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Investigate Prevalence of (*blaIMP, blaOXA-40,* and *blaGES)* Genes in Carbapenems Resistance *Pseudomonas aeruginosa* Isolates

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Abstract: *Pseudomonas aeruginosa*, a Gram-negative bacterium, is renowned for its versatility and adaptability, Morphology and Structure of *P. aeruginosa* is a rod-shaped bacterium, typically 0.5 to 3 microns in length, with a polar flagellum for movement. Is to determine resistance to antibiotics, especially carbapenems, and to determine the presence of the genes *blaIMP, blaOXA-40 and blaGES* in *Pseudomonas aeruginosa* bacteria, Multidrug-resistant *Pseudomonas aeruginosa* poses a global concern, particularly for individuals susceptible to burns, blood bacteremia, and wound infections. This study aimed to gather and analyze 150 clinical samples from various Baghdad hospitals, detecting and confirming *Pseudomonas aeruginosa* isolates, evaluating antibiotic susceptibility, exploring resistance-linked genes, and estimating the presence and prevalence in *Pseudomonas aeruginosa* isolates meropenem. Samples underwent examination at the University of Baghdad, Institute of Genetic Engineering and Biotechnology. Identification methods included chemical tests, microscopy, cetrimide agar, VITEK-2 system, and 16S-rRNA analysis. Conventional PCR identified target genes (*blaIMP, blaOXA-40, blaGES*). Among 150 samples, 38 were *P. aeruginosa* isolates. Molecular analysis revealed *blaIMP* absence, with *blaOXA-40 and blaGES* presence in 20 and 18 isolates, respectively. It was concluded that target genes likely contribute to meropenem resistance.

Keywords: *blaIMP, blaOXA-40, blaGES, P. aeruginosa, PCR.*

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Introduction

Pseudomonas aeruginosa, a Gram-negative bacterium, is renowned for its versatility and adaptability, Morphology and Structure of *P. aeruginosa* is a rod-shaped bacterium, typically 0.5 to 3 microns in length, with a polar flagellum for movement. It is surrounded by a slimy polysaccharide layer called the extracellular matrix or biofilm (1), This bacterium is a facultative anaerobe capable of utilizing a wide range of organic and inorganic compounds as carbon sources (2), compounded by the scarcity of effective antimicrobial treatments. Notably, the rise of carbapenem-resistant Enterobacteriaceae (CRE), extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, and multidrug-resistant (MDR) *Pseudomonas aeruginosa* is acknowledged as a critical public health issue by the centers of diseases control and prevention (CDC). Immediate and assertive actions are imperative to mitigate their dissemination and devise innovative therapies to combat these

resilient pathogens (2). The increasing resistance of *Pseudomonas aeruginosa* to multiple antibiotic classes is a major global concern. The widespread use of broad-spectrum antibiotics in burn units and Intensive Care Units (ICUs) has exerted selective pressure on bacteria, leading to the emergence of multidrug-resistant (MDR) strains. Currently, MDR *Pseudomonas aeruginosa* is involved in 4–60% of nosocomial infections worldwide (3). The rise of multidrug-resistant (MDR) strains (4), displaying resistance to almost all antibiotics except for one or two classes, is becoming a significant public health challenge. This development is leading to increased morbidity, mortality rates, and extended hospitalizations (5,6).

Unfortunately, *Pseudomonas aeruginosa* possesses a wide range of mechanisms that contribute to multidrug resistance (MDR). These include the presence of outer membrane barriers like porin OprD, increased expression of multidrug efflux pumps, and the deactivation of natural antimicrobials. Consequently, selecting effective treatment options presents a significant challenge in hospitals worldwide (7,8).

