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Emerging Problems in Infectious Diseases

Molecular study of carbapenem-resistant *Pseudomonas aeruginosa* causing wound infection in an Egyptian tertiary hospital

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Abstract

Introduction: *Pseudomonas aeruginosa* is a bacterial pathogen that causes various acute and chronic human infections, including wound and burn infections, with serious consequences. This study aimed to determine the antimicrobial resistance profile of *P. aeruginosa* isolated from wound infections and investigate the molecular mechanism of carbapenem resistance.

Methodology: Forty-nine *P. aeruginosa* wound infection isolates were collected from a tertiary care hospital in Cairo, Egypt, from September 2022 to September 2023. The resistance profile of *P. aeruginosa* isolates was determined by the disc diffusion method, minimum inhibitory concentration (MIC) of meropenem susceptibility, and detection of metallo-β-lactamase (MBL) production by imipenem-EDTA combined disc test. Polymerase chain reaction (PCR) was utilized to identify the carbapenem resistance genes, bla_{KPC}, bla_{NDM-1}, and bla_{OXA-48} among carbapenem-resistant *P. aeruginosa* (CRPA) isolates. The ERIC-PCR was used to assess the genetic diversity and relatedness among CRPA isolates. The results were presented as descriptive statistics in percentages and relative frequencies.

Results: The findings revealed that 44.9% (22/49) of *P. aeruginosa* isolates were multidrug-resistant (MDR), meropenem resistant, and MBL producers. PCR assays showed that out of 22 CRPA isolates, six isolates (6/22, 27.3%) harbored the bla_{NDM-1} gene, and three (3/22, 13.6%) carried the bla_{OXA-48} gene, while none of the isolates had the bla_{KPC}. ERIC-PCR-based genotyping demonstrated a significant molecular heterogeneity, indicated by 16 ERIC-based patterns or fingerprints among 22 CRPA isolates.

Conclusions: The resistance profile demonstrated by *P. aeruginosa* in wound infections suggests the need for effective hospital infection control and antibiotic policies in developing countries. The CRPA isolates were polyclonal, highlighted by their substantial genetic heterogeneity.

Key words: *Pseudomonas aeruginosa*; multidrug resistant; carbapenem; wound; infection.

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Introduction

Microbial infection is the primary cause of delayed wound healing, particularly in primary closure, traumatic wounds, burns, and chronic skin ulcers [1]. It has been found that the infection of wounds with *Pseudomonas aeruginosa* (*P. aeruginosa*) can lead to more serious consequences. Compared to other types of wounds, those infected with *P. aeruginosa* are both larger and more persistent [2]. *P. aeruginosa*, a common opportunistic human pathogen, causes various chronic and acute illnesses, such as burn and wound infections, sepsis, soft tissue infections, bacteremia, urinary tract infections, and ventilator-associated pneumonia. Additionally, *P. aeruginosa* is likely to infect patients with cystic fibrosis and those with traumatic injuries [3]. Moreover, *P. aeruginosa* is widely recognized as a leading bacterial species implicated in hospital-acquired infections due to its remarkable adaptability to thrive in various environments, including medical devices and surfaces [4,5].

The issue of antimicrobial resistance (AMR) has become a critical public health challenge on an international level. The extensive and frequently inappropriate use of antimicrobial drugs, including carbapenems, in agriculture and healthcare has been strongly linked to the increased occurrence of MDR pathogens, leading to more than 70,000 deaths yearly [6,7]. *P. aeruginosa* is an essential member of the six highly virulent and antimicrobial-resistant ESKAPE bacterial pathogens group, which includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* spp. Notably, the World Health Organization (WHO) has designated carbapenem-resistant *P. aeruginosa* (CRPA as one of

the top priority or "critical" bacterial pathogens that urgently require the development of new antimicrobial therapies [8]. Despite inherent resistance to a wide range of antimicrobial agents, *P. aeruginosa* can develop resistance to different antimicrobial classes through mutations or the acquisition of antimicrobial resistance encoding genes [9]. The antimicrobial resistance mechanisms in *P. aeruginosa* include efflux pumps mediated, β-lactamase production, biofilm formation, porin-related resistance, and target site alteration [10]. Consequently, CRPA may exhibit resistance to other antimicrobial classes; such infections are associated with limited therapeutic choices and high mortality and morbidity rates [11].

Carbapenem drugs are the last therapeutic option to treat MDR *P. aeruginosa* infections [12]. Carbapenems are β-lactam drugs that act by binding to penicillin-binding proteins (PBPs), thereby preventing bacterial cell wall synthesis [10]. There are three types of carbapenemase enzymes: A, D (serine carbapenemases), and B (metallo-β-lactamases). The development of resistance to carbapenem drugs is attributed to these enzymes. It is important to highlight that *P. aeruginosa* has been found to carry genes that encode for carbapenemases class A (bla_{KPC}), class B (bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{SIM}, $bla_{SPM,}$ and bla_{GIM}), and class D (bla_{OXA-48}). Genes encoding carbapenemases are commonly found on mobile genetic elements that can rapidly disseminate among bacterial populations [11].

