed with nicotinamide and adenosine monophosphate. **Conclusion:** Most *P. aeruginosa* isolates recovered from burn infections produce ExoA and generally resist recently used antibiotics and some MDR isolates.

Keywords: Exotoxin A, multidrug resistance, PCR-DNA sequencing, *Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogenic bacterium that can live in various environments.[1] The presence of diverse resistance mechanisms has an important clinical influence, as it limits therapeutic options for *P. aeruginosa* infection, impairs the effectiveness of antiapoptotic agents, and makes it very difficult to treat bacterial infections.[2–4] *P. aeruginosa* strains are not only resistant to a wide range of presently available antimicrobial agents, such as fluoroquinolones but also third-generation cephalosporins and carbapenems, which are chosen options in the treatment of serious infections caused by multidrug-resistant (MDR) strains.[3,5]

Pathogenic *P. aeruginosa* has different virulence factors that increase infection severity, such as proteases, toxins, siderophores, alginate, lipopolysaccharide, flagellum, and MDRs. Exotoxin A (ExoA) is the most prevalent and toxic virulence agent among pathogenic *P. aeruginosa* species that acquire adenosine diphosphate-ribosyltransferase (ADP-ribosyltransferase) activity.[6,7] This is the reason for the high mortality rates among experimentally infected animals in which a single 80 ng injection was sufficient to induce acute necrosis and swelling of the liver and hemorrhage in the lungs and kidneys within 48 h of exposure.[8]

P. aeruginosa is intrinsically resistant to a wide range of antimicrobialsmainlyduetolowoutermembranepermeability, the expression of efflux pumps, and the production of an inducible AmpC cephalosporinase. Moreover, it can also

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Address for correspondence: Mr. Zahraa Hamza Merza, Department of Medical Laboratory Techniques, Al-Mustaqbal University, 51001 Hillah, Babylon, Iraq.
E-mail: zahraa.hamza@uomus.edu.iq

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easily develop resistance to antimicrobials commonly used to treat *P. aeruginosa* infections, such as piperacillin (PRL)/tazobactam, ceftazidime, carbapenems, fluoroquinolones, or aminoglycosides.[9] In addition, a study in India showed that the incidence of *P. aeruginosa* was 14.3% in diabetic foot ulcers, which is significant compared to previous studies. In accordance with previous studies.[10,11]

Pseudomonas ExoA (PE) has enzymatic activity and belongs to the mono-ADP-ribosyltransferase family.[12] With regard to its function, it is specified as NAD+-diphthamide-ADP-ribosyltransferase (EC 2.4.2.36).[12,13] Later, it was characterized as a two-component AB toxin family, containing an A domain with enzymatic activity and a B domain as a cell binding subunit,[14] with respect to its function, has been identified as NAD^+-diphthamide-ADP-ribosyltransferase.[15] Chromosomally *tox A* gene of *P. aeruginosa* expressed to PE as a single pro-protein chain of 638 amino acids with 66 kDa molecular weight contains a highly hydrophobic leader sequence of 25 amino acids at its N-terminal, and it is removed during secretion. A mature toxin of 613 amino acids is secreted into its extracellular environment or the culture medium.[15]

X-ray crystallography studies of functional or mature PE molecule revealed the presence of three domains. The N-terminal receptor-binding domain is the first domain consisting of two nonadjacent regions, Ia (1–252 aa) and Ib (365–404), composed of antiparallel ß-sheets. The second domain is the membrane translocation (253–364 aa) with six consecutive α-helices, enabling the toxin to translocate across cell membranes. The third domain is the ADP-ribosylation of elongation factor 2 (405–613 aa) at the C-terminus of the polypeptide. There are also four disulfide bridges, two located in domain Ia, one in domain Ib, and one in domain II.[15]

PE is secreted into the extracellular environments via the general secretary pathway, a two-step mechanism highly conserved in Gram-negative bacteria.[16,17] After cytoplasmic expression as an unfolded precursor protein, PE is transported to the periplasm using the Sec machinery.[6] During translocation through the inner membrane, the N-terminal signal peptide is cleaved off, and PE is released into the periplasmic space. In the hydrophilic environment of the periplasm, PE is folded to a mature conformational protein in a manner that can be recognized by the type II secretion system, specifically called Xcp in *P. aeruginosa*, for secretion into the extracellular space.[16,17]

Once secreted, the terminal lysine (aa 613) of PE can be cleaved from the toxin in the extracellular environment, presumably by the plasma carboxypeptidases of the host. This leads to forming of a C-terminal motif from REDLK (one of the C-terminal motif from/*Pseudomonas aeruginosa*) (aa 609–613) to REDL (one of the C-terminal motif from/*Pseudomonas aeruginosa*) (aa 609–612), which enables the toxin to bind to KDEL (one of the receptor mediated pathway / *Pseudomonas aeruginosa*) receptors at the Golgi apparatus during subsequent intracellular trafficking. On the host cell surface, PE specifically binds via domain Ia to CD91, also known as alpha2-macroglobulin receptor/low-density lipoprotein receptor-related protein (α2MR/LRP).[18,19] Then, there are two pathways open for PE to reach the Endoplasmatic Reticulum: the KDEL receptor-mediated pathway and the lipid-dependent sorting pathway. Once inside the cytosol, the enzymatically active C-terminal domain catalyzes the ADP-ribosylation and inactivation of EF2, which inhibits protein synthesis within the affected cell through its inability to lengthen polypeptide chains, and then irreversibly causes cell death.[20,21] This study aims to investigate ExoA in multidrug resistance *P. aeruginosa* isolated from burn infections.

