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Research Article

Prevalence of *BlaOXA-48, BlaDIM-1*, and *BlaKPC1* genes in carbapenem -resistant *Pseudomonas* species isolated from wastewater and clinical samples from Baghdad hospitals

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Abstract
Antibiotic resistance poses a significant threat to public health, driven by misuse and overuse of antibiotics in healthcare and agriculture. Among Gram-negative bacteria, carbapenem-resistant *Pseudomonas* species are particularly worrisome due to their ability to harbor resistance genes such as *blaOXA-48*, *blaDIM-1*, and *blaKPC1*. The present study aimed to determine the prevalence of *blaOXA-48*, *blaDIM-1*, and *blaKPC1* genes in *Pseudomonas* isolates from wastewater and clinical samples collected from two Baghdad hospitals. Sixty-nine (69) samples, including blood, urine, wounds, ear swabs, and wastewater, were cultured and subjected to morphological and biochemical analyses. Antibiotic susceptibility testing was conducted using the VITEK 2 system. Molecular analysis was conducted through the polymerase chain reaction (PCR) to detect the most common resistance genes (*blaOXA-48*, *blaDIM-1*, and *blaKPC1*). Out of sixty-nine samples, thirty-two *Pseudomonas* isolates were identified, with *Pseudomonas aeruginosa* accounting for 65.62% of isolates. Other isolates included *P. putida* (21.5%), *P. sutzeri* (21.5%), *P. fluorescens* (6.25%), and *P. lutrola* (3.13%). Antibiotic susceptibility testing revealed 31.25% of isolates were resistant to carbapenems, classifying them as multidrug-resistant (MDR). Polymerase chain reaction analysis demonstrated the presence of *blaOXA-48* and *blaDIM-1* genes in 15.62 and 6.25% of isolates, respectively, with one isolate harboring both genes. No *blaKPC1* gene was detected. The present study highlights the alarming dissemination of carbapenem resistance genes in clinical and environmental settings, posing a significant challenge to effective treatment. Findings underscore the importance of molecular epidemiological surveillance to inform targeted interventions and mitigate the spread of resistance in resource-limited settings like Baghdad.

Keywords: Antibiotic resistance, Baghdad, *blaDIM-1, blaKPC1*, *blaOXA-48,* Carbapenem, *Pseudomonas* spp.

INTRODUCTION

Antibiotic resistance (AR) is the ability of bacteria to resist the effects of drugs that were once effective for treating infections caused by them. This resistance develops through various mechanisms, including genetic mutations and the horizontal transfer of resistance genes, and is exacerbated by the misuse and overuse of antibiotics in healthcare and agriculture (Walsh *et al*., 2023). The implications of AR are severe. Antimicrobial resistance kills people worldwide, lowers the quality of life in general, and threatens to undo much of the progress made in modern treatments especially over the last century. Unless something is done, up to ten million more lives will be lost quickly every year due to AR by 2050 (Aboksour *et al.,* 2024). An antibiotic is a bioactive substance produced by a living organism, often a microorganism, that has detrimental effects on other

microorganisms. Antibiotics are commonly produced by soil microorganisms, such as bacteria and fungi, to limit the proliferation of competing bacteria in complex environments like soil (Aslam *et al.,* 2018).

Antibiotics are pharmaceutical substances that can either eradicate or impede the growth of germs. Physicians employ antibiotics to treat bacterial illnesses. They achieve this by eradicating microorganisms and inhibiting their growth (Muhsin *et al.,* 2023 .(For these "wonder drugs" to continue being effective, it is crucial to employ presently existing antibiotics wisely and promptly create new antimicrobial molecules. Certain bacterial species have developed resistance to all antibiotics effective in treating infections caused by multidrug-resistant (MDR) pathogens (Aslam *et al.,* 2018).

A rise in healthcare-associated infections caused by Gram-negative bacilli has been widely reported, and the pathogens responsible, which included both Enterobacteriaceae members and non-glucose-fermenting bacilli, were frequently MDR strains (Aboksour *et al.,* 2024). Extended-spectrum beta-lactamases (ESBLs) are enzymes that confers resistance to most beta-lactam antibiotics, notably penicillins, cephalosporins, monobactam, and aztreonam. Infections with organisms that produce ESBL have been linked to negative results. Extended-spectrum beta-lactamase-producing Enterobacteriaceae are common in the community and hospitals worldwide (Partridge *et al.,* 2018). Because accurate identification of organisms that produce ESBL in clinical laboratories is difficult, their frequency is likely understated. Carbapenems are the most effective antibiotic agent against infections triggered by such pathogens. In the entire world, the high infection rates caused by ESBL producing Enterobacteriaceae that are resistant to 3rd generation cephalosporins but susceptible to carbapenems have been reported for many years (Partridge *et al.,* 2018).