Understanding the resistance profiles of *P. aeruginosa*, particularly in the context of wound infections, is crucial for implementing strategies to combat antimicrobial resistance. Furthermore, recognizing CRPA and the prevalence of carbapenemase genes might help select appropriate antibiotics for treating infections caused by these resistant strains. Additionally, investigating the genetic relationships among CRPA isolates can aid in implementing effective antibiotic stewardship programs to preserve the effectiveness of carbapenems and other critical antibiotics. Accordingly, the current study aimed to determine the resistance profile of *P. aeruginosa* causing wound infections against diverse antimicrobial classes and the phenotypic identification of CRPA among the collected *P. aeruginosa* isolates. The study also aimed to find the most common carbapenemases encoding genes, bla_{KPC}, $bla_{NDM-1,}$ and bla_{OXA-48}, besides determining the genetic relationship among CRPA isolates using enterobacterial repetitive intergenic consensus (ERIC)-based genotyping.

Methodology

Isolation and identification of P. aeruginosa from wound infection clinical samples

For the present study, 49 non-repetitive *P. aeruginosa* isolates originating from wound infections were collected from the microbiology laboratory at El-Demerdash Tertiary Care Teaching Hospital, Ain Shams University, Cairo, Egypt, over the period from September 2022 to September 2023. Isolates were identified through the utilization of biochemical tests, colonial morphology, Gram staining, catalase production, oxidase production, growth on triple-sugar-iron agar, motility determination via the semisolid-agar stabbing method, and indole, fluorescein, and pyocyanin production on cetrimide agar (Oxoid, Basingstoke, Hants, England) [13]. Additionally, the identification of isolates as *P. aeruginosa* was confirmed by the automated system VITEK 2 (bioMérieux, France). All isolates were preserved in brain heart infusion broth (Oxoid, Basingstoke, Hants, England) supplemented with 30% glycerol (Merck, Germany) at - 20 °C until required for further analysis.

Antimicrobial susceptibility profiling

Disc diffusion method

The antimicrobial resistance profile of *P. aeruginosa* isolates was determined using the Kirby-Bauer disc diffusion method on Mueller Hinton agar (Oxoid, Basingstoke, Hants, England) according to Clinical and Laboratory Standards Institute (CLSI) criteria [14]. The standard antimicrobial discs employed in this study were as follows: ceftazidime (CAZ 30 µg), aztreonam (ATM 30 µg), piperacillin-tazobactam (TZP 100 µg/10 µg), imipenem (IPM 10 µg), meropenem (MEM 10 µg), levofloxacin (LEV 5 µg), amikacin (AK 30 µg) and polymyxin B (300 units) (Bioanalyse Co., Ltd., Ankara, Turkey). *P. aeruginosa* isolates were classified as MDR /or non-MDR isolates according to Magiorakos *et al*. definition of MDR bacteria when they are resistant to at least one antimicrobial agent from three or more different antimicrobial classes [15].

Determination of the minimum inhibitory concentration (MIC) of meropenem

For the susceptibility testing of *P. aeruginosa* isolates to meropenem (Sigma-Aldrish- Schnelldorf, Germany), the MIC of meropenem was determined using broth microdilution method and interpreted following the CLSI criteria including sensitive (S) when MIC ≤ 2, intermediate (I) when MIC = 4 and resistant (R) when MIC ≥ 8 [14].

Phenotypic detection of Metallo-β-lactamase (MBL) production by combined disc test (CDT)

The combined disc test (CDT) was used for phenotypic detection of MBL production. The IMP-EDTA CDT was carried out following an established protocol [16]. Briefly, *P. aeruginosa* isolates were inoculated onto Mueller Hinton agar plates. Then, one IMP disc (10 g) was put on one side of the agar plate, and an IMP-EDTA disc (750 g) (Bioanalyse Co., Ltd., Ankara, Turkey) was placed on the other side. After an 18-hour incubation at 35 °C, the inhibition zones of imipenem and imipenem-EDTA discs were measured and analyzed. An isolate was considered to produce MBL positively if the inhibition zone with the IMP-EDTA disc exhibited an increase of more than 7 mm compared to the inhibition zone with the IMP disc alone [16].