Materials and Methods

Specimens

The study included 70 swabs of burned infections collected from the hospitals of Babylon Province for 4 months, from November 2021 to January 2022. These swabs were transferred in the cooling box to the Advanced Biotechnology Laboratory at the Department of Biology, College of Science, University of Babylon, for bacterial isolation and identification. All swabs were initially cultivated on blood agar and nutrient agar plates, then the single colonies were grown on MacConkey agar and Cetrimide agar, and *Pseudomonas* chromogenic agar (Condalab/Spain) and then confirmed as *P. aeruginosa* by VITEK® 2 system and they were preserved until used to complete the study.

In addition, 62 pure *P. aeruginosa* isolates were recovered from glycerol stocks preserved in the laboratory. These isolates were previously isolated from burned infections collected from hospitals in Babylon, Najaf, and Karbala governorates for 8 months in 2020. These isolates were previously identified using microbial, cultural, and biochemical characteristics and were confirmed their diagnosis using VITEK® 2 system. The pseudomonad strains confirmed their purity by staining using Gram stain and recultured on selective media such as MacConkey agar and Cetrimide agar, and *Pseudomonas* chromogenic agar plats.

Antibiotic susceptibility test

The Kirby–Bauer disc diffusion method on Mueller–Hinton agar was used to test different antibiotic susceptibilities including (µg/disc): gentamycin (CN: 10), amikacin (AK: 30), meropenem (MEM: 10), imipenem (IPM: 10), ciprofloxacin (CIP: 5), and PRL: 100. Antibiotic susceptibility results compared with the standard of clinical and laboratory standards institute, 2020 after measuring the inhibition zones around the disks.

Bacterial genomic DNA extraction

Genomic DNA of *Pseudomonas aeruginosa* was extracted using the FavorPrep™ Genomic Kit (Favorgen/Taiwan)

according to the manufacturer's instructions and DNA was confirmed by migration of samples on electrophoresis gel device. The methods included 1% agarose mixed with 0.5 µL of safe DNA stain (IntronBio/Korea) under conditions: 1× Triss-buffer EDTA buffer, 70 V, and 20 mA for 1 h, and then the gel were visualized using a gel imaging system under (Cleaver Scientific—UK). DNA concentration and purity (ABS_{260nm}/ABS_{280nm}) were determined using a NanoDrop spectrophotometer (Optizen POP NanoBio/Mecasys Co., Ltd., Korea), and the DNA extract was stored at -20°C until used.

Molecular detection for *tox A* gene by polymerase chain reaction (PCR)-DNA sequencing

Target DNA was performed by conventional PCR using T Professional TRIO—Thermal Cycler (PCR ((Biometra/Germany). The specific primer pairs for the target region of *tox A* gene were utilized according to Sabharwal *et al.*[22] method includes forward primer: 5′-GGAGCGCAACTATCCCACT-3′ and reverse primer: 5′-TGGTAGCCGACGAACACATA-3′ (Macrogen/Korea). The reaction mixture was composed of 4 µL DNA template (concentration 5 ng/µL and purity 1.9), 12.5 µL (Green master mix/USA/Promega), 2 µL for each of forward and reverse primer pairs at concentration 10 pmol/µL and 4.5 µL of nuclease-free water to reach a final volume 25 µL. The reaction was performed for 30 cycles after the initial denaturation at 95°C (2 min) as follows: the denaturation at 95°C (30 s), annealing at 58.1°C (30 s), extension at 72ºC (20 s) and the final step at 72ºC for 5 min. The amplified product of the target site (150 bp) was electrophoresed through an agarose gel 2% at an appropriate time interval and was visualized and documented by the gel imaging system.

The amplified DNA was (PCR product) purified by gel electrophoresis according to the protocol suggested by Macrogen Sequencing Corporation (Macrogen/Korea), then delivered 11 samples that showed positive *tox A* gene band to the company of sequencing. The DNA sequence data of the studied genes were analyzed and aligned according to program version 7.2.5. and MEGA-X program and compared with reference sequences available in the GenBank (NCBI) database for identification of polymorphisms and phylogenetic tree construction.

Statistical analysis

Data for this study were statistically analyzed using statistical program software suite (SPSS, 23/2021, Hilla, Babil Governorate) version 19 by *t*-test and one-way analysis of variance, and the *P* value ≤0.05 was statistically significant.

Ethical considerations

The approvals were obtained from all the participants, and they agreed to study scientifically and morally the hospitals of Hillah city and the Public Health Laboratory in Babylon. The following information is recorded (patient name, age, sex, date of infection, and chronic disease). According to the document with the number 8220903 on September 28, 2022, the study protocol, subject information, and agreement were examined and approved by a local ethics committee of the Biology Department at the College of Science, University of Babylon.

