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Molecular identification and detection of *Opr1* and *OprD* virulence genes among *Pseudomonas aeruginosa* isolated from burn infections

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ABSTRACT

Bacterial infection, especially *Pseudomonas aeruginosa*, is the leading cause of complications acquired from injuries that burn patients develop. This study aimed to evaluate the rate of infection in burn wounds caused by *P. aeruginosa* and to estimate the prevalence of some virulence genes. A total of 110 burn samples were collected from the Burn and Plastic Surgery Hospital in Duhok City, Iraq. *P. aeruginosa* isolates were identified using phenotypic and conventional biochemical tests and confirmed by amplifying a species-specific primer. The antibiotic susceptibility was determined by disk diffusion method, and Polymerase chain reaction was used for the detection of the two virulence genes *Opr1* and *OprD*. Out of the 110 tested samples from burn infection, 71 were found to be positive for *P. aeruginosa*, with a rate of (64.54**%**). Females showed higher infection rates than males (59.15% vs 40.84%). The most effective antibiotic tested was found to be Colistin, with a 100% rate, while the highest level of resistance was detected with Piperacillin at a rate of (98.59%). The most common virulence gene encountered was *OprD*, with a rate of (61.11%) followed by *Opr1* at a rate of (57.4%). It is recommended that an antibiotic susceptibility test be performed prior to therapy to improve the treatment options for patients.

Keywords: Antibiotic resistance, burn infection, *Opr1*, *OprD*, *Pseudomonas aeruginosa*

1. Introduction

The skin is the body's largest organ, playing a crucial role in physiological functions such as preserving homeostasis, controlling body temperature, enabling sensory perception, and shielding against external elements (Roy et al., 2024). Man's skin serves as his physical defense against infection. Therefore, conditions that result in a loss of skin integrity have several serious consequences (Jiao et al., 2024). Burn infection is a widespread health issue in many nations throughout the world (López-Jácome et al., 2020). Significant risk factors for the onset of infection were a damaged skin barrier, extensive burn area, immunosuppressive effects of burns, and extended hospitalizations (Gupta et al., 2019). The World Health Organization (WHO) reports that every year, 265,000 people lose their lives due to burn injuries most burns in adult males happen outdoors, whereas most burns in adult females happen at home (Pujji et al., 2019).

Burn incidents result in losing the skin barrier, making the condition favorable for the growth and colonization of the injury site by microorganisms. Otherwise, for each individual, skin serves as the primary defense line (Bhardwaj et al., 2021). In case of the presence of skin damage, the infecting microbe can easily enter and reach the internal body tissues, making the etiology more complex (Jeschke et al., 2020). Hence, the high morbidity and mortality rates in burn patients are caused by microbial infections, in particular those caused by multidrug-resistant (MDR) bacteria, such as *Pseudomonas aeruginosa* (Norbury et al., 2016). *P. aeruginosa* is a multidrug-resistant (MDR) bacterium that attacks the respiratory tract, urinary tract, cardiovascular system, and central nervous

system and results in severe morbidity and mortality. Nosocomial infections are primarily caused by *P. aeruginosa* strains, which are a serious concern to hospitalized patients who are immunocompromised (Pang et al., 2019; Abdelrahman et al., 2020).

P. aeruginosa is well-known for a wide variety of virulence factors, including its elastase production, motility, surface hemagglutinin, adhesins, rhamnolipid, biofilm formation, production of pyocyanin, alkaline protease, type Ill secretion system, lipopolysaccharide, colonization pili, and flagella (Moradali et al., 2017). Protein channels called porins, and in particular *OprD*, are responsible for the translocation of carbapenems across lipid bilayer membranes. *P. aeruginosa* isolates lacking *OprD* contribute to their resistance to carbapenems (Mirsalehian et al., 2017). Although *P. aeruginosa* is a well-recognized pathogen in burn infections, particularly due to its multidrug resistance and diverse virulence factors, limited data are available regarding its molecular characteristics in the Duhok region. In particular, studies investigating the prevalence of the *Opr1* and *OprD* virulence genes in clinical burn isolates are scarce. This study aims to address this gap by combining phenotypic antibiotic susceptibility testing with molecular detection of *Opr1* and *OprD* genes in *P. aeruginosa* isolates collected from burn patients. To the best of our knowledge, this is one of the few studies in Duhok, offering valuable epidemiological and molecular insights that may support targeted infection control strategies and guide local antimicrobial stewardship efforts. The current study aimed to assess the activity of different antibiotics against *P. aeruginosa* isolated from burn patients and detect the presence of *Opr1* and *OprD* virulence genes.