Molecular study for the detection of carbapenem resistance genes

DNA extraction

The DNA extraction from *P. aeruginosa* isolates was performed utilizing the boiling method, as previously described [12]. A few colonies were suspended in TE (Tris-EDTA) buffer and boiled at 100 °C for 10 minutes. Subsequently, the mixture was centrifuged at 14000 rpm using a cooling centrifuge for 3 minutes. The supernatant containing DNA was utilized as a template for polymerase chain reaction (PCR) amplification. The extracted DNA concentration was measured using a biophotometer (Eppendorf, Germany) at a wavelength range of 260 − 280 nanometres and stored at - 20 °C until use [13].

PCR-based detection of carbapenem resistance encoding genes

The oligonucleotide primers of three carbapenem resistance genes representing the three main carbapenemases, class A (bla_{KPC}), class B (bla_{NDM-1}), and class D (bla_{OXA-48}), were used for PCR experiments [17]. The primer's sequence and amplicon size are presented in Table 1. PCR protocol was carried out employing a 25 µL reaction mixture consisting of 2.4 µL of DNA, 0.8 µL of 20 µM each forward and reverse primers, 12.5 µL of PCR master mix (Thermo Scientific™ DreamTaq Green PCR Master Mix (2x) (Fermentas, Thermo Fisher Scientific, Schwerte, Germany) and 8.5 µL of DNAase-free water. Thermocycling was conducted in a thermal cycler (Bio Cycler TC-S, BOECO, Germany) with the following conditions: 95 °C for 5 minutes, then 35 cycles of 94 °C for 60 seconds, 56 °C for 60 seconds, and 72 °C for 60 seconds, and a final extension step at 72 °C for 7 minutes.

ERIC-PCR fingerprinting

ERIC-PCR can be utilized to determine genetic diversity, phylogenetic relationships, and genomic DNA fingerprints in Gram-positive and Gram-negative bacterial pathogens. Recently, ERIC-PCR has been used to analyze opportunistic infections such as *P. aeruginosa* and *A. baumannii* infections, which can cause epidemics in hospitals worldwide [18]. ERIC-PCR amplification was performed on extracted DNA using previously reported ERIC primers (Table 1) [19]. The PCR reactions were prepared in 25 µL total volumes, with 12.5 µL of PCR master mix kit (Thermo Scientific™ DreamTaq Green PCR Master Mix (2X) (Fermentas, Thermo fisher scientific, Schwerte, Germany), 1 µL of template DNA, 1 µL of ERIC-1 primer, 1µL of ERIC-2 primer, and 9.5 µL of nuclease-free water. The PCR amplifications were performed in a thermal cycler (Biometra UNO-Thermoblock, Germany) set for an initial denaturation at 94 °C for 5 minutes and 40 cycles of denaturation at 95 °C for 1 minute, primer annealing at 45 °C for 1 minute, extension at 72 °C for 8 minutes, and final extension at 72 °C for 10 minutes [20].

Detection of amplified PCR products by agarose gel electrophoresis

PCR products were resolved by gel electrophoresis using agarose (0.8 % high biology grade) (GIBCO Bethesda Research Lab., Life Technologies, Grand Island, NY, USA) in 1 × Tris-borate-EDTA (TBE) buffer (Thermo Scientific, Waltham, MA, USA). DNA fragments were electrophoresed (at 100 V and 90 mA for 30 minutes) in a horizontal gel electrophoresis

Table 1. Sequences of PCR oligonucleotide primers of carbapenem-resistance encoding genes and ERIC-based PCR.

Gene	Primer sequence (5' – 3')	Amplicon size (bp)	Reference
bla_{NDM-1}	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	621	[17]
bla_{OXA-48}	F: GCGTGGTTAAGGATGAACAC R: CATCAAGTTCAACCCAACCG	438	
bla_{KPC}	F: CGTCTAGTTCTGCTGTCTTG R: CTTGTCATCCTTGTTAGGCG	798	
ERIC PCR	ERIC-1: ATGTAAGCTCCTGGGGATTCAC ERIC-2: AAGTAAGTGACTGGGGTGAGCG	≥ 200	[19]

system (Cole Parmer, Germany), stained with ethidium bromide (Alliance Bio, Bothell, Washington, USA), and photographed directly using a UV transilluminator (Biometra, Göttingen, Germany). Gene Ruler 1 Kb DNA ladder (Thermo Scientific, Waltham, Massachusetts, USA) was employed to determine the sizes of the separated DNA fragments.

ERIC fingerprint analyses and dendrogram construction

The DNA banding patterns were entered into a database in BioNumerics 7.1 software (Applied Maths, Sint-Martens-Latem, Belgium). The ERIC-PCR patterns were interpreted and compared as described by Codjoe *et al.* [19]. For cluster analyses, similarity analysis was calculated using the Dice coefficient and the unweighted pair group method with arithmetic mean (UPGMA). The threshold for related clones or categorization into the same type was 80% or higher matching bands [19].