Results

Of the 70 patients examined with second and third-degree burns, 45 (64.3%) showed positive bacterial growth and 25 (35.7%) negative growth. Of the 45 positive cases, only 30 (66.67%) had pure cultivation, while the other 15 (33.33%) had mixed growth. About 73 bacterial isolates were primarily diagnosed according to microbial and cultural characteristics and found 18 (24.66%) Gram-positive isolates, mostly *Staphylococcus* spp. and 55 (75.34%) Gram-negative isolates. The dominant Gram-negative bacteria were *P. aeruginosa* 27 (49.1%), followed by *Klebsiella* sp. 14 (25.45%), *Escherichia coli* 10 (18.18%), and the remaining 4 (7.27%), including 7.27% (4) other Gram-negative bacteria.

Only 27 *P. aeruginosa* isolates were selected to complete this study; others were excluded. These Pseudomonad isolates were identified according to microbial, cultural characteristics, and biochemical properties tested according to the VITEK® 2 system to confirm the bacterial diagnosis.

The total number of *P. aeruginosa* was 89 isolated from burn infections, including 27 isolates of this study isolated during four months (November 2021 to January 2022), in addition to 62 *P. aeruginosa* previously isolated during 2020–2021 obtained from hospitals in Babylon Najaf and Karbala governorates which preserved as glycerol stock in the biotechnology laboratory. They all examined phenotypically for antibiotic susceptibility (CN, AK, MEM, CIP, and PRL) and molecularly for detecting toxin A encoding gene.

Antimicrobial susceptibility results showed that *P. aeruginosa* isolates had variable abilities to resist the studied antimicrobial drugs, including six antibiotics belonging to four different classes with different patterns or mechanisms inhibiting the growth or killing of microorganisms. Table 1 showed the antibacterial susceptibility test of 89 *P. aeruginosa* isolated from burn infections had a higher percentage of antibiotic resistance against AK (58.42%). In comparison, the higher percentage of intermediate resistance of antibiotics was (23.60%) for PRL, and the higher percentage of sensitivity of antibiotics was (47.19%) for MEM. Most isolates were sensitive (94.38) for IPM.

Figure 1 shows the number of isolates and resistant percentage, where there are (19 out of 89) isolates sensitive to antibiotics at 21.34%, (21 out of 89) at 23.6% resistant to one type of antibiotic, (11 out of 89) at 12.36% resistant

Table 1: Antibiotics susceptibility *P. aeruginosa* isolated from burn infections

Drug class	Drug	Breakpoints (mm)			Resistant no. (%)	Intermediate no. (%)	Sensitive no. (%)	Total no. (%)
		R ≤	I	S ≥				
Aminoglycosides	AK	14	15–16	17	52 (58.42)*	10 (11.24)	27 (30.34)	89 (100%)
	CN	12	13–14	15	34 (38.20)	19 (21.35)	36 (40.45)	
Carbapenems	MEM	19	20–22	23	34 (38.20)	13 (14.61)	42 (47.19)	
	IPM	19	20–22	23	0	5 (5.62)	84 (94.38)*	
Penicillins	PRL	13	14–16	17	43 (48.31)	21 (23.60)*	25 (28.09)	
Quinolones	CIP	21	22-25	26	46 (51.69)	10 (11.24)	33 (37.07)	

AK: amikacin (30 µg), CN: gentamicin (10 µg), MEM: meropenem (10 µg), IPM: imipenem (10 µg), PRL: piperacilline (100 µg), CIP: ciprofloxacin (5 µg), R: resistant, I: intermediate, S: sensitive

*significant *P* value at ≤0.05

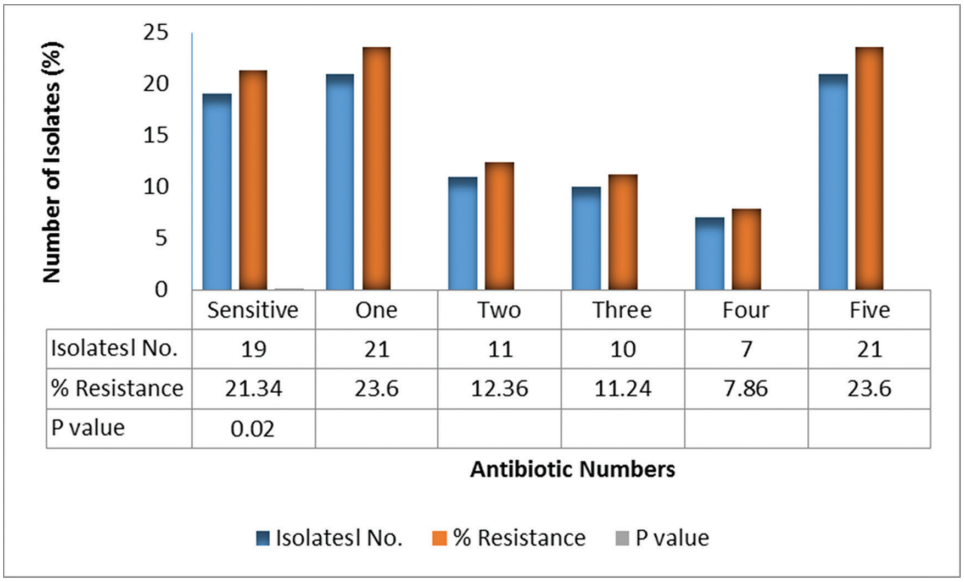

Figure 1: Frequency of antibiotic resistance of *P. aeruginosa* isolates

to two types antibiotic, (10 out of 89) at 11.24% resistant to two types antibiotic, (7 out of 89) at 7.86% resistant to two types antibiotic, and (21 out of 89) at 23.6% resistant to two types of antibiotic.