Gram-negative bacterial resistance involves a wide range of mechanisms; as a result, some of these Gram-negative bacteria are resistant to third and fourth-generation cephalosporins, necessitating carbapenem treatment, while others are carbapenem-resistant (Ahmed *et al.,* 2023). Ineffective empirical antibiotic is a choice for patients suffering from healthcare-associated infections caused by Gram-negative bacteria (Khadim and Al-Marjani, 2019). An ineffective empirical antibiotic choice for patients suffering from healthcare-associated infections caused by Gram-negative bacteria may result in a poor outcome (Muslim *et al.,* 2023; Aboksour 2018). Moreover, it is apparent that focusing solely on discovering new antibiotics will not solve the current, global epidemic any time soon (Aboksour, 2018). Rapid and accurate laboratory diagnostics are equally important for detecting pathogens on time and optimizing anti-infective therapy (Alraddadi *et al.,* 2022). It is important for the microbiology laboratory to quickly give proof of the presence of multidrug resistance pathogens in clinical samples in both routine diagnostics and anti-infective clinical trials (Shibl *et al.,* 2013). The rise of carbapenemase-producing colistin-resistance in Gram-negative bacteria that have achieved tolerance to all viable human antibiotics, is of special concern. There are many phenotypic tests available (Kazmierczak *et al.,* 2021). The present study was conducted to investigate the prevalence of *BlaOXA-48, BlaDIM-1*, and *BlaKPC1* genes in carbapenem-resistant *Pseudomonas* species isolated from wastewater and clinical samples in Baghdad hospitals.

MATERIALS AND METHODS

Sample collection

Sixty-nine samples were collected from Baghdad Teaching Hospital and Fatima Al-Zahraa Women's Hospital during the period from January 3rd and March 14th, 2024. The samples included blood, urine, wound, ear swabs, and wastewater from these hospitals. The collected samples were cultured on nutrient agar and MacConkey media and incubated at 37ºC for 18 hrs. Subsequently, the mixed cultures were purified by subculturing onto the same medium and incubating at 37°C overnight (AL-Bayati *et al*., 2024).

Ethical approval

Ethical approval for the present study was obtained from the Ethics Committee of the University of Mustansiriyah, College of Science, Baghdad/ Iraq.

Bacterial identification

Morphological and microscopic examinations were performed to identify the bacterial isolates, followed by biochemical tests; catalase, oxidase, indol test, methyl red test, Voges-Proskauer, citrate utilization, glucose fermentation, and nitrate reduction tests (Shahnaz *et al.,* 2020). In addition, the VITEK 2 system, using Gram -negative bacteria identification cards, was employed to identify the suspected isolates at the species level according to the manufacturer's instructions.

Antibiotic susceptibility testing

To evaluate the antibiotic potential, the susceptibility of bacterial isolates to fifteen antibiotics was assessed using the VITEK 2 system, following the manufacturer's instructions. This system provides fast and accurate results for antibiotic sensitivity testing. The tested antibiotics, representing the first line of treatment, included Ampicillin, Amoxicillin/Clavulanic acid, Ampicillin/ Sulbactam, Piperacillin/Tazobactam, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Imipenem, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, and Trimethoprim/Sulfamethoxazole.

Bacterial DNA extraction

A commercial purification technology (ABIO Pure^{TM} Total DNA extraction kit) was used to extract total genomic DNA from all positive isolates of *Pseudomonas* sp. Each isolate's DNA was extracted using the Gram-negative bacterial technique (Muhammad *et al.,* 2022).

Detection of resistance genes in *Pseudomonas* isolates

Detection of *blaOXA-48*, *blaDIM-1*, and *blaKPC-1* genes was performed using simple polymerase chain reaction (PCR) amplification with primers described in previous studies (Khadim & Marjani 2019), as shown in Table 1. Commercial purification technology and the Presto Mini gDNA Bacteria kit were used in all carbapenem-resistant *Pseudomonas* strains on a thermal cycler instrument (Agilent, USA). The primers used in the present study were lyophilized and then dissolved in sterile deionized distilled water according to the manufacturer's instructions. The amplification system was performed separately for each primer (OXA-48, NDM-1, and KPC-1). An aliquot of 12.5 µl of Master mix (2X), 1 µl of both forward and reverse primer, 4 µl of DNA template, and 6.5 µl nuclease-free water was added to complete the volume to 25 µl.