Statistical analysis

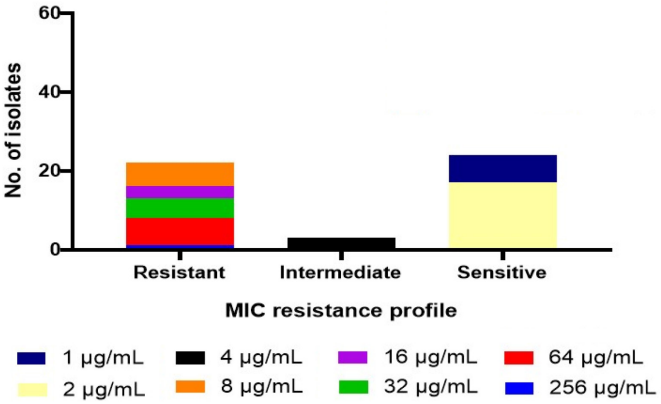
The results were presented as descriptive statistics in percentages and relative frequencies using GraphPad Prism software (GraphPad version 8.0.2., available at http://www.graphpad.com/prism/prism.htm). The Dice similarity coefficients were utilized to analyze ERIC-based fingerprints, and a dendrogram was constructed using the UPGMA algorithm.

Results

Antimicrobial susceptibility profile of P. aeruginosa isolates

According to the CLSI interpretive criteria for the disc diffusion assay, the resistance rates of the 49 *P. aeruginosa* isolates examined in this study to β-lactam antibiotics were as follows: 85.7% (42/49) exhibited resistance to ceftazidime, 20.4% (10/49) to piperacillin/tazobactam, 26.6% (13/49) to aztreonam, 44.9% (22/49) to imipenem, and 53.1% (26/49) to meropenem. For non-β-lactam antibiotics, the resistance rates were 49% (24/49) to amikacin, 71.4% (35/49) to levofloxacin, and only 4.1% (2/49) of isolates were resistant to polymyxin (Table 2). Of the *P. aeruginosa* isolates, 22 (22/49, 44.9%) were identified as MDR, exhibiting resistance to three or more classes of antimicrobials. Among the 22 MDR *P. aeruginosa* isolates, 19 isolates (86.4%) were MDR along with resistance to both imipenem and meropenem, and one MDR isolate (4.5%) was resistant to meropenem only. Of the non-MDR isolates, four isolates (4/27, 14.8%) were resistant to imipenem and meropenem, two isolates (2/27, 7.4%) were resistant to meropenem, and one isolate (1/27, 3.7%) was resistant to imipenem.

Figure 1. MIC profiles of carbapenems (meropenem) against *P. aeruginosa* isolates.



The MIC results for meropenem susceptibility testing revealed that the 22 MDR isolates were meropenem resistant (22/49, 44.9%); MICs ranged from 8 µg/mL to 256 µg/mL. The 22 MDR isolates were thus classified as CRPA for subsequent analysis. Additionally, 3 (3/49, 6.1%) isolates showed intermediate resistance (MIC = 4 µg/mL) while the other *P. aeruginosa* isolates, 24 (24/49, 49%), demonstrated susceptibility to meropenem with MICs of 1 − 2 µg/mL, as shown in Figure 1.

Metallo-β-lactamase (MβL) production among P. aeruginosa wound isolates

The IMP-EDTA-CDT assay was conducted on 22 (22/49, 44.9%) CRPA isolates. The findings indicated that the 22 CRPA isolates were MBL producers, as

Table 2. Antimicrobial resistance profiles of all *P. aeruginosa* isolates included in the study.

Antimicrobial agent	Susceptible (S)	Intermediate (I)	Resistant (R)
	No. (%) *	No. (%)	No. (%)
Ceftazidime	7 (14.3)	0 (0)	42 (85.7)
Aztreonam	28 (57.1)	8 (16.3)	13 (26.6)
Piperacillin-tazobactam	23 (46.9)	16 (32.7)	10 (20.4)
Imipenem	25 (51)	2 (4.1)	22 (44.9)
Meropenem	16 (32.6)	7 (14.3)	26 (53.1)
Levofloxacin	14 (28.6)	0 (0)	35 (71.4)
Amikacin	19 (38.8)	6 (12.2)	24 (49)
Polymyxin B	47 (95.9)	0 (0)	2 (4.1)

*Percentage was calculated to the total number of *P. aeruginosa* (n = 49).

evidenced by an increase in the inhibition zone to ≥ 7 mm around the IMP-EDTA disc, leading to the conclusion that these isolates are phenotypically positive for MBL production.