Figure 2 shows the frequency of antibiotic resistance of *P. aeruginosa,* which appeared to have variable resistance to antibiotics. About 21 (23.6%) isolates were resistant to five antibiotics, and 19 (21.34%) isolates were sensitive to all antibiotics. Therefore, about (31 out of 89) isolates were MDR at 34.8%. In addition (33 out of 89) isolates were non-MDR at 37.1%, then (25 out of 89) isolates were sensitive at 28.1%.

Molecular detection of *tox A* gene by PCR

Figure 3 shows the amplified band (150 bp) of *tox A* gene on agarose gel electrophoresis. About 95.5% of *P. aeruginosa* isolates harbor *tox A* gene. These isolates included 17 (89.47%) sensitive to antibiotics and 34 (94.45%) non-MDR isolates. All of the MDR isolates harbor *tox A* gene [Figure 4]. The presence of the *tox A* gene is not associated with antibiotic resistance ($P = 0.45$). Still, the MDR isolates became more resistant when they produced ExoA.

Three *P. aeruginosa* isolates were selected randomly from three groups, MDR *P. aeruginosa* isolates 13-NR2, non-MDR *P. aeruginosa* isolates 61-NR1, and Sensitive *P. aeruginosa* isolates 18-GF to analyze further using PCR-DNA sequencing technique. The results revealed several mutations in *tox A* gene sequences of *P. aeruginosa* isolate 18-GF. It had an amino acid sequence identity of about 84% compared with the ExoA protein of standard strains [Figure 5B]. In contrast, there are three mutations in the non-MDR *P. aeruginosa* isolate gene 61-NR1, with an amino acid sequence identity of about 94%. There is one mutation in the *tox A* gene of the isolate MDR *P. aeruginosa* isolate 13-NR2, as shown in Figure 5A. It had an amino acid sequence identity of about 100% compared with the ExoA protein of standard strains [Figure 5B]. This indicates the mutation does not change the amino acid (serine: AGC>AGT). Whereas the mutations in other isolates changed the amino acids, as shown in Figure 5B, which may be the effect on exotoxin functions in the *P. aeruginosa* isolate 18-GF and slight

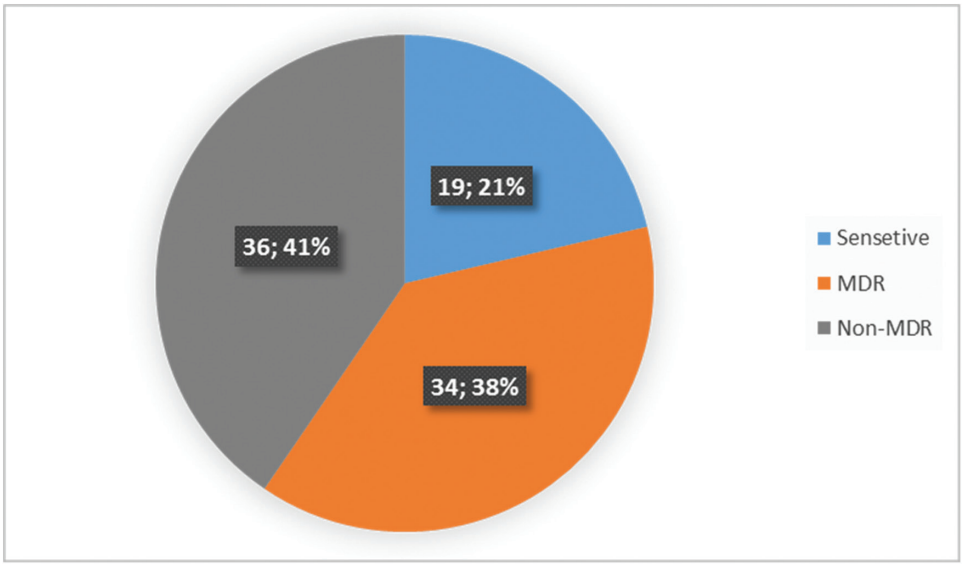

Figure 2: Distribution of *P. aeruginosa* isolates according to MDR and non-MDR

MDR: multidrug resistance *P. aeruginosa*, which resist at least three class of antibiotics. Non-MDR: *P. aeruginosa*, which resist less than three class of antibiotics