The PCR amplification program of *NDM*-1 gene was carried out according to a pervious study (Loucif *et al.,* 2022): initial denaturation at 95°C for 5 min, 35 cycles of 95°C of denaturation for 45 seconds, 52°C of annealing for 45 seconds, 72°C of extension for 60 seconds, and 72°C of final extension for 8 minutes (Krystyna *et al*., 2021). For *OXA*-48 gene, the PCR amplification was performed as initial denaturation at 95°C for 10 minutes, followed by 36 cycles of denaturation at 95°C for 35 seconds, annealing at 52°C for 40 seconds, extension at 72°C for 50 seconds, and final extension at 72°C for 5 minutes (Xue *et al.,* 2020). Finally, the PCR amplification of *KPC-1* gene detection was conducted by initial denaturation at 95°C for 6 minutes, followed by 35 cycles of denaturation at 95 °C for 60 second, annealing at 58°C for 30 seconds, extension at 72°C for 60 seconds, and final extension at 72°C for 10 minutes (Robledo *et al.,* 2011). The PCR products were fractionated on 1.5% agarose gel electrophoresis. Subsequently, the gel was stained, viewed under UV light, and photographed using a gel documentation system.

Statistical analysis

Statistical Package for Social Sciences (SPSS; IBM Inc., Chicago, IL, USA; version 22.0) was used to conduct statistical analysis of data. Descriptive statistics were used for analysis, including frequencies, proportions, means, and standard deviations. The Chi-square test was conducted to assess the associations between resistance gene-carrying samples and the general and clinical characteristics of the patients.

RESULTS AND DISCUSSION

Identity of *Pseudomonas* species isolates

Out of sixty-nine clinical and environmental samples, thirty-two isolates of *Pseudomonas* sp. were isolated and identified according to microscopic biochemical tests and the VITEK 2 Gram-negative cards. The bacteria appeared as red rods (Gram-negative) under microscopic examination, non-capsulated and non-spore forming. They were positive for catalase, oxidase, methyl red, citrate utilization, and nitrate reduction tests (Badr *et al*., 2022), while negative for the indole, Voges -Proskauer, and glucose fermentation tests (Badr *et al*., 2022). VITEK 2 test was performed to confirm that these bacterial isolates belonged to *Pseudomonas* sp. Out of the thirty-two *Pseudomonas* isolates which were isolated in the present study, ten isolates (31.24%) were from wound samples, nine (28.13%) and seven (21.87%) isolates were from blood and wastewater samples, respectively. Meanwhile, three *Pseudomonas* isolates (9.37%) were from both urine and ear swaps samples. The isolated bacteria included five different species. Twenty-one (65.62%) of them were *Pseudomonas aeruginosa*, four isolates (12.5%) each of *Pseudomonas putida* and *Pseudomonas sutzeri*, two isolates (6.25%) of *Pseudomonas fluorescens*, and one isolate (3.13%) of *Pseudomonas lutrola*, as shown in Table 2. The isolation percentage of *Pseudomonas* spp. in this study was 33.30%.

Table 1. Primers of carbapenemase genes

No.	Genes	Primer sequence	Size	Ref.
1	*bla*$_{OXA-48}$	OXA-F: GCGTGGTTAAGGATGAACAC	438 bp	Loucif *et al*., 2022
		OXA-R: CATCAAGTTCAACCCAACCG		
2	*bla*$_{NDM-1}$	NDM-1-F: GGTGCATGCCCGGTGAAATC	661 bp	Xue *et al.,* 2020
		NDM-1-R: ATGCTGGCCTTGGGGAACG		
3	*bla*$_{KPC-1}$	KPC-1-F: TGTCACTGTATCGCCGTC	1010 bp	Loucif *et al*., 2022
		KPC-1-R: CTCAGTGCTCTACAGAAAACC		

Table 2. Identification of *Pseudomonas* spp. from wastewater and clinical samples