2. Materials and methods

Sample collection

The current study was conducted from July 2021 to May 2022, during which a total of 110 clinical samples were collected from hospitalized patients suffering from burn injuries attending the Burn and Plastic Surgery Hospital in Duhok City, Iraq. A sterile cotton swab was used to take a sample from the infected deep burn sites immediately after the dressings were changed. After taking the swabs, they were transported to the laboratory by placing them in bottles containing 5 ml of brain heart infusion broth.

Identification of *P. aeruginosa*

Following sample collection and delivery to the laboratory, the obtained swab samples were incubated for 24 h at 37 °C. Then a loop was taken from the incubated samples and cultured on MacConkey agar and again incubated at 37 °C for 24 h. The following day, a loop was taken of plates showing growth and subcultured on MacConkey agar to obtain pure colonies. The obtained pure colonies were cultured on several selective and differential culture media, such as Nutrient agar, Blood agar, and Cetrimide agar plates. The suspected isolates were initially identified based on morphological characteristics such as Gram stain and India ink and were further confirmed by performing several different biochemical tests, including catalase, coagulase, indole, citrate, oxidase, and kligler iron agar (KIA). After phenotypically identifying *P. aeruginosa* isolates, the genotype of each identified *P. aeruginosa* strain was confirmed using a species-specific gene (*16S rDNA*).

Antimicrobial susceptibility test

The susceptibility of the isolated *P. aeruginosa* towards several different antibiotics was investigated using the Kirby-Bauer disk diffusion method. Ten antibiotic discs supplied by Bioanalyses (Turkey) were used. The tested antibiotics included: Ceftazidime (CAZ; 30 mg), Cefepime (CPM; 30 mg), Piperacillin (PI; 100 mg), Meropenem (MEM; 10 mg), Imipenem (IPM; 10 mg), Colistin (CL; 10 mg), Levofloxacin (LEV; 5 mg), Ciprofloxacin (CIP; 5 mg), Gentamicin (CN; 10 mg) and Amikacin (AK; 30 mg). The result of the antibiotic sensitivity test and measuring the inhibition zone around the antimicrobial discs used were determined according to Clinical and Laboratory Standards Institute (CLSI) instructions.

Bacterial DNA extraction and PCR conditions

The genomic DNA of all the isolated and identified *P. aeruginosa* was extracted using a commercial DNA Purification Kit supplied by (Favorgen-Taiwan). The concentration and purity of the extracted DNA were evaluated

using a NanoDrop Spectrophotometer. Polymerase chain reaction (PCR) was used to amplify the three primers used in this study. For the molecular confirmation of *P. aeruginosa*, a species-specific primer (*16S rDNA*) was used, and two primers for the amplification of the two virulence genes investigated among *P. aeruginosa* isolates. Used primers are listed in Table 1. The final volume of the PCR amplification reaction was in a 20 µl reaction tube, which contained 10 µl PCR Master mix, 1 µL of forward primer (10 pmol/µL), 1 µL of reverse primer (10 pmol/µL), 3 µL of DNA and 5 µl of deionized distilled water. The amplification conditions of the used primers are described in Table 2. The amplified product was visualized by running on 1.5% (w/v) of agarose gel prepared in 1X TBE buffer. For determining the molecular weight of the PCR product, a marker of 100-1500 bps was used.

Ethics declarations

The study protocol was received and approved by the Duhok Directorate General of Health, Directorate of Planning, Scientific Research Division, Institutional Ethics Committee (approval No. 13072021-7-10).

Table 1. Sequence and molecular weight of amplified primers.