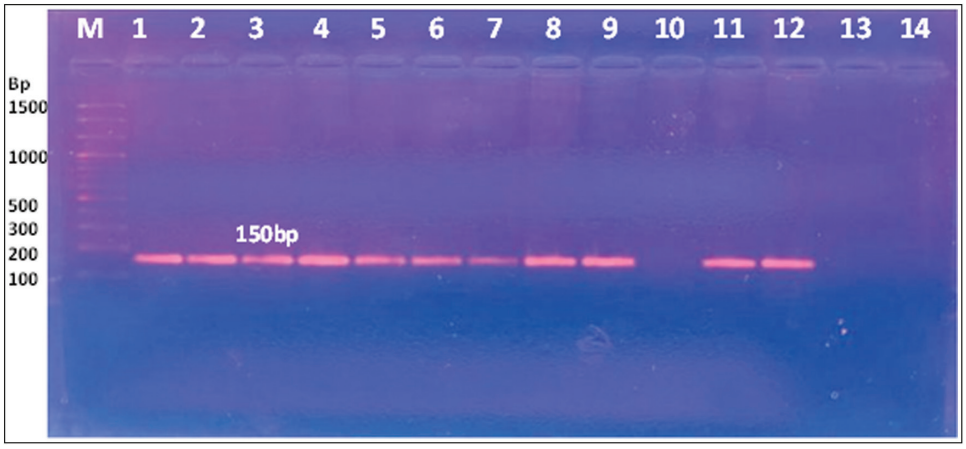

Figure 3: Agarose gel electrophoresis of *tox A* amplified product patterns of *P. aeruginosa* isolates

M: refers to DNA size marker (100–1500 bp); lanes 1–9 and 11–12 refer to positive results of PCR product (150 bp) of *tox A* and lanes 10, 13, and 14 refer to negative results for *tox A* of *P. aeruginosa* isolated from burn infections. Electrophoresis conditions: 2% agarose concentration; 100 V, 20 mA for 60 min. Staining method; precast Red Safe stain

impact in the *P. aeruginosa* isolate 61-NR1 by affecting protein conformation of domain III [Figure 6], which is participating in forming exotoxin complexed with nicotinamide and adenosine monophosphate.

Discussion

In the current study, *P. aeruginosa* was proved to be the main cause in burn patients, accounting for 49.1% of Gram-negative isolates and 27 (36.99%) of the total isolates (73) and was found in 75%, 64.3% of the positive growths. This result was in agreement with those of Song *et al.*[23] and Al-Habib *et al.*,[24] who reported that 25% of the total bacterial isolates of burn samples were Gram-positive. In comparison, 75% were Gram-negative bacterial isolates, especially *P. aeruginosa*. However, other studies have reported a lower prevalence of *P. aeruginosa* in burn infections.[25-27] On the other hand, Mansour and Klantar[28] recorded a higher isolation rate (68.3%). The second most recovered organism in this work is *Klebsiella pneumoniae* (25.45%), which is in agreement with the result obtained by Kehinde *et al.*,[29] about 34.3%, and Al-Habib *et al.*,[24] about 26.7%, and in contrast to others Gad *et al.*[30] who report. In addition, *E. coli* recovered 18.18% of total Gram-negative and 13.7 of the total cases; this rate was more than (10%) of what was discovered by Daher *et al.*[31] and Al-Habib *et al.*[24]

The difference in the prevalence of bacterial isolates can be attributed to the environmental conditions of a

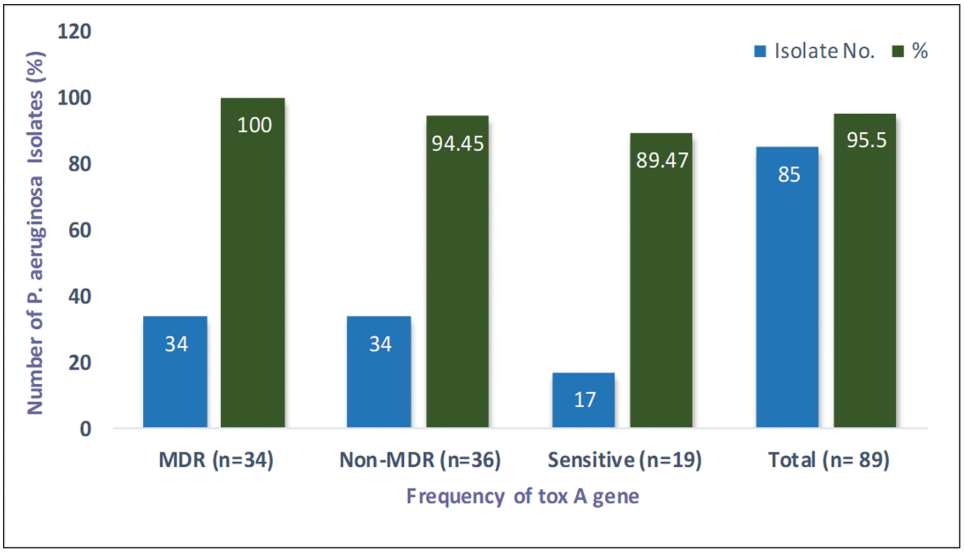

Figure 4: Frequency of the presence of *tox A* in *P. aeruginosa* isolated from burn infections

MDR: multidrug resistance *P. aeruginosa*, which resist at least three class of antibiotics. Non-MDR *P. aeruginosa*, which resist less than three class of antibiotics