No.	Bacterial isolate	Gender	Age	Isolate symbol	Source
1	*P. aeruginosa*	Male	45	BT1	Wound
2	*P. aeruginosa*	Female	34	FZ1	Wound
3	*P. putida*	-	-	FZ2	Wastewater
4	*P. aeruginosa*	Male	9	FZ3	Wound
5	*P. aeruginosa*	Female	21	FZ4	Blood
6	*P. lutrola*	Male	5	FZ5	Urine
7	*P. aeruginosa*	Male	11	FZ6	Wound
8	*P. fluorescens*	-	-	BT2	Wastewater
9	*P. aeruginosa*	Female	40	FZ7	Blood
10	*P. putida*	Male	48	BT3	Blood
11	*P. stutzeri*	Female	36	FZ8	Blood
12	*P. aeruginosa*	Male	28	BT4	Ear swaps
13	*P. aeruginosa*	Male	64	BT5	Wound
14	*P. stutzeri*	-	-	FZ9	Wastewater
15	*P. aeruginosa*	Female	29	FZ10	Urine
16	*P. aeruginosa*	Male	65	BT6	Blood
17	*P. aeruginosa*	Female	52	FZ11	Wound
18	*P. aeruginosa*	Male	47	BT7	Blood
19	*P. aeruginosa*	Female	34	FZ12	Wound
20	*P. aeruginosa*	-	-	FZ13	Wastewater
21	*P. aeruginosa*	Female	50	FZ14	Blood
22	*P. putida*	Male	61	BT8	Wound
23	*P. aeruginosa*	Female	23	FZ15	Ear swaps
24	*P. stutzeri*	Female	38	FZ16	Blood
25	*P. aeruginosa*	Male	52	BT9	Blood
26	*P. aeruginosa*	-	-	BT10	Wastewater
27	*P. aeruginosa*	Male	12	FZ17	Wound
28	*P. aeruginosa*	Female	41	FZ18	Wound
29	*P. stutzeri*	-	-	BT11	Wastewater
30	*P. aeruginosa*	Male	8	FZ19	Urine
31	*P. putida*	Male	59	BT12	Ear swaps
32	*P. fluorescens*	-	-	BT13	Wastewater

Antibiotic susceptibility profile of *Pseudomonas* isolates

Fig. 1 illustrates the antibiotic susceptibility profile of *Pseudomonas* isolates, which revealed that ampicillin/ sulbactam exhibited the highest activity, with 87.5% susceptibility. This was followed by amoxicillin/ clavulanic acid, cefazolin, and nitrofurantoin, each showing 81.25% resistance. The lowest activity was observed against gentamicin and tobramycin, with 28.12% resistance for each. The antibiotic results showed that, out of thirty-two *Pseudomonas* isolates, ten isolates (BT_1, FZ_4, FZ_7, BT_4, FZ_{10}, BT_7, FZ_{14}, FZ_{16}, FZ_{17}, and FZ_{19}) were resistant to imipenem, classifying them as carbapenem-resistant *Pseudomonas*, with a resistance percentage of 31.25%. Moreover, nine of these resistant isolates belonged to *P. aeruginosa*; one isolate (FZ_9) was *P. stutzeri*. All thirty-two *Pseudomonas* isolates collected from clinical and environmental samples in the present study were MDR, with a resistance rate of 100%.

Presence of carbapenem resistance genes in *Pseudomonas* isolates

Detection of carbapenem resistance genes (*blaOXA*-48, *blaDIM*-1, and *blaKPC*−1) showed that out of ten carbapenem-resistant *Pseudomonas* isolates, two isolates (BT_1 and BT_7) possessed *NDM-1* gene, five isolates (FZ_4, FZ_7, BT_4, FZ_{10}, and FZ_{19}) contained *OXA-1* gene, while none of these isolates had *KPC-1* gene (Fig. 2). Also, the results illustrated that isolate BT_1 possessed both *blaOXA-48* and *blaNDM-1* genes. It was observed that the isolation percentage of *Pseudomonas* isolates in this study was higher compared to previous studies, such as those conducted in China between 2007 and 2014, which reported an increase in the isolation percentage of *Pseudomonas* from 10 to 26% (Xue *et al*., 2020). This rise may be attributed to increased resistance to antimicrobial agents or the acquisition of strategies to counteract antibiotic effects. In addition, it may result from irregular and excessive in-