Primer	Primer sequence (5' –3')	Amplified product (bp)	References
16S rDNA	**F:** GGGGGATCTTCGGACCTCA **R:** TCCTTAGAGTGCCCACCCG	956	(Spilker *et al*., 2004)
Opr1	**F:** ATGAACAACGTTCTGAAATTCTCTT **R:** CTTGCGGCTGGCTTTTTCCAG	249	(De Vos *et al.*, 1993)
OprD	**F:** CGCCGACAAGAAGAACTAGC **R:** GTCGATTACAGGATCGACAG	1413	(Rodrigues- martinez *et al*., 2009)

Table 2. The amplification condition of tested primers

Genes	Temperature (°C) /Time				
	Initial Denaturation	Cycling condition			Final Extension
		Denaturation	Annealing	Extension	
16S rDNA	95 °C; 2min one cycle	94 °C; 20 sec	54 °C; 20 sec	72 °C; 40 sec	72 °C; 5min one cycle
		25 cycles			
Opr1	95 °C; 2min one cycle	94 °C; 40 sec	57 °C; 50 sec	72 °C; 20sec	72 °C; 5min one cycle
		25 cycles			
OprD	95 °C; 5min one cycle	95 °C; 45 sec	61 °C; 45 sec	72 °C; 2min	72 °C; 10min one cycle
		30 Cycles			

3. Results

During the present study, 110 samples were collected from hospitalized burn patients attending the Burn and Plastic Surgery Hospital in Duhok City, Iraq. Out of the samples examined, 71 of them were found to be positive for *P. aeruginosa* at a rate of (64.54**%**) followed by other bacterial species at lower rates, as shown in Fig. 1. The infection rate was higher in females than males (59.15% vs 40.84%) as indicated in Table 3. The antibiotic sensitivity pattern of the isolated *P. aeruginosa* against the 10 tested antibiotics is listed in Table 4. *P. aeruginosa* showed high levels of resistance towards the majority of tested antibiotics, with the highest level of resistance being detected against Piperacillin at a rate of (98.59%) followed by Cefepime (88.73%), Ceftazidime (85.91%), and Meropenem (78.87%). While moderate resistant levels were detected against the other antibiotics, such as Amikacin (74.64%), Gentamicin (71.83%), Ciprofloxacin and Levofloxacin both (69.01%), and Imipenem (61.97%). However, the antibiotic *P.*

aeruginosa was most sensitive to was found to be Colistin since all of the tested isolates 71/71 showed a total sensitivity at a rate of (100%). Based on the results of the antibiotic sensitivity test (54/71) of *P. aeruginosa* was found to be multidrug-resistant (MDR) at a rate of 76.06%. All of the phenotypically identified *P. aeruginosa* isolated 71/71 were further confirmed genetically by using a species-specific primer (*16S rDNA*) and producing a positive single band with a molecular weight of 956 bps as shown in Fig. 2. Depending on the results from the antibiotic susceptibility test, out of the 71 *P. aeruginosa* isolates, 54 of them were detected to be MDR and suspected to harbor genes coding for virulence factors such as *Opr1* and *OprD*. The distribution of the investigated virulence genes among isolated *P. aeruginosa* is shown in Table 5 and Fig. 3 and 4.

The most common gene detected out of the 54 suspected multidrug-resistant *P. aeruginosa* was *OprD*, with a rate of (61.11%) followed by *Opr1* at a slightly lower rate (57.4%). While the presence of both genes in the same bacterial isolate was detected in 24/54 of the isolates at a rate of (44.44%).

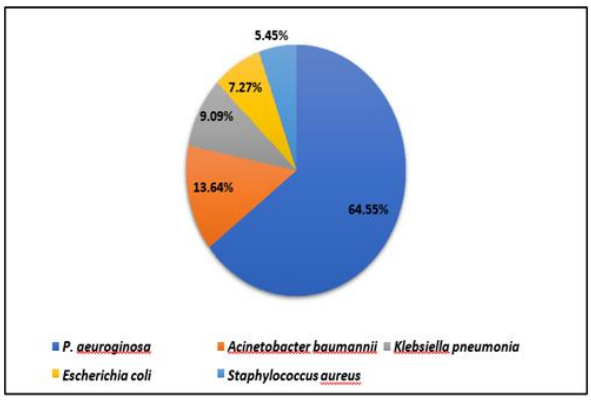

Fig. 1. Percentage of bacterial species isolated from burn patients.