Extended-spectrum β-lactamases (ESBLs) belonging to the Guiana extended-spectrum β-lactamases (GES) family are notably prevalent and significant in clinical *Pseudomonas aeruginosa* isolates. GES, categorized as carbapenemases, are commonly found in these isolates and can be inhibited by various compounds such as clavulanate, tazobactam, avibactam, relebactam, and vaborbactam. GES-1, in particular, exhibits more efficient hydrolysis of ceftazidime compared to cefotaxime. These genes are typically harbored on transposons, facilitating their transfer among Gram-negative bacteria (9). The role of blaIMP gene is to hydrolyze almost all types of β-lactam antibiotics by inactivating metal chelating agent as it is considered B metallo-β-lactamases (10) while blaOXA-40 gene role is majorly hydrolyzing cephalosporins, cephems and monobactams plus aiding bacterial dissemination (11) as for blaGES gene exhibiting strong activity against most beta-lactams and regarded one of extended-spectrum lactamse (12). The aim of study is to determine resistance to antibiotics, especially carbapenems, and to determine the presence of the genes blaIMP, blaOXA-40 and blaGES in *Pseudomonas aeruginosa* bacteria.

Materials and methods

Bacterial isolation and identification

A total of 150 specimens were procured from the Center Health Laboratory at Medical City, Baghdad, Iraq, between October 2022 and March 2023. These specimens underwent meticulous cultivation on suitable culture media. To corroborate this identification, we employed a dual approach utilizing both the VITEK-2 system and a molecular technique targeting the 16s-rRNA gene. This comprehensive methodology validated that all 38 isolates indeed belonged to *P. aeruginosa.* The combined verification process underscores the accuracy and reliability of our identification protocol.

Antibiotic susceptibility test

The evaluate antibiotic susceptibility, we employed AST-N222 cards along with the VITEK 2 Compact Instrument(13). This comprehensive assessment included a range of antimicrobial agents such as Amikacin,

Aztreonam, Cefepime, Ceftazidime, Trimethoprim/Sulfamethoxazole, Gentamicin, Imipenem, Ticarcillin/clavulanate, Tobramycin, Rifampicin, Colistin, Pefloxacin, Ciprofloxacin, Meropenem, Piperacillin, Minocycline, and Piperacillin/tazobactam. The analysis provided valuable insights into antibiotic sensitivity (14,15).

DNA extraction

Genomic DNA extraction from bacterial cultures was performed using the Transgene Genomic DNA Purification Kit method. This versatile kit facilitates DNA isolation from various sample types. Following the kit's guidelines, the bacterial protocol tailored for gram-negative bacteria was utilized during extraction. This precise method ensured the effective isolation of genomic DNA from the bacterial samples analyzed.

Conventional PCR for *16S-rRNA* and *blaIMP, blaOXA-40, and blaGES* genes

Each target gene was identified using the conventional polymerase chain reaction (PCR) method with specific primers. These primers, supplied in lyophilized powder, were reconstituted to a final concentration of 100 picomoles/ml. Notably, the primers targeting the 16S-rRNA gene(16), as well as the bla-oxa, bla-ges, and bla-imp genes, were selected based on previous studies outlined in (Table 1).

Table (1): Primer sequences used PCR in this study.

No	Primer name	Sequence	Product size	Vendor
1	*16S-rRNA-F*	5-CCTACGGGAGGCAGCAG-3	215 bp (16)	Macrogen/ Korea
2	*16S-rRNA-R*	5-ATTACCGCGGCTGCTGG-3		
3	*blaIMP-F*	5`- TTGACACTCCATTTACDG-3`	139 bp (17)	
4	*blaIMP-R*	5`- GATYGAGAATTAAGCCACYCT-3`		
5	*blaGES-F*	.5`- ATGCGCTTCATTCACGCAC -3`	846 bp (18)	
6	*blaGES-R*	5`- CTATTTGTCCGTGCTCAGG -3`		
7	*blaOXA-40-F*	5′- CACCTATGGTAATGCTCTTGC-3′	491 bp (19)	
8	*blaOXA-40-R*	5′- GTGGAGTAACACCCATTCC-3′		

A total volume of 50 µl for each PCR reaction was prepared by adding nuclease-free water to reach the desired final volume. The reaction mixture comprised 1 µl of each primer at a concentration of 10 picomoles/µL, along with 25 µl of Go Taq® green master mix 2X obtained from Transgene (China). Furthermore, 5 µl of DNA template at a concentration of 100 ng/µl was included. Following thorough mixing and a brief centrifugation of the extracted DNA, the samples were stored at 4°C to maintain stability.