Genotypic detection of carbapenem resistance encoding genes

The 22 CRPA isolates that showed positive in IMP-EDTA-CDT were further investigated for the presence of three distinct carbapenem resistance genes: class A (*bla*$_{KPC}$), class B (*bla*$_{NDM-1}$), and class D (*bla*$_{OXA-48}$). Not all the phenotypically confirmed CRPA isolates carried the examined genes. PCR assays showed that out of 22 CRPA isolates, six isolates (6/22, 27.3%) harbored the *bla*$_{NDM-1}$ gene, and three isolates (3/22, 13.6%) carried the *bla*$_{OXA-48}$ gene, while none of the isolates harbored the *bla*$_{KPC}$ gene. Two isolates co-harbored *bla*$_{NDM-1}$ and *bla*$_{OXA-48}$ genes (Figure 2). Figure 3 shows the correlation between the phenotypic and genotypic resistance profiles of 22 CRPA isolates.

ERIC-PCR-based genotyping of the selected CRPA isolates

ERIC-PCR-based genotyping of the 22 CRPA isolates showed the significant molecular heterogeneity

Figure 2. PCR analysis of *P. aeruginosa* isolates on agarose gel electrophoresis. A, PCR products for amplification of metallo-β-lactamase gene *bla*$_{NDM-1}$ at 621 bp. B, PCR products for amplification of metallo-β-lactamase gene *bla*$_{OXA-48}$ at 438 bp.

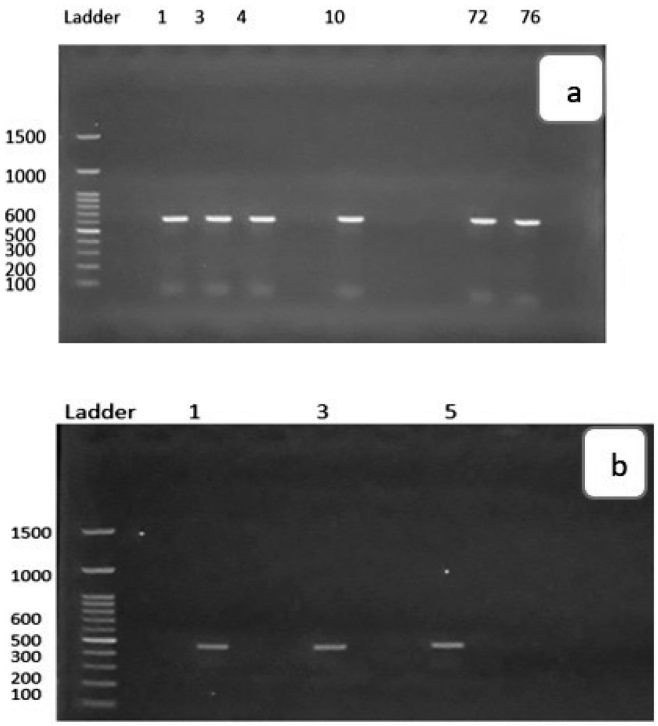
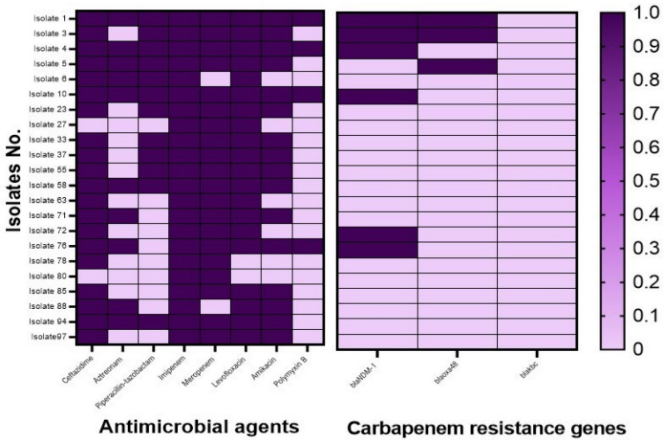


Figure 3. Distribution of screened phenotypic and genotypic resistance profiles among 22 CRPA isolates (Presence of phenotypic antimicrobial resistance and PCR genes indicated by deep violet; Absence of phenotypic antimicrobial resistance and PCR genes indicated by light violet). The gene *bla*$_{NDM-1}$ was the most prevalent carbapenemase-encoding gene among isolates. Thirteen phenotypically confirmed CRPA isolates did not carry any of the examined genes.

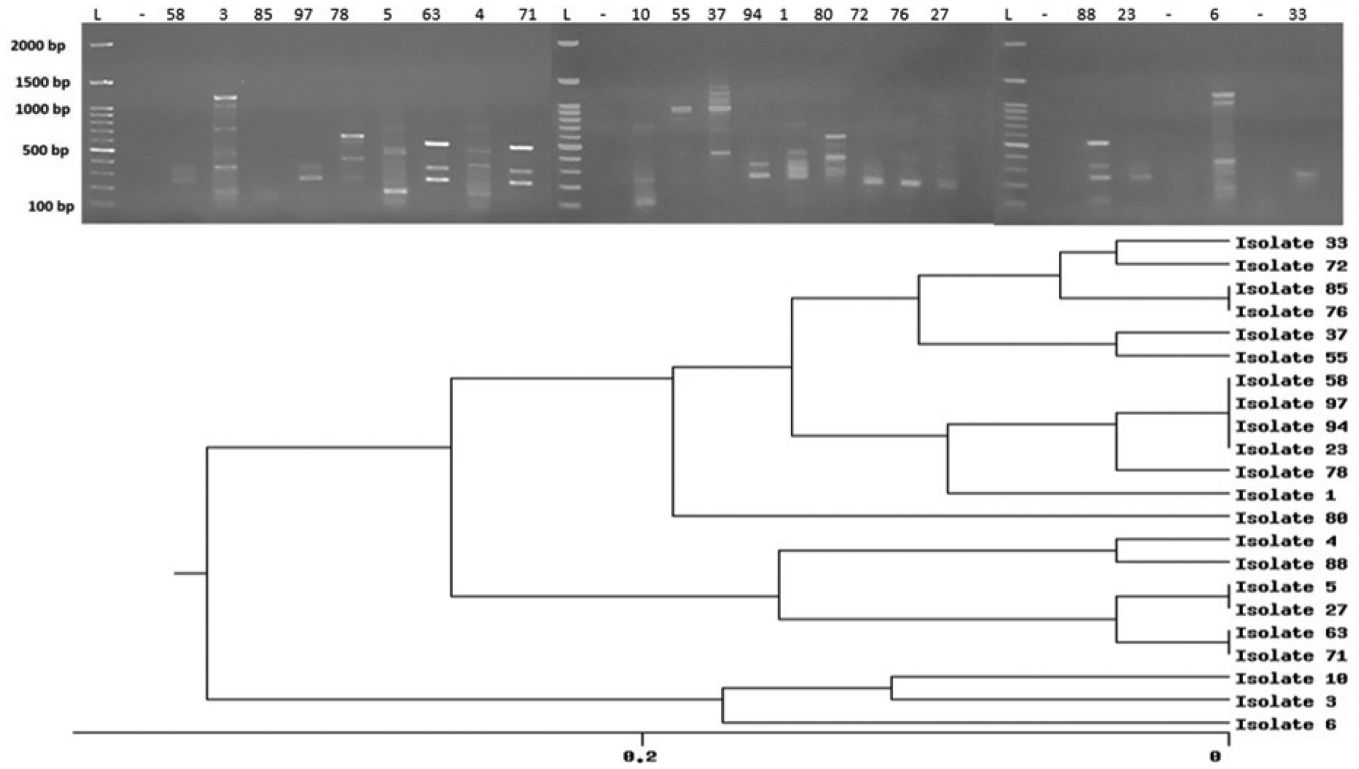


of these isolates, as evidenced by the identification of 16 distinct ERIC-based patterns or fingerprints. These fingerprints were arbitrarily numbered from 1 to 16 systematically. One pattern comprised four isolates; three patterns contained two isolates each, while all other patterns were represented by only one isolate (Figure 4).

Discussion

Wound infections are prevalent, which increases hospitalized patients' mortality and morbidity and raises healthcare costs [21]. Infections of wounds can cause delays in healing, leading to increased healthcare visits and prolonged hospital stays. Consequently, wound infections result in higher financial costs and negatively impact the quality of life for both patients and their families. Hence, the accurate and timely diagnosis of wound infection is crucial to effective wound infection management [22]. MDR *P. aeruginosa* presents a significant medical challenge as it is responsible for several diseases, including the majority of burn and wound infections, for which there are few effective treatments due to its harboring resistance mechanisms to multiple antimicrobial classes. Gaining insights into resistance mechanisms paves the way for developing new and effective therapies [23]. Accordingly, this study aimed to investigate the resistance profile of *P. aeruginosa* isolated from wound infections and phenotypic and genotypic identification of the resistance mechanism to carbapenems among CRPA isolates.

Figure 4. ERIC-based PCR patterns of the 22 MDR *P. aeruginosa* isolates representing different antibiotypes.



P. aeruginosa has been markedly associated with persistent wound infections and worse outcomes [24]. The current study included 49 *P. aeruginosa* isolates from wound infection clinical samples. Several factors contribute to wound infections with *P. aeruginosa*, including the severity of the condition, exposure to water, and the setting in a warm environment [24]. Furthermore, multiple studies have demonstrated that *P. aeruginosa* virulence traits can influence wound healing efficiency and interact with, and potentially enhance, the virulence factors of other pathogens at the same infection site [25-27].