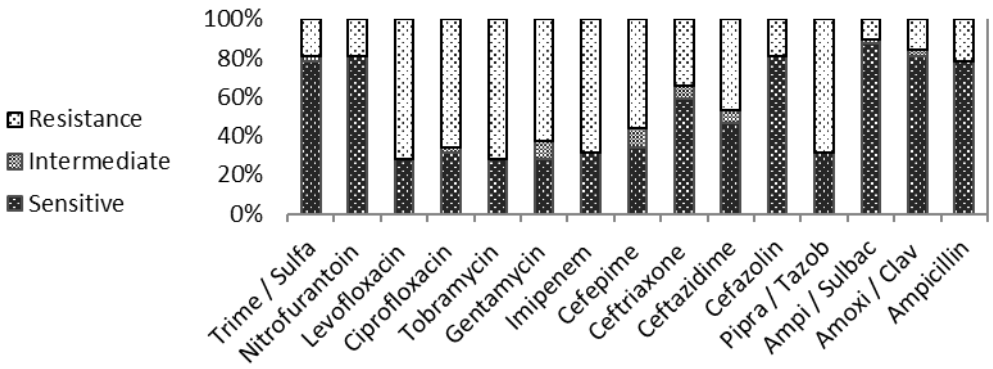

Fig. 1. *Antibiotic susceptibility of Pseudomonas isolates*

discriminate use of antibiotics and antimicrobial agents (Badr *et al.,* 2022).

The high prevalence of *P. aeruginosa* can be attributed to its opportunistic nature. This finding aligns with expectations, given the elevated incidence of *P. aeruginosa* within the community. Its prevalence may be associated with the growing number of immunocompromised patients in Iraq, resulting from various diseases and environmental contamination, particularly in hospitals. Individuals with prolonged hospital stays are especially vulnerable (Moser *et al.,* 2021). This is consistent with the findings of various investigations done by previous studies that the occurrence of *P. aeruginosa* in urinary tract infections (UTIs) is lower compared to its infection rate in burn cases, with only 4 isolates (20%) identified (Ross *et al*., 2022). The buildup of varied biofilms on the synthetic interface of the catheter or another implant is primarily responsible for these recurring infections. An embedded biofilm on a urinary catheter is made up of adhering microorganisms, their extracellular products, and host components. Biofilm on urinary catheters causes long-term antibiotic-resistant infections (Glen and Lamont, 2021).

Many researchers indicated that penicillin had no significant effect on *Pseudomonas* isolates, but their studies showed a high resistance of *Pseudomonas* against different types of penicillin (Glen, 2021). Based to these and the present study, it is obvious that penicillin antibiotics are not good antibiotics for the treatment of *Pseudomonas* infections, despite their usage as a first antibacterial agent in the normal treatment of *Pseudomonas* infections (Glen and Lamont, 2021).

The present study demonstrated that Augmentin (amoxicillin/clavulanic acid) exhibited no significant activity against both environmental and clinical isolates. Clavulanic acid, a potent β-lactamase inhibitor, mimics the chemical structure of β-lactam antibiotics, enabling it to interact with β-lactamase enzymes secreted by certain microbes. This interaction helps confer resistance to β-lactam antibiotics (Manyi-Loh *et al*., 2023). A similar finding was reported by Manyi-Loh *et al*. (2023), who observed that *Escherichia coli* isolated from patients with UTIs exhibited a 94% resistance rate to this antibiotic. However, a study by Rheimaet *et al*. (2021) reported a lower resistance rate, with only 37% of ESBL-producing isolates showing resistance. The generation of mutant β-lactamase enzymes is a key factor responsible for resistance to β-lactam antibiotics and accounts for most cases of β-lactam/β-lactamase inhibitor resistance (Rheima *et al.,* 2021). Because of its resistance to degradation by β-lactamase or cephalosporinase, imipenem is regarded as one of the most effective β-lactams against ESBLs. Most isolates exhibited a wide range of susceptibility to imipenem. Unlike many previous studies, the findings of the present study highlighted imipenem's remarkable efficacy against *Pseudomonas,* with a susceptibility rate reaching 98%. In general, imipenem resistance developed due to penicillin binding protein mutations, the formation of metallo-β-lactamases, or resistance to passage across the bacterial outermost membrane (Manyi-Loh *et al*., 2023).

Multi-drug-resistant isolates have become a significant health concern in Iraq, driven by factors such as the misuse of antibiotics and exposure to biocides. The observed MDR rate of 100% in this study was significantly higher than previously reported rates in Iraq, which ranged from 24 to 47% (Loucif *et al.,* 2022). This could show a higher resistance rate in cases of infections. Other global studies revealed even lower MDR rates; 5.9% in Canada, 35% in Germany, 35 and 20% in both Nepal and Malaysia, respectively (Djeffal *et al*., 2022). This alarming MDR rate underscores the urgent need for strict antibiotic prescription policies in the country. The findings also revealed that levofloxacin and imipenem were the most effective antibiotics against *Pseudomonas* (Al-Bayati and Samarasinghe, 2019).