Negative control experiments, devoid of DNA template, comprised the same components as the standard PCR reaction. The PCR protocols were performed using the Thermal Cycler Gradient PCR system by Thermo Fisher (USA). The amplification conditions were as follows: an initial denaturation step at 94°C for five minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, and specific annealing temperatures of 56°C, 56°C, 54°C, and 54°C for 30 seconds each (corresponding to the 16s rRNA, bla-

oxa, bla-ges, and bla-imp genes, respectively). Subsequently, an extension step was conducted at 72°C for one minute, repeated in each cycle for 30 cycles, and finalized with an additional extension at 72°C for 10 minutes. The resulting PCR products were visualized through agarose gel electrophoresis using a 2% agarose gel medium.

Estimation of extracted DNA concentration

The concentration of the extracted DNA was determined using the Quantus™ Fluorometer Single-Tube Format Protocol.

Ethical approval

The study adhered to ethical principles based on the Declaration of Helsinki. Prior to sample collection, verbal and written consent was obtained from all patients. The study protocol, along with the informed consent form, was reviewed and approved by a local ethics committee (document number: EC/3141A, dated 4/12/2022).

Results and discussion

***P. aeruginosa* distribution based on type of sample**

The prevalence of *Pseudomonas aeruginosa* was observed 38 out of 150 were *P.aeruginosa*. These samples were sourced from diverse medical contexts such as wounds, burns, and blood from hospitalized patients.

Sample collection

Samples of blood source were 50 (33.3%) and showed about 10 (6.6%) positive while samples collected from wounds condition patients are 80 (53.3%) and positive ones prevailed 16(10.6%), as for burns samples number is 20 (13.3%) and positive results revealed 12(8%). The difference in number of collected samples from various sources is due to limited time duration within hospitals in Baghdad and restricted government criteria to collect specially for burn section unit. All data mentioned above are detailed in (Table 2).

Table (2): Prevalence of *P. aeruginosa* isolates among clinical samples. No (150).

Source	No. of sample	Positive sample
Blood	50 (33.3%)	10(6.6%)
Wound	80 (53.3%)	16(10.6%)
Burns	20 (13.3%)	12(8%)
total	150 (100%)	38 (25.3 %)

Identification of *P. aeruginosa*

Following microscopic scrutiny and biochemical assays, 38 isolates were determined to be Pseudomonas aeruginosa. The identification of 38 clinical isolates as *Pseudomonas aeruginosa* underwent a rigorous validation process. This involved a dual approach: utilizing the Gram-negative strain identification card within the VITEK 2 system, and confirming with cetrimide selective media, where the colonies exhibited a characteristic blue-greenish color showed in figure (1). Additionally, examination of the *16s-rRNA* gene further (20) facilitated this confirmation process.

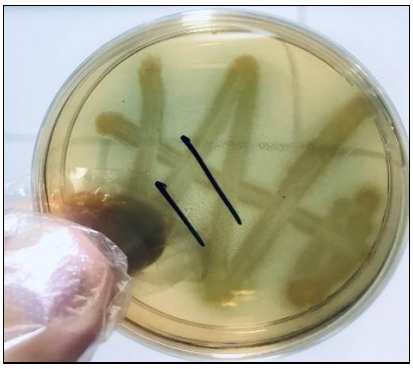

Figure (1): *P. aeruginosa* colonies on cetrimide agar after 24hours incubated.

Susceptibility to antibiotic test

We systematically evaluated the efficacy of 14 different antibiotics against various bacterial strains using the Gram-negative susceptibility card integrated within the VITEK 2 Compact instrument. The aim was to assess their potential to inhibit bacterial growth (21) (see Table 3 for details).

Presence of *blaIMP, blaOXA-40 and blaGES*

Out of the 38 isolates, 20 were consistently identified as *P. aeruginosa* through culture, biochemical assays, and VITEK-2 testing. Confirmation was further established via PCR analysis targeting the *16s-rRNA* gene, with clear bands at 215 bp observed on agarose gel electrophoresis. Additionally, PCR was used to evaluate the presence of efflux pump regulatory genes in all 20 multidrug-resistant *P. aeruginosa* isolates. Results indicated that all isolates (100%) harbored the *blaOXA-40* gene, while 18 (90%) possessed the *blaGES* gene, as shown in (Figure 2). A study in Egypt found blaGES present in 50% of isolates resistant to meropenem (22). Importantly, the *blaIMP* gene was absent in all 20 isolates in our samples.