In the current study, the resistance rates to β-lactam tested antibiotics among *P. aeruginosa* isolates varied from 20.4% for the piperacillin/tazobactam combination to 85.7% for ceftazidime. On the other hand, resistance rates for non-β-lactam antibiotics were 71.4% and 49% for levofloxacin and amikacin, respectively, with only 4.1% of isolates exhibiting resistance to polymyxin. Similar findings were reported, but higher resistance rates compared to ours, in an Iranian investigation, including 99 *P. aeruginosa* isolates from burn and wound infections, particularly against the anti-pseudomonal β-lactam (68% vs 85,7% for ceftazidime, 62% vs 44.9% for imipenem, 62% vs 53.1% for meropenem, 55.33% vs 49 % for amikacin and 66.67% vs 26.4% for aztreonam [28]. In addition, high resistance rates of *P. aeruginosa* isolates to several antimicrobials, including 65% vs 53.1% for meropenem, 62.5% vs 44.9% for imipenem, 60% vs 49 % for amikacin, 55% vs 71.4% for levofloxacin and 70% vs 85.7% for ceftazidime, were reported in a previous Egyptian study [29]. Besides, the results of the current study were relatively consistent with the Sambrano *et al*. [23] study from Brazil, which noted lower resistance rates among *P. aeruginosa* collected from different infection sites, including wounds and ulcers: 12% vs 85.7% for ceftazidime, 33% vs 44.9% for imipenem, 25% 53.1% for meropenem, and 2% vs 49 % for amikacin. Likewise, Kamali *et al*. [30] study from Iran revealed low resistance pattern of 22.5% vs 44.9% for imipenem, 15% vs 53.1% for meropenem, 17.5% vs 85.7% for ceftazidime, 12.5% vs 20.4% for piperacillin/tazobactam, 12.5% vs 49 % for amikacin and 23.75% vs 71.4% for levofloxacin [30]. These findings may indicate the resistance of *P. aeruginosa* bacteria from wound infections to the most clinically used antibiotics in developing countries, such as Egypt and Iran, where antibiotics are extensively used.

P. aeruginosa can develop resistance simultaneously to multiple antimicrobials that result in multidrug-tolerant persister cells through intrinsic and acquired mechanisms [31-33]. Overall, in the current study, the prevalence of MDR *P. aeruginosa* was

44.9%. This finding was comparable to the Sambrano *et al*. study in Brazil, which determined that 59% of the *P. aeruginosa* isolates were MDR [23]. A study conducted in Iran reported a prevalence rate of 66% for MDR *P. aeruginosa* [28]. Furthermore, a study in Egypt found that 69.1% of clinical *P. aeruginosa* isolates were MDR [32]. Another Egyptian study demonstrated that 21% of the *P. aeruginosa* isolates analyzed were MDR [13].

Globally, there has been an alarming increase in reduced carbapenem susceptibility in *P. aeruginosa* bacteria during the last decade [8]. In the current study, 44.9% of *P. aeruginosa* wound infection isolates showed resistance to meropenem antibiotic by broth microdilution assay (MIC ranged from 8 to 256 μg/mL). This finding closely resembles that of a recent study from Egypt, which found that 55.9% of *P. aeruginosa* isolates exhibited MIC values of at least 256 μg/mL for one or more of the examined carbapenems [13]. This rate of resistance raises the threat of restricting available medications. This might be explained by the increased use of carbapenems in recent years, as well as selective pressure from more carbapenem drug prescriptions. Many earlier investigations in Egypt indicated substantial carbapenem resistance rates among *P. aeruginosa* isolates [29,34].

Different phenotypic tests for identifying MBL production have been suggested as the initial screening step before verifying with molecular assays. It has been found that the IMP-EDTA-CDT test is a reliable, valuable, accurate, and cost-effective method that can be implemented in any laboratory setting without requiring specialized equipment [35]. The IMP-EDTA-CDT testing in the present study indicated that all carbapenem-resistant isolates were MBL producers, consistent with previous studies [13,36]. Remarkably, aztreonam and polymyxins are the therapeutic options for treating critically infected patients with such CRPA-producing MBL due to their stability against hydrolysis by MBL and lower resistance rates, as they are not commonly used for treating infections in Egypt [13].