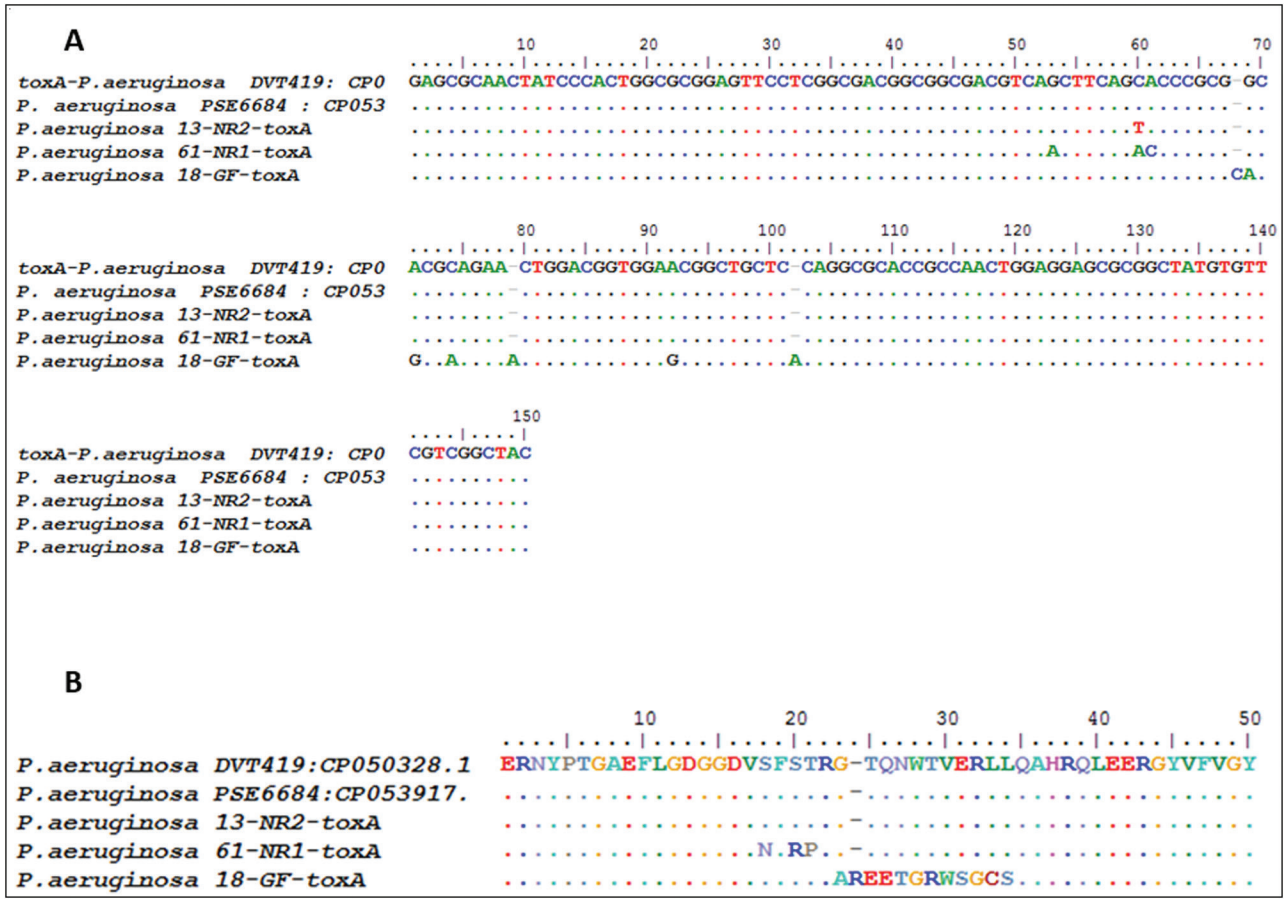

Figure 5: Nucleotides sequence and amino acid alignment of the *tox A* gene among the local *P. aeruginosa*

(A) Nucleotide sequence alignment of the *tox A* gene among the local *P. aeruginosa* isolates and nucleotide sequence of those available in databanks. The local isolates, including *P. aeruginosa,* isolate 13-NR2, 61-NR1, and 18-GF. Data indicated that nucleotide positions differ among isolates, and identical data for all isolates are not shown by Bio Edit program version 7.2.5. (B) Pair sequence alignment of the amino acid of the *tox A* gene among the local *P. aeruginosa* isolates 13-NR2, 61-NR1, and 18-GF and amino acid sequences of those available in databanks. Draw based on the alignment of Bio Edit program version 7.2.5