Ticarcillin shows a high level of resistance with 73.68% (28 out of 38) of the isolates being resistant, while only 26.32% (10 out of 38) are sensitive. Interestingly, when combined with Clavulanic acid, Ticarcillin/Clavulanic acid exhibits a dramatic increase in sensitivity, with 89.47% (34 out of 38) of the isolates being sensitive and only 10.53% (4 out of 38) being resistant.

Piperacillin/Tazobactam also shows a significant level of sensitivity, with 71.05% (27 out of 38) of the isolates being sensitive and 28.95% (11 out of 38) being resistant. Piperacillin alone performs slightly better, with 81.58% (31 out of 38) of the isolates being sensitive and 18.42% (7 out of 38) being resistant, Ceftazidime displays a sensitivity rate of 76.32% (29 out of 38), while 23.68% (9 out of 38) are resistant. Cefepime shows a more balanced profile, with 73.68% (28 out of 38) sensitivity, 13.16% (5 out of 38) intermediate resistance, and 13.16% (5 out of 38) resistance.

Imipenem is one of the antibiotics with a higher resistance rate, showing 60.53% (23 out of 38) resistance and 39.47% (15 out of 38) sensitivity. In contrast, Meropenem demonstrates higher efficacy with 76.32% (29 out of 38) sensitivity, 18.42% (7 out of 38) resistance, and 5.26% (2 out of 38) intermediate resistance. Amikacin exhibits a high sensitivity rate of 81.58% (31 out of 38), while 18.42% (7 out of 38) are resistant. All data mentioned above are illustrated in (Figure 2).

Table (3): Antimicrobial Susceptibility of *Pseudomonas aeruginosa* Isolates to Different Antimicrobial Agents.

Antibiotic	Resistant	Intermediate	Sensitive
Ticarcillin	28 (73.68%)	0 (0.0%)	10 (26.32%)
Ticarcillin/Clavulanic acid	4 (10.53%)	0 (0.0%)	34 (89.47%)
Piperacillin/Tazobactam	11(28.95%)	0 (0.0%)	27 (71.05%)
Piperacillin	7 (18.42%)	0 (0.0%)	31 (81.58)
Ceftazidime	9 (23.68%)	0 (0.0%)	29 (76.32%)
Cefepime	5 (13.16%)	5 (13.16%)	28 (73.68%)
Imipenem	23 (60.53%)	0 (0.0%)	15 (39.47%)
Meropenem	7(18.42%)	2 (5.26%)	29 (76.32%)
Amikacin	7 (18.42%)	0 (0.0%)	31 (81.58%)
Gentamicin	5 (13.16%)	20 (52.36%)	13 (34.21%)
Tobramycin	11 (28.95%)	9 (23.68%)	18 (47.37%)
Ciprofloxacin	25 (65.79%)	5 (13.16%)	8 (21.05%)
Colistin	14 (36.84%)	0 (0.0%)	24 (63.16%)

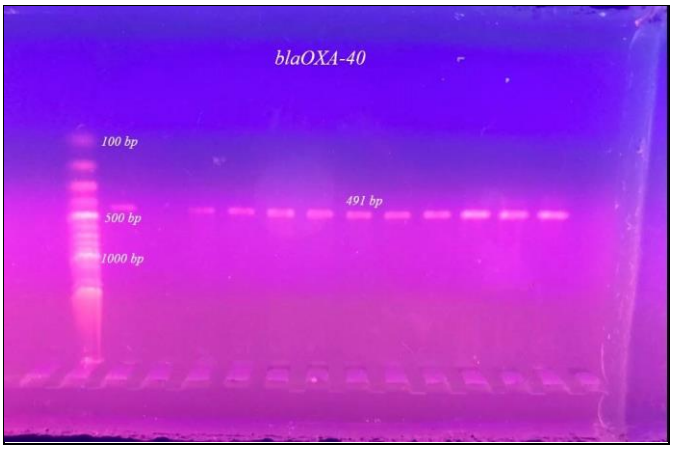

Figure (2): The results of the amplification of *blaOXA-40* gene of *Pseudomonas aeruginosa* samples were fractionated on 2% agarose gel electrophoresis stained with red-save stain. L: 100bp ladder marker. Lanes 1-9 resemble bp PCR product.