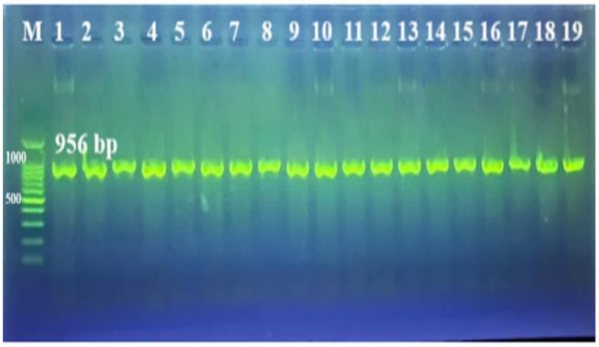

Fig. 2. Illustrating *16S rDNA* gene amplification by PCR with 1.5% agarose gel electrophoresis, showing amplicons each of 956 bp. Lane M: DNA marker with 100 bp, with lanes (1-19) showing positive bands produced.

Table 3. Distribution of *P. aeroginosa* among both genders

Examined burn samples	(+ve) sample for *P. aeruginosa* No. (%)	Gender	
		Female No. (%)	Male No. (%)
110	71 (64.54)	42 (59.1)	29 (40.8)

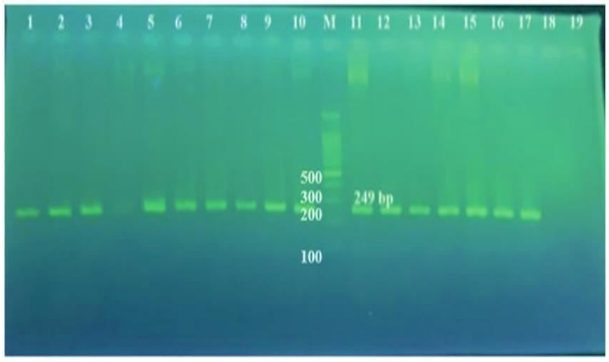

Fig. 3: Illustrating *Opr1* gene amplification by PCR with 1.5% agarose gel electrophoresis with an amplicon size of 249 bp. Lane M: DNA marker of 100 bp, lanes (1-17) showing the produced bands.

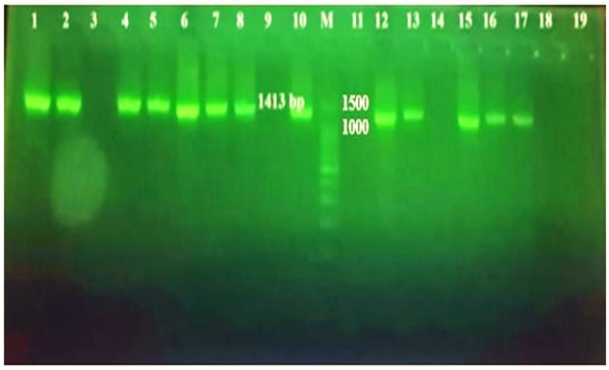

Fig. 4: Illustrating *OprD* gene amplification by PCR with 1.5% agarose gel electrophoresis, with an amplicon size of 1413 bp. Lane M: DNA marker of 100 bp, lanes 1- 17 showing the produced bands.

Table 4. Antibiotic sensitivity patterns of *P. aeruginosa.*

Antibiotic disc	Code	Resistance No. (%)	Sensitive No. (%)
Colistin	CL	0	71 (100)
Imipenem	IMP	44 (61.97)	27 (38.02)
Amikacin	AK	53 (74.64)	18 (25.35)
Ciprofloxacin	CIP	49 (69.01)	22 (30.98)
Levofloxacin	LEV	49 (69.01)	22 (30.98)
Gentamicin	CN	51 (71.83)	20 (28.16)
Meropenem	MEM	56 (78.87)	15 (21.12)
Ceftazidime	CAZ	61 (85.91)	10 (14.08)
Cefepime	FEP	63 (88.73)	8 (11.26)
Piperacillin	PI	70 (98.59)	1 (1.40)

Table 5. Distribution of virulence genes.

Virulence genes	No. (%)
Opr1	31/54 (57.4)
OprD	33/54 (61.11)
Opr1 + OprD	24/54 (44.44)

Discussion

The skin functions as a physical barrier that keeps out harmful bacteria that can be compromised in burn injuries. Another significant factor contributing to the development of microbes in burn wounds is disrupting the vasculature beneath the skin (Church et al., 2006). In numerous underdeveloped nations, burn injuries pose a significant threat to public health, causing a great deal of suffering, illness, and even death (Lami and Al Naser, 2019).

Based on the results obtained during the present study, out of the collected and examined burn swab samples, a rate of (64.54**%**) were found to be positive for *P. aeruginosa.* Similarly high rates of *P. aeruginosa* were reported in some studies on infected burns in Iraq. Al-Salihe (2022) reported a rate of 54.54% in Al-Najaf, in Baghdad, a rate of 60.9% by Al Abood (2022), and in Basra, 97.6% by Alkhulaifi and Mohammed (2023a). In Algeria, a high rate 62% of burn infections caused by *P. aeruginosa* have been reported by Meradji et al. (2016). However, results from worldwide studies showed lower occurrence of *P. aeruginosa* in infected burns, including Egypt (19.8%), Morocco (15.1%), Ghana (30.7%), and Pakistan (24.95%) (Mahmoud et al., 2013; Essayagh et al., 2014; Richcane et al., 2017; Chaudhary et al., 2019), respectively. On the other hand, a study conducted in India revealed that the most prevalent pathogen contaminating burn wounds was *Acinetobacter* (Roopa and Sanath, 2015). While other studies stated that the most common organism detected in burn infections was *S. aureus* (Altoparlak et al., 2004; Erol et al., 2004).

This variation in the types of encountered bacteria could be caused due to several factors such as various hospital infection control methods, abuse of antibiotics, environmental factors and hygiene (Kulkarni et al., 2015). In burn infections, *P. aeruginosa* was the most prevalent, tolerant, and dangerous microorganism. This bacterium can produce a variety of virulence factors, including extracellular and cell-associated virulence factors such as elastases, Type III protein secretion, alginate, and pyocyanin. All these factors contribute to its high rate of pathogenicity (Crousilles et al., 2015).

Regarding the gender of infected patients, the highest rate of infected burns (59.15%) among burn patients was found to be in females. Similar results were reported in a study carried out in Duhok/city, which also recorded the highest rate at 55% of burn infections among females (Oumeri and Yassin, 2021). Similarly, Al-Salihe (2022) in Al-Najaf, Iraq, reported a higher rate of 60.90% of infected burns among females. Furthermore, studies from other countries also reported higher rates of burn infections among females, such as 76.4% in India (Datta et al., 2016), 70% in Nepal (Pujji et al., 2019), and 76.8% in India (Roy et al., 2022). The explanation behind this could be that women are more likely responsible for cooking duties, a common practice in Eastern countries that exposes them to the risk of burns.

However, in another study conducted in Duhok, Iraq, by Qader et al. (2020) reported that 63.5% of males were affected by burns which is inconsistent with the current study. Also, in studies from other countries like Ghana by Richcane et al. (2017) and Pakistan by Chaudhary et al. (2019) were males had a higher infection rate with burns, 59.3% and 55%, compared to females, which has been reported. This may be attributed to the fact that

men are more likely than women to experience burn injuries caused by work hazards (Chaudhary et al., 2019).

According to the antibiotic sensitivity test results all *P. aeruginosa* isolates showed 100% sensitivity to Colistin. This outcome is consistent with research done in Bahrain and Egypt that found all *P. aeruginosa* isolates were Colistin-sensitive (Joji et al., 2019; Mohamed et al., 2022). Some studies reported slightly lower sensitivity rates of *P. aeruginosa* to Colistin, in Al-Najaf province 96.2%, in Wasit 98.03%, and in Erbil 96% (Rasool, 2015; Hussein and Shamkhi, 2018; van Burgh et al., 2019), respectively.

According to this study, Colistin might be the best medication for treating *P. aeruginosa* infections, since a 100 % susceptibility rate was recorded. The last treatment option in infections caused by gram-negative bacteria that are resistant to antibiotics has been Colistin, especially those that produce carbapenemase *Enterobacterales*, *P. aeruginosa*, and *Acinetobacter baumannii* (Azzopardi et al., 2013). Since 1980, the use of Colistin has been discontinued because of dose-related nephrotoxicity (Rasool, 2015). Levels of *P. aeruginosa* sensitivity towards Colistin reaching up to 100% have been reported in the majority of countries in the Middle East and North Africa (Al-Orphaly et al., 2021). It is recommended to use Colistin in conjunction with other antimicrobials primarily as a salvage treatment.