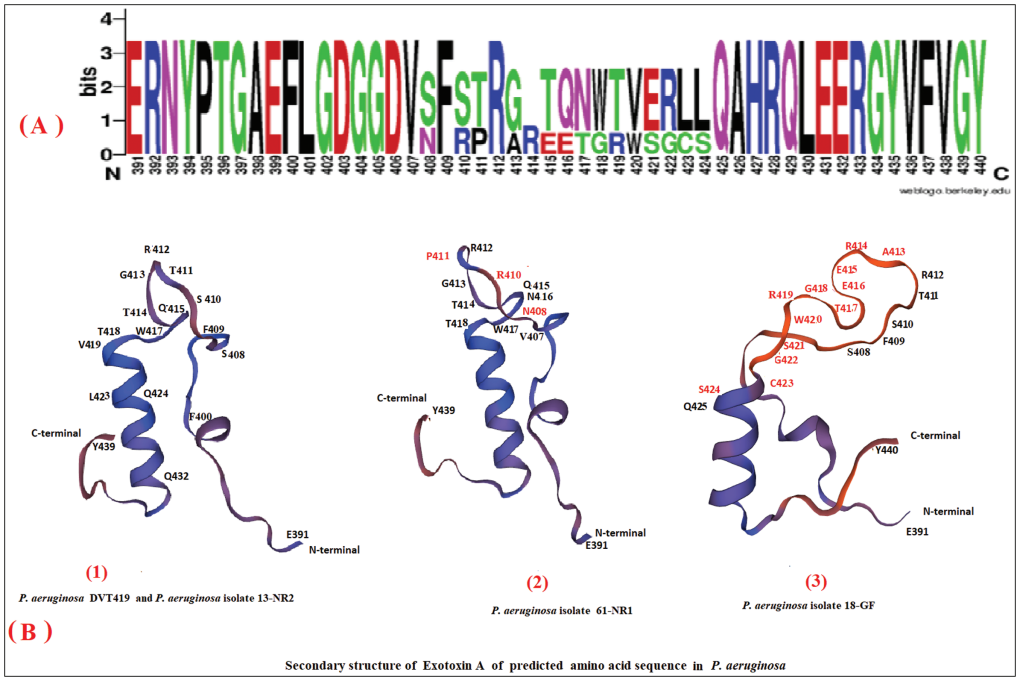

Figure 6: Secondary structure of *P. aeruginosa* exotoxin A of predicted amino acid sequence

(A) The Weblogo of amino acids repeats of the studied region (391–439) composed of the end sequence of binding domain Ib (365–404) and the beginning sequence of Domain III ADP-ribosylation of elongation factor 2 (405–613 aa) of exotoxin A. (B) Secondary structure of the studied region of an Exotoxin A in *P. aeruginosa* isolates; 1—3D structure of standard strain *P. aeruginosa* DVT419 and *P. aeruginosa* 13-NR2; 2—3D structure of *P. aeruginosa 61-NR1;* 3—3D structure of *P. aeruginosa* 18-GF according to https://swissmodel.expasy.org/

particular area and the contamination of the burning units. Due to the increasing antibiotic resistance and the spread of infection in the hospital environment, there is a clear change in the bacterial spectrum. A few decades ago, the dominant bacteria were *Streptococcus* which was later followed by *Staphylococcus aureus*, but frequent use of topical antibiotics, fungi, and viruses become more prevalent[24] but frequent use of topical antibiotics, fungi, and viruses become more prevalent. Also, Gram-negative and Gram-resistant bacteria are becoming more prevalent due to using a wide range of antibiotics. This growing antibiotic resistance poses a challenge to burn care units as it reduces treatment efficacy and may increase morbidity and mortality.

Although burn patients survive, complications of infection remain the leading cause of morbidity and mortality. Although invasive bacterial burn wound infection has been controlled, strict isolation techniques and infection control policies have significantly reduced the incidence of burn wound infection.[32] The current study showed a high prevalence of bacterial infection among burn patients, which agrees with the findings of other researchers[24] but in contrast to another study.[33] In the present study, a burn infection swab resulted in positive bacterial growth in 64.3% of the cases examined, similar to other researchers' observations.[24,29,31,34] Single isolates were found in 56.7% of the studied cases, matching the result reported by Daher *et al*.,[31] who obtained pure isolates in 58.7% of their patients. Various types of Gram-positive and Gram-negative microorganisms were detected in the present study, including Gram-negative bacteria. It constituted (75.34%) Gram-positive (24.66%). This result is consistent with that of Gram-negative bacteria constituted (72%) and (83.8%) of their isolates, respectively. However, other researchers have reported lower incarceration rates of 33%–51.1%.[27,29,31] Moreover, hospital infections with Gram-negative bacilli, especially *P. aeruginosa*, have increased over the last decade. Other studies have described *P. aeruginosa* as the common cause of hospital burn infection.[25]

Skin infections with pathogenic bacteria are widespread, especially in burns and wound patients, due to the contamination of the area with this microorganism called a hospital-acquired infection, so conducting antibiotic susceptibility testing for bacterial isolates and identifying the genes responsible for resistance is necessary to reduce the severity of infection.[35-37] This is consistent with the current study, which was conducted on *P. aeruginosa* bacteria, considered one of the most pathogenic causes of skin diseases in burn patients, as it is classified as one of the most bacterial species that have antibiotic resistance genes.