The identification of carbapenem resistance by the conventional disc diffusion method presents a challenge. The Clinical and Laboratory Standards Institute (CLSI) has recommended several inhibition-based phenotypic methods for carbapenemase detection, including the combination disc test, modified Hodge technique (MHT), and double-disc synergy assay. However, the PCR-based molecular technique is recommended as a reliable method for identifying the most prevalent genes in carbapenem-positive clinical isolates [11]. PCR-based tests for identifying carbapenemases encoding genes revealed that 27.3% of CRPA isolates harbored the $bla_{\text{NDM-1}}$ gene, and 13.6% carried the $bla_{\text{OXA-48}}$ gene. Two isolates co-harbored bla_{NDM1} and $bla_{\text{OXA-48}}$ genes. However, the bla_{KPC} gene was not detected in any of the isolates. This finding is comparable to an Egyptian study, which reported that $bla_{\text{NDM-1}}$ was the most prevalent carbapenemase-encoding gene among CRPA [13]. Similarly, a study in South India reported that $bla_{\text{NDM-1}}$ was detected in 17.3% of CRPA isolates; however, bla_{KPC} or $bla_{\text{OXA-48}}$ genes were not found [37]. A study in Colombia detected only the bla_{KPC} gene in one CRPA isolate [38]. Two other studies in China and Dubi indicated that no CRPA isolates carried the $bla_{\text{NDM-1}}$ or bla_{KPC} genes [39,40]. Conversely, a recent study in Egypt showed increased prevalence rates of carbapenemase-encoding genes with bla_{KPC}, $bla_{\text{NDM-1}}$, and $bla_{\text{OXA-48}}$ genes being identified in 67.7%, 30.3% and 26.7% of CRPA isolates, respectively [41]. Furthermore, research in Saudi Arabia detected a greater incidence of carbapenem resistance genes among CRPA isolates: $bla_{\text{OXA-48}}$ (46.88%), bla_{NDM} (37.5%), $bla_{\text{NDM-1}}$ (15.63%), while bla_{KPC} was detected in only one isolate [11]. Some CRPA isolates did not harbor the investigated genes, which may indicate the presence of other genes or mechanisms of resistance.

The findings of this study indicate a concerning rise in CRPA bacteria producing NDM-1 in Egypt, warranting serious attention. The first documentation of *P. aeruginosa* carrying $bla_{\text{NDM-1}}$ in Egypt was in 2014, when the authors demonstrated that two *P. aeruginosa* isolates out of 33 (6%) were carbapenem-resistant and possessed $bla_{\text{NDM-1}}$ [42]. The inappropriate and excessive carbapenem prescribing in Egyptian hospitals has resulted in selective pressure and the emergence of CRPA [43]. Carbapenemases can induce resistance to carbapenems and other β-lactam antibiotics, including β-lactam/β-lactamase inhibitor combinations [44]. This broad resistance spectrum may be responsible for worse outcomes in patients with CRPA infections. Furthermore, because most quick diagnostic techniques for carbapenem resistance rely on identifying carbapenemase genes, the efficacy of these tests for detecting CRPA is dependent upon the prevalence and types of carbapenemases present in these organisms [44,45].

ERIC-PCR-based genotyping aids in the epidemiological inquiry and the better understanding of disease acquisition and transmission, as well as determining if there was patient-to-patient transmission or a common source of infection as an exogenous source or whether it was predominantly an endogenous

source [18]. ERIC-PCR-based genotyping of 22 CRPA isolates, which represent the highly resistant antibiotypes, showed considerable molecular heterogeneity of these isolates, as evidenced by 16 distinct ERIC-based patterns or fingerprints. Additionally, one pattern included four isolates, three patterns each included two isolates, and the remaining patterns contained only one isolate each. The banding patterns of the isolates from patients exhibited substantial variation, indicating a significant genetic diversity of CRPA isolates in our investigation. This suggests that these bacterial isolates were polyclonal as they exhibited varied genetic fingerprint patterns, and there was no shared source of *P. aeruginosa* wound infections. This finding was consistent with a study in Egypt, which reported that most of the tested CRPA isolates were genetically unrelated by ERIC-PCR typing [41]. Furthermore, Iranian research revealed that most isolates showed unique patterns, i.e. 50 single patterns among their tested MDR *P. aeruginosa* isolates, indicating that the rate of transmission of resistant strains was low in their study hospitals [28]. The current study's limitation is that it did not address clinical data or the therapy regimen and response related to the obtained isolates, preventing a complete demonstration of the CRPA infections and wound healing outcomes picture. According to the study findings, the incidence of *P. aeruginosa* in wound infections was not addressed in this study since *P. aeruginosa* isolates were the only ones aimed for, and the total number of wound isolates within a given period was unavailable.

Conclusions

P. aeruginosa bacteria causing wound infections have a high frequency of carbapenem resistance, revealed by a 44.9 % resistance rate to imipenem in this study. The increasingly serious situation with diminished susceptibility of *P. aeruginosa* to carbapenems emphasizes the necessity of an in-depth look into this problem. Studying the molecular mechanisms underlying antimicrobial resistance will help in efficiently managing CRPA-infected patients and adopting effective infection control strategies. The $bla_{\text{NDM-1}}$ gene was the most prevalent among CRPA isolates, while the bla_{KPC} gene was not detected at all. CRPA isolates exhibited significant molecular heterogeneity; therefore, the ERIC-PCR-based genotyping approach could be employed to investigate the genetic diversity or relatedness of *P. aeruginosa* isolates in clinical settings.

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Conflict of interests

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