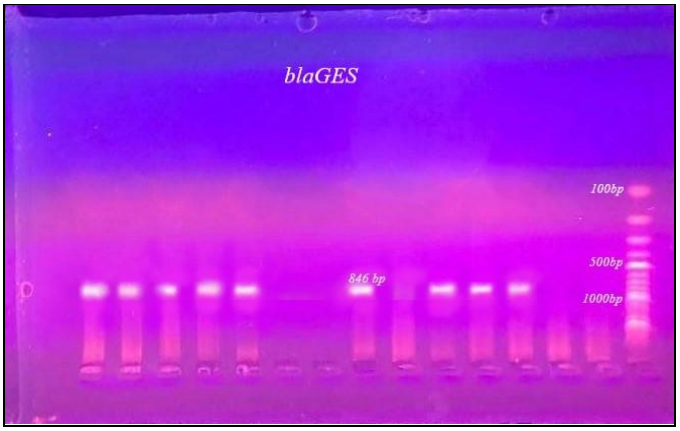

Figure (3): The results of the amplification of *blaGES* gene of *Pseudomonas aeruginosa* samples were fractionated on 2% agarose gel electrophoresis stained with red-save stain. L: 100bp ladder marker. Lanes 1-9 resemble bp PCR product.

Figure (4): The results of the amplification of *blaIMP* gene of *Pseudomonas aeruginosa* samples were fractionated on 2% agarose gel electrophoresis stained with red-save stain. L: 100bp ladder marker.
Lanes 1-9 resemble 139 bp PCR product the result were no detection of the target gene.

Gene detection discussion

Treatment failures for infections caused by carbapenem-resistant *Pseudomonas aeruginosa* are becoming more likely due to the undetected presence of carbapenemases (23). In a study conducted by (24), out of 192 cases, 171 were resistant to carbapenems. Among these, at least 47 cases were found to harbor at least one carbapenemase-encoding gene, with GES being one of them, accounting for 23.4% of the cases. Another study reported the highest incidence of extensively drug-resistant/multidrug-resistance (XDR/MDR) strains in isolates carrying the blaGES gene (25). Results indicated that all isolates (100%) harbored the blaOXA-40 gene, while 18 (90%) possessed the blaGES gene, as shown in (Figure 2). A study in Egypt found blaGES present in 50% of isolates resistant to meropenem (23). Research conducted in Iran revealed that *blaOXA*24/40 was detected in 2.24% of strains, with a co-occurrence observed in 2.23% of *Pseudomonas aeruginosa* strains. Interestingly, no significant correlations were found between antibiotic resistance and the presence of resistance genes represented as Among the 171 carbapenem-resistant cases, at least 47 (27.5%) harbored one or more carbapenemase-encoding genes. This suggests that nearly a third of the resistant cases possess these genes, highlighting their potential role in resistance mechanisms.

Specifically, the GES gene was present in 23.4% of the resistant cases, underscoring its significant contribution to carbapenem resistance while All isolates (100%) in one study harbored the blaOXA-40 gene, indicating its ubiquitous presence among the tested strains. Additionally, 18 out of 20 isolates (90%) possessed the blaGES gene, demonstrating its high prevalence and potential impact on resistance profiles, aligning with our study's findings (6). Hence, the observed of presence of target gene in the resistant strain and sensitive strain the role of these gene is not exclusive but may play slight role in meropenems resistance.

Conclusion

For rapid detection of *P.aeruginosa*, Cetrimide agar medium gave credible, accurate and clear results. This study demonstrated the role of *16S-rRNA* genes for molecular detection *P.aeruginosa* at the level of genus and species respectively. Target genes (*blaOXA-40, blaGES)* play role in

meropenem resistance because this is the latest carbapenem antibiotic generation which *P. aeruginosa* has not yet developed resistance for it that is why showed sensitivity to it. The gene *blaIMP* didn't detected in this study may due to geographical distribution. The need for more investigation and understanding to this resistance at molecular level.

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