MEM was the most effective antibiotic in this study, with 47.19% against bacteria. Other studies have reported variable IPM resistance rates, such as 37% in Tunisia, 43%

in Libya, and 76% in Iran.[38,39] Another study consistent with this showed that the most effective antibiotic was MEM, with 37% resistance.[40] The rate of resistance against six types of antibiotic used in the present study ranged between 38.2% and 58.42% for burn isolates, in which 58.42% of the isolates were resistant to AK, 51.69% were resistant to CIP, 48.31% were resistant to PRL, and 38.2% were resistant to MEM and gentamicin. In contrast, the results of some studies made in some European countries showed the highest rates of resistance to Carbapenems and Aminoglycosides.[41] In Iraq, Baban's investigation in Erbil revealed that 50 (62.5%) *P. aeruginosa* isolates had an antibiotic resistance profile, with 4% pan-drug resistant, 20% extensively drug-resistant, and 76% MDR.[42]

In this study, PRL was the effective agent against *P. aeruginosa*, which yielded the least resistance percentage (48.31%). This could be explained on the basis that PRL is not commonly prescribed against *Pseudomonas* infection in this locality. However, higher resistance to this drug (86.2%) was reported by other investigators, Strateva *et al.*,[43] where the use of this antibiotic is more frequent in their locality. The sensitivity test of the six antibiotics used in this study against *P. aeruginosa* isolates was relatively low compared to the sensitivity pattern to the antibacterial drugs used in many other studies. This is due to the selective pressure exerted on bacteria for many reasons, such as noncompliance with the hospital's antibiotic policy and the excessive and indiscriminate use of extensive antibiotics, in addition to the antibiotic sensitivity test against clinical isolates of *Pseudomonas* bacteria, especially those that have shown a high inhibition activity, IPM, and MEM may help in the prevention and treatment of MDR pathogens in burn and wound infections.[44]

Antibiotic resistance test of *Pseudomonas aeruginosa* isolates showed that most It was resistant to many antibiotics that are widely used to treat bacterial infections, such as gentamicin and ceftriaxone, which makes the bacteria more virulent, and this is what many researchers mentioned who noticed this high antibiotic resistance.[24,27,43] MDR *P. aeruginosa* is currently a major problem. The present study accounted for 44.94% of the total *P. aeruginosa* recovered. This result agreed with Strateva *et al.*[43] and Al-Habib *et al*.[24] about 44.4%, although it was higher than that reported by other workers.[28] The relationship between MDR *P. aeruginous* and antimicrobial consumption was also analyzed in this study. A statistically significant relationship was found with Ceftriaxone, MEM, Ceftazidime, and AK, which agreed with Al-Habib *et al.*[24] and in contrast to the study by Messadi *et al*.,[45] who found that a significant association was with CIP use.[46] This discrepancy in results may be due to different antibiotic use in different settings.

The production of beta-lactamase enzymes is the mechanism by which *Pseudomonas* are resistant to antibiotics. Moreover, the inducible β-lactamase producers were the MDR, and this result reflects the role of inducible β-lactamase in antibiotic resistance.[47-49] This increased rate of MDR may be attributed to the inhibitory concentration of antibiotics in vivo due to the administration of an inappropriate dose of beta-lactam antibiotics or regular administration of aminoglycosides in combination with beta-lactam drugs that provide optimal conditions for the persistence of MDR *P. aeruginosa* strains. These results highlighted the need for greater attention to disinfecting the nonliving hospital environment and controlling contact between staff and patients to reduce *P. aeruginosa* transfer in burn units. Furthermore, the use of some antimicrobial agents should be restricted due to the presence of high resistance. Combined effective antibiotics are also recommended. In conclusion, bacteria isolated from burn unit patients are the best examples for studying pathogenic bacterial species, especially *P. aeruginosa*, other enteric bacilli, and *Staphylococcus*. The doctrine that is often responsible for human colonization. Also, *P. aeruginosa* and other Gram-negative bacilli are frequently associated with nosocomial burn infection. Moreover, most of the *P. aeruginosa* isolates from the flaring units are producers of β-lactamases, and most of these isolates were MDR *P. aeruginosa*.

ExoA of *P. aeruginosa* is an important virulence factor belonging to the class of exotoxins secreted by pathogenic bacteria that cause human diseases such as cholera, diphtheria, pneumonia, and whooping cough. The third domain of EoxA is involved in forming a catalytic complex composed of ExoA with an elongation factor 2 and proper NAD(+), suggesting a direct role of two rings of the active site in ExoA during the catalytic cycle. Mutational studies of the two rings in the ExoA identify several important residues of catalytic activity, particularly Glu 546 and Arg 551, clearly supporting new complex structures that contribute to forming the transition state model of the toxin-catalytic reaction.[50] in addition, most *P. aeruginosa* isolates that recovered from burn infections produce ExoA and generally resist recently used antibiotics and some MDR isolates.[15]

In the current study, we performed a scan of the Toxa gene and compared it with the genetic sequence in the Gen Bank, where we found some new mutations related to this toxin compared to the previous gene, which indicates an increase in the susceptibility of pathogenic bacteria. This is what Cotar *et al.*[51] and his group referred to, who mentioned that this poison has an important role in this enzyme in interaction with the proteins of the immune defense system, human blood cells, especially multinucleated leukocytes, natural killer cells, and immunoglobulins of type IgA, IgG. Thus, the *P. aeruginosa* strains producing this enzyme are among the strongest immunity inhibitors, making the body more susceptible to infection with other microorganisms.

Conclusion

We concluded that most burn isolates were *P. aeruginosa* bacteria, characterized by their high ability to resist antibiotics because they possess many resistance genes, including ExoA, which has a major role in increasing the susceptibility of pathogenic bacteria and increasing their resistance to antibiotics.

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Conflicts of interest

The others declare no conflicts of interest.

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