This study represents the first molecular epidemiological investigation of carbapenemase genes in western Baghdad, a region that has previously lacked data on antimicrobial resistance factors. The setting presents significant challenges, including the lack of surveillance systems, unrestricted over-the-counter access to antibiotics, inadequate oversight of their use, limited awareness of infection prevention practices, and the absence

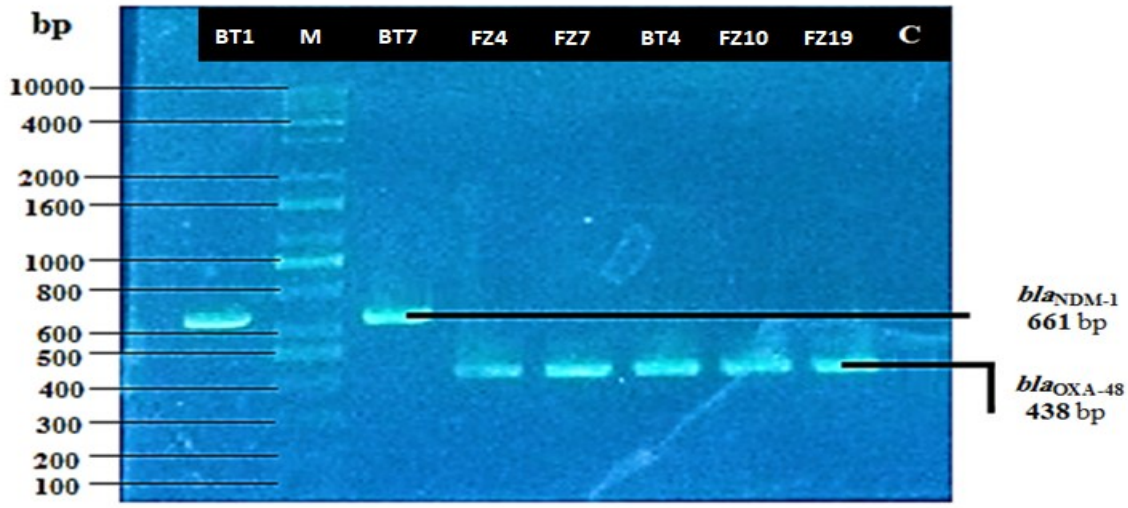

Fig. 2. *Agarose gel electrophoresis of polymerase chain reaction (PCR)-amplified fragment of the carbapenem resistance genes. Lane M: DNA ladder; Lane C: negative control; Lanes 1-7: bacterial isolates.*

of a national antibiotic stewardship policy (Al-Bayati and Samarasinghe, 2019). The significant consequences of carbapenemase gene propagation have led to the argument that early identification of strains bearing these genetic markers is required for efficient prevention strategies and informed therapy alternatives. Although the existence of carbapenemase genes in populations other than Acinetobacter cannot be assumed to result in substantial resistance to carbapenem antibiotics. The results obtained from the present study clearly showed a spreading reservoir for such resistance determinants (Aboksour, 2018). Given the needs, resources, and context of this environment, the findings of this study support the continued use of biological tools to understand better the extent, severity, and progression of carbapenemase gene circulation and MDR in the region. Recent studies indicate that the *blaOXA-48* gene, along with other related genes, becomes active and spreads to other Acinetobacter species via conjugative plasmids (Potron *et al*., 2013; Woerther *et al*., 2018; Al-Bayati and Samarasinghe, 2019).

Conclusion

This study highlights the alarming prevalence of carbapenem-resistant *Pseudomonas* species, with significant rates of MDR and the detection of *blaOXA-48* and *blaDIM-1* genes among clinical and environmental isolates. The absence of the *blaKPC-1* gene contrasts with global trends. These findings underscore the urgent need for stricter antibiotic stewardship, improved surveillance, and early detection of resistance genes in Baghdad hospitals. Also, routine molecular characterization assays in hospital laboratories are strongly recommended to accurately identify resistance-determinant-carrying bacterial infections and better understand antibiotic resistance mechanisms. This study provides valuable insights for future research on predictors of carbapenemase-producing microorganism acquisition, though larger sample sizes and regression analysis are needed for more precise conclusions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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