High levels of *P. aeruginosa* resistance towards Piperacillin (98.59%) were detected in the current study. Reports of variable resistance rates of *P. aeruginosa* to Piperacillin in previous studies such as 94%, 91.94%, 88.46%, 85%, and 74.8% have also been detected (Adjei et al., 2018; Dash et al., 2019; Mahdi, 2020; Qader et al., 2020; and Haghighifar et al., 2021), respectively. An important reason why *P. aeruginosa* can become resistant to beta-lactams is that it produces enzymes known as beta-lactamase, such as Penicillinase, which can result in the breakdown of the beta-lactam ring in the nucleus of Penicillins and Cephalosporins, rendering them useless as antibiotics. Additionally, it produces broad-spectrum enzymes called ESBLs (Jalil et al., 2017).

The level of *P. aeruginosa* resistance to Ceftazidime detected in the current study was 85.91%. While variable rates of resistance of this bacterium to Ceftazidime have been reported in other studies in various Iraqi provinces, such as 91% in Duhok (Qader et al., 2020) in Baghdad 85.50% (Al Abood, 2022) and in Basrah 98.95% (Alkhulaifi and Mohammed, 2023b). The resistance rate of Cefepime was 88.73%. Somewhat this rate is close to the rate reported by Mahdi (2020) in Basrah which was 84.61%. While high resistance levels 93% in Duhok was reported by Qader et al. (2020). Several recent studies have demonstrated that Carbapenem resistance is expanding globally (Vatansevera et al., 2020; Mimura et al., 2020). The level of Meropenem resistance detected in this study was 76.92%. While variable rates of to Meropenem in different provinces in Iraq have been detected, such as 95% in Duhok (Qader et al., 2020), in Basrah 19.23% (Mahdi, 2020), in Diyala 41.7% (Saleh, 2021) and in Baghdad 26.0% (Al Abood, 2022).

According to this study, the rate of Imipenem resistance was 61.97%. However, studies conducted in Iraq revealed varying resistance rates to this antibiotic, such as 47% in Duhok (Qader et al., 2020), in Basrah 30.76% (Mahdi, 2020), in Diyala 50% (Saleh, 2021), in Baghdad 28.98% (Al Abood, 2022) and in Basra 68.40% (Alkhulaifi and Mahmmed, 2023b). The resistance is caused by some mechanisms. The most frequent mechanism might be due to intrinsic loss or a decrease in the porin protein OprD, which is important in the uptake of carbapenem (Richardot et al., 2015). Furthermore, by ejecting these substances into the extracellular environment, overexpression of efflux pumps belonging to the resistance-nodulation-division (RND) family, particularly the MexAB-OprM efflux pump, contributes ominously to the resistance of *P. aeruginosa* to carbapenems (Aguilar-Rodea et al., 2022).

The level of MDR *P. aeruginosa* 76.06% detected in the present study was found to be higher than the results reported in other studies such as in Iran (16.5- 41%), and Iraq (12.4%)

(Mirzaei et al., 2020; Ahmadian et al., 2020; Alkhudhairy and Al-Shammari, 2020). While it is consistent with other studies such as Brazil (71.4%) and Egypt (70%) (De Almeida et al., 2017; Kishk et al., 2020). However, rates higher (89.24%) of MDR *P. aeruginosa* than the results from the present study have been reported in Iran (Khoshnood et al., 2019).

There are three main methods through which *P. aeruginosa* can develop resistance against antibiotics: intrinsic, acquired, and adaptive, *P. aeruginosa* possesses inherent resistance in the form of reduced permeability of the outer membrane, ejecting antibiotics out of the cell through the production of efflux pumps, in addition to the generation of enzymes that render drugs inactive. The horizontal transfer of resistance genes or mutations are the mechanisms through which *P. aeruginosa* can acquire resistance (Breidenstein et al., 2011; Hong et al., 2016). One possible explanation for the global rise of multidrug-resistant *P. aeruginosa* is the extensive use of antibiotics in both healthcare settings and the general public, which has led to several resistance mechanisms being developed (Horcajada et al., 2019). The result of this study indicated that all of the 71 isolates analyzed were found to be *P. aeruginosa*, and they produced an amplicon size of 956 bps for the 16S rDNA gene. This strategy has been utilized in multiple investigations due to its reliability and speed. All of *P. aeruginosa* isolates tested in the present study were found to be positive for the 16S rDNA gene, which is in agreement with a local investigation conducted by Qader et al. (2020) in Duhok also. Similarly, in studies performed in other parts of Iraq, they used this gene for *P. aeruginosa* identification, including Baghdad (Jaafar et al., 2014; Al Abood, 2022), Basrah (Jalil et al., 2017), and Diyala province (Al-Saadi, 2020 and Alhayali, 2021). Due to its simplicity and reliability, the 16S rDNA gene is used in the identification of *P. aeruginosa*. The high molecular identification rate suggests that genomic experiments were crucial in determining the precise taxonomic role of *P. aeruginosa* (Mohammed et al., 2015).

The conventional PCR results for the *Opr1* gene in the current study showed that 57.4% of *P. aeruginosa* isolates were found to be carrier of this gene. The findings of this study are comparatively lower than those reported in Baghdad, Iraq, by Ahmed (2017), and Duhok, Iraq, by Qader et al. (2020), which were 87.5%. Conversely, investigations carried out in Egypt and Baghdad/Iraq recorded a high percentage (100%) of this gene (Khattab et al., 2015; Al-Mayyahi, 2018; Al Abood, 2022) respectively. The *Opr1* gene produces lipoprotein, which is a constituent of *P. aeruginosa* outer membranes. Because directly it affects the permeability of bacterial cell membranes, this virulence factor gene also functions as a component of the efflux pump systems for antibiotics and toxins that impact bacterial cells, this prevents antibiotics and toxins from harming bacterial cells. Consequently, there is a rise in resistance (Firoved et al., 2004). Furthermore, the integrity of the bacterial membrane and the shape of the cell are preserved by the *Opr1* gene, which encodes for outer membrane proteins (Lin et al., 2010). Therefore, the gene presence and their effective expression in bacteria results in increased resistance, and as a result, chronically infected patients are more likely to develop a severe *P. aeruginosa* infection due to the bacteria's weak resistance mechanisms and inability to cross their outer membrane barrier (Al Abood, 2022).

According to the study's findings, 61.11% of *P. aeruginosa* isolates had the *OprD*. These findings are higher than those from Tehran/Iran, which revealed that 56.6% of *P. aeruginosa* isolates have *OprD* (Mirsalehian et al., 2017). Conversely, the results of this study are much lower than other studies such as 100% in Baghdad/Iraq (Al-Hashimy et al., 2019), 90% in Nigeria (Nmema et al., 2019), and 83.3% in Diyala/Iraq by Saleh (2021). In *P. aeruginosa*, *OprD* encodes the outer membrane protein (*OprD*) porin, whose absence results in resistance to carbapenems, particularly imipenem (Nmema et al., 2019). The findings of the study indicated that *P. aeruginosa* is becoming more resistant to imipenem. Additionally, they showed that among *P. aeruginosa* isolates, point mutations were the most frequent reason for the inactivation of the *OprD* coding area upstream.

It appears that this process works well to make isolates resistant to imipenem (Kiani et al., 2021). The loss of the *OprD* porin is one of the processes by which clinical isolates are resistant to imipenem. Alterations such as substitutions, deletions, insertions, or mutations in *OprD* can alter the structure of *OprD* porin or prevent it from existing, leading to the development of carbapenem resistance (González-Vázquez et al., 2021).

Conclusion

Pseudomonas aeruginosa remains the most frequently isolated pathogen from burn patients. The findings of this study indicate that *P. aeruginosa* exhibits a high level of resistance to most of the tested antibiotics, with the exception of colistin, to which all isolates remained sensitive. Additionally, a high prevalence of multidrug-resistant (MDR) strains was observed among the isolates. The presence of virulence genes such as ***Opr1*** and ***OprD*** contributes to the organism's adaptability, its ability to cause persistent infections, and its various mechanisms of antibiotic resistance, all of which complicate treatment strategies. Given the limited number of effective antibiotics available, it is essential to perform antimicrobial susceptibility testing prior to initiating treatment. This approach not only aids in selecting appropriate therapy but also helps reduce the indiscriminate use of antibiotics, minimizes the risk of resistance development in burn units, and supports the formulation of effective treatment plans.

Conflicts of Interest: The authors have no conflicts of interest to declare for this study.

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