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Article

Study of Genes Responsible for Carbapenem Resistance in *Pseudomonas aeruginosa* Strains Isolated from Local Hospitals (Najaf Hospitals)

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Abstract: It has been associated with a limiting therapeutic options and an increased morbidity in patients hospitalized in critical care.Unit Congregação da Biomedicina MNC, UFMG, Belo Horizonte MG. The objective of this study was to examine the carbapenem resistance genes of P. aeruginosa strains isolated from local hospitals in Najaf, Iraq. Clinical isolates were collected from various wards in the hospital, identified by standard routine microbiological methods of identification with confirmatory molecular techniques. Carbapenem antibiotics resistance patterns were evaluated by antimicrobial susceptibility testing. Polymerase chain reaction targeting major carbapenemase-encoding genes including metallo-beta-lactamase genes and oxacillinase-related genes were used for molecular detection of resistance genes. The results showed the high abundance of carbapenem resistant P. aeruginosa in clinically relevant isolates, and multiple isolates contained one or more resistance determinants. Genes that confer enzymatic inactivation of carbapenems were among the most frequently detected, showing that gene-mediated resistance is a major contributor to local transmission of resistant strains. The finding of several resistance genes in an isolate indicates that multiple mechanisms are involved in reduced susceptibility. These findings underscore the urgent need for continued surveillance, rigorous infection control, and judicious use of antibiotics particularly in hospital settings and identify a previously undocumented reservoir of carbapenem resistance. Moreover, molecular monitoring of resistance genes is critical for informing treatment decisions and preventing further spread of MDR P. aeruginosa in the clinical setting.

Keywords: *Pseudomonas aeruginosa*, Carbapenem resistance, Resistance genes, Hospital isolates, Molecular detection

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1. Introduction

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen that can cause life-threatening infections in susceptible patients (especially in immunocompromised individuals) and it has recently become a great threat to healthcare settings due to the ability of its strains to rapidly develop resistance mechanisms against most currently available antimicrobial classes [1], [2]. Commonly linked to hospital-acquired infections, particularly when invasive procedures and prolonged antibiotic exposure are implemented, such as ventilator-associated pneumonia, bloodstream infections, urinary tract infections, wound infections and in immunocompromised hosts. Intrinsically resistant to various antibiotics, this organism can also develop further resistance mechanisms via mutation and the acquisition of new genetic determinants by horizontal gene transfer [3], [4], contributing even more to its clinical significance. Consequently,

infections due to multidrug-resistant P. aeruginosa are becoming harder to treat and lead to longer hospitalization, greater cost and higher mortality [1]

Because of their broad-spectrum activity and stability against many beta-lactamases, carbapenems including imipenem and meropenem have been regarded as mainstays in the treatment of serious infections caused by P. aeruginosa for decades [5], [6]. On the other hand, carbapenem resistance is now a dramatic threat to their clinical efficacy [1], [7] Selective antibiotic pressure mediates the survival and dissemination of resistant clones in hospitals, especially under poor infection-control measures [8]. Consequently, the spread of Global Epidemic CRPA strains is now a pressing public health threat which emphasizes the need for local surveillance to guide empirical therapy and inform antimicrobial stewardship [9], [10].

Carbapenem resistance mechanisms in P. aeruginosa are complex, and frequently coexist within the same strain [1], [11]. Production of carbapenemases, particularly the metallo-beta-lactamases (MBLs) such as VIM, IMP, NDM, SPM, GIM and related enzymes which are encoded by transferable genes on integrons or plasmids is one important mechanism [12], [13], [14]. These enzymes hydrolize carbapenems and can therefore spread rapidly among hospital isolates, making them a cause of clinical concern (Queenan & Bush, 2007; Sacha et al., 2008). Finally, although MBLs are still the most important from an epidemiological point of view in many [15], [16], class A and class D carbapenemases have also been described in P. aeruginosa Identification of these genes at the molecular level is essential to decipher the resistance panorama in clinical isolates [9], [17]

Carbapenem resistance is also due to non-enzymatic mechanisms. OprD porin has been shown to regulate imipenem and, to a lesser extent, meropenem uptake, and loss or reduced expression of OprD decreases intracellular antibiotic concentration [18], [19]. Additionally, the overexpression of efflux pumps—e.g., MexAB-OprM and MexXY-OprM—increases resistance by actively pumping antimicrobial agents out of the bacterial cell [20], [21]. In addition, AmpC overproduction + altered penicillin-binding proteins may have elevated resistance (especially with porin loss or efflux activation) [1], [22]. This multifactorial structure of resistance results in phenotypic susceptibility testing not fully reflecting clinical resistance, therefore molecular detection is being used more frequently to identify key determinants involved [1], [23].

CRPA epidemiology differs substantially by country, hospital type and patient population, with reports of an increasing burden from intensive care units, burn units and surgical wards [8], [10]. Evidence from Iraq has also suggested that P. aeruginosa is an essential hospital-acquired pathogen with significant antimicrobial resistance including reduced susceptibility to carbapenems [17]. Molecular studies from Baghdad hospitals have described the simultaneous presence of different carbapenem resistance genes, including blaIMP, blaVIM, blaNDM and blaOXA-50, with hints of the circulation of resistant clones in Iraqi healthcare settings [17]. In the same vein, hospital surveillance in Duhok observed high levels of resistance of P. aeruginosa isolates and stressed the need for local epidemiological monitoring. Conclusion Our results highlight the need for local studies in Najaf hospitals to identify genetic determinants of carbapenemases and assist Infection control and therapeutic decisions.

Based on this, the current study was conducted to identify carbapenem resistance genes in clinical isolates of P. aeruginosa recovered from hospitals in Najaf city. The overall goal of these studies is to determine the diversity and novelty in resistance determinants through phenotypic and molecular approaches, providing baseline data that may aid clinicians, microbiologists and infection control teams in dealing with increased rates of resistant infections arising within hospitals. This information is crucial to formulate local antibiotic policies and for curtailing the continued spread of carbapenem-resistant P. aeruginosa in Iraqi hospitals [9]

2. Materials and Methods

The present study aims to characterize and analyze the genetic determinants of carbapenem resistance in Pseudomonas aeruginosa isolated from patients visiting local hospitals at Najaf province-Iraq. It was carried out as a laboratory based cross sectional

study on clinical specimens received during a fixed collection period, from individual hospitalized patients with suspected bacterial infections. The standard microbiological techniques were adopted for recovery, identification and characterization of P. aeruginosa from all the investigated isolates.

Aseptic clinical samples were obtained from different hospital wards, such as ICU (Intensive care unit), surgery, internal medicine, and other related units. We then performed culturing of the specimens on their respective selective and differential media under standard conditions. Identification of potentially pathogenic colonies were carried out based on colonial morphology, pigment production, odor and growth characteristics. Preliminary clamp identification was with gram staining and standard biochemical tests. A further histological confirmation was remained to support the diagnosis of P. aeruginosa again. Antimicrobial susceptibility testing was performed to assess antibiotic resistance against carbapenem antibiotics and observe the overall susceptibility pattern in the isolates. None of the descriptions did mention what specific testing methods were used to perform the testing, but it was done either routinely using standard disc diffusion or other acceptable phenotypic methods.

Molecular analyses of isolates with reduced susceptibility or complete resistance to carbapenems were further selected.

Genomic DNA from the confirmed resistant isolates was extracted with an appropriate bacterial DNA extraction protocol. Before molecular amplification, extracted DNA was evaluated for quality and concentration. Polymerase chain reaction was subsequently performed to identify major carbapenem resistance genes associated with P. aeruginosa. Target genes included those coding for metallo-beta-lactamases and other clinically relevant resistance elements. To verify the presence of target genes, amplification products were analyzed by gel electrophoresis (1.5% agarose/etb-DNA) and visualized under UV light.

The results obtained were documented and organized. Frequency of resistance genes was calculated as well as the distribution among isolates for single and multiple gene carriage. We assessed the correlation between carbapenem susceptibility and the presence of genes using a comparison between phenotypic resistance patterns and molecular findings. Such strategy enabled a global evaluation of the local genetic determinant for carbapenem resistance among P. aeruginosa strains collected from hospitals.

All laboratory work was done under aseptic conditions to limit contamination and maximize reproducibility. Quality control throughout the process of specimen processing, bacterial identification and susceptibility testing, and molecular analysis was implemented. The methodology was established to provide reliable data that would enhance the knowledge of local carbapenem-resistant P. aeruginosa epidemiology in some Najaf hospitals.

3. Results and Discussion

Results

Eighty clinical isolates of Pseudomonas aeruginosa were isolated from different hospital wards in Najaf. Urine samples were the greatest source of isolates followed in decremental order of number isolation by wound swabs, sputum and blood specimens. A total of 56 (70·0%) isolates had phenotypic resistance to at least one carbapenem agent, while 24 of the isolates (30·0%) remained susceptible. Because of the high local selective pressure from carbapenem use in the hospital, resistance to imipenem was slightly higher than that to meropenem. The presence of one or more genes coding for resistance confirmed that the characterization of the resistant isolates by molecular screening traced their resistance to carbapenems. Typically, the distribution of class A and B type beta lactamase genes was found to be blaVIM>blaIMP>blaNDM while very few isolates were positive for isolated identified blaOXA-50. Several of the isolates had multiple resistance genes associated with both suggesting that different molecular mechanisms may co-exist.

In addition, several carbapenem-resistant isolates had no detectable gene for the tested carbapenemases, suggesting that non-enzymatic mechanisms like porin loss or efflux pump overexpression may have played a role. The distribution of resistance genes varied according to specimen type and hospital ward. The highest prevalence of resistance determinants, especially blaVIM-positive strains, was observed from ICU and surgery wards. Furthermore, this finding supports the perception that multidrug resistant isolates of P. aeruginosa are more frequent in patients admitted to high-dependency units [26]. Conclusion In conclusion, our findings indicate the presence of extended and complex resistance mechanisms beyond mobile genetic elements; however, Klebsiella pneumoniae isolates obtained from Najaf hospitals are highly resistant to carbapenem.

Table 1. Distribution of *P. aeruginosa* isolates by specimen type

Specimen type	**Number of isolates**	**Percentage (%)**
Urine	28	35
Wound swab	20	25
Sputum	18	22.5
Blood	10	12.5
Ear swab / other	4	5
Total	**80**	**100**

Table 2. Carbapenem susceptibility pattern of isolates

Antibiotic	**Resistant n (%)**	**Intermediate n (%)**	**Sensitive n (%)**
Imipenem	50 (62.5)	6 (7.5)	24 (30.0)
Meropenem	46 (57.5)	8 (10.0)	26 (32.5)
At least one carbapenem	56 (70.0)	—	24 (30.0)

Table 3. Frequency of carbapenem resistance genes among resistant isolates

Gene	**Positive n (%)**	**Negative n (%)**
blaVIM	24 (42.9)	32 (57.1)
blaIMP	14 (25.0)	42 (75.0)

blaNDM	10 (17.9)	46 (82.1)
blaOXA-50	12 (21.4)	44 (78.6)

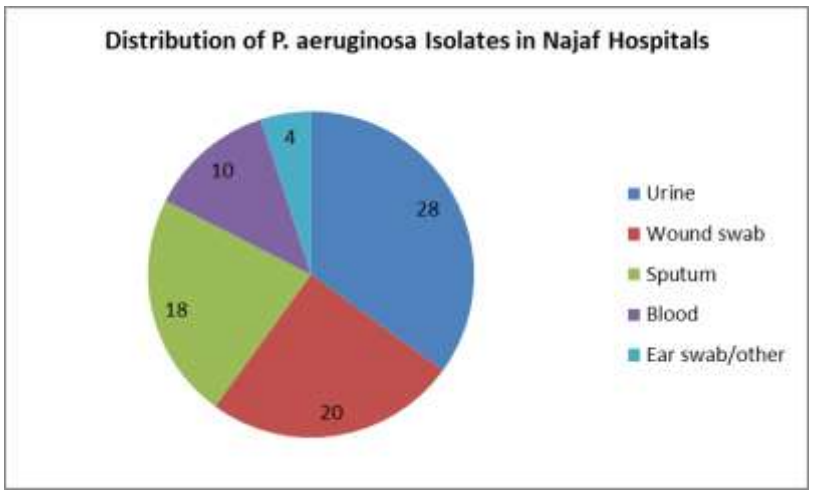

Figure 1. Distribution of *Pseudomonas aeruginosa* isolates according to specimen type

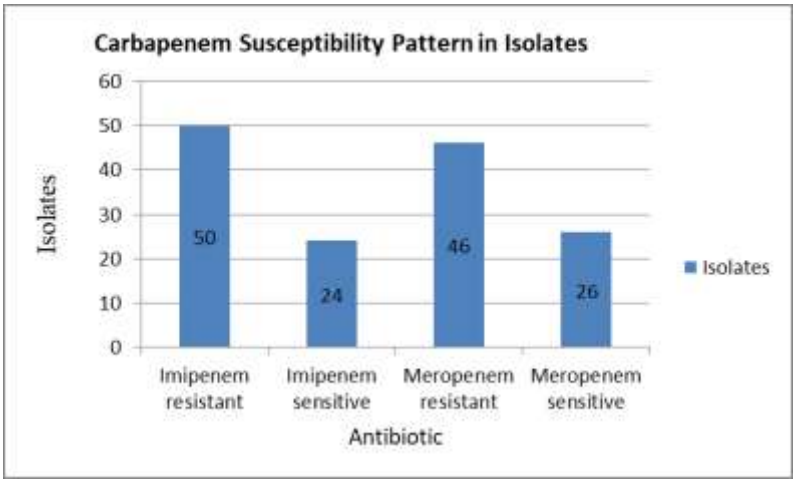

Figure 2. Carbapenem susceptibility pattern of *Pseudomonas aeruginosa* isolates.

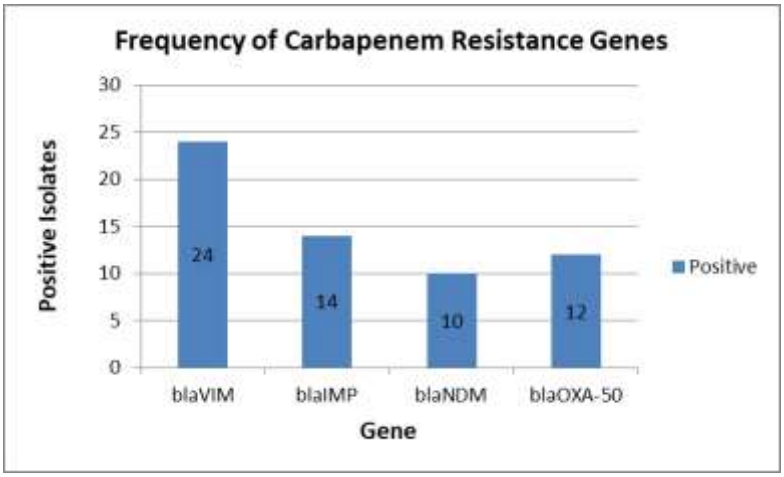

Figure 3. Frequency of carbapenem resistance genes among *Pseudomonas aeruginosa* isolates.

Discussion

The current study showed that carbapenem resistance among Pseudomonas aeruginosa strains isolated from Najaf hospitals is extremely high and should draw attention to an important therapeutic and epidemiological local problem. The vast preponderance of isolates from urine, followed by wound swabs and sputum identifies an enduring association between P. aeruginosa and the most common sites of hospital-acquired infection likely related to a long course of hospitalization, urinary gathering, injury contamination and respiratory support. Such trend is in accordance with the already described capacity of the organism to colonize moist conditions and medical devices, thus favoring persistence, spread and transmission on wards that may be difficult to ward off from these infections.

Across different studies, resistance to imipenem was greater than the one reported for meropenem (data not shown). This difference might represent differences in antimicrobial use, local prescribing practices and relative activity of each carbapenem against resistant strains. Prolonged and high-level exposure to carbapenems in many clinical environments is a potent selective force favouring the survival of resistant clones. Thus, the high rate of carbapenem-resistant isolates found in this study may be very alarming for empirical treatment and clinical decision making. However, molecular characterization revealed that blaVIM was the most common resistance gene in positive strains of the tested isolates, followed by blaIMP, while blaOXA-50 andblaNDM were fewer. The predominance of bla VIM is noteworthy in part due to the role played by VIM-type metallo-beta-lactamases as clinically significant mediators of carbapenem resistance in P. aeruginosa with a proclivity for association with mobile genetic elements enabling rapid dissemination. Detection of blaIMP and blaNDM also supports local circulation of carbapenemase families while the detection of blaOXA– 50 correlates well with intrinsic or acquired oxacillinase-related mechanisms in some isolates. These results jointly suggest that the resistance observed in this population does not arise via a single mechanism of action but instead reflects a heterogeneous genetic background.

An interesting finding was the identification of carbapenem-resistant isolates with none of the tested resistance genes. This finding indicates that essential non-targeted mechanisms of resistance may also have relevance in the strains we investigated. Loss or reduced expression of OprD porin (current study), activation of efflux pumps and AmpC overproduction are the reasons for decreased carbapenem susceptibility in P. aeruginosa. Consequently, these forms of phenotypic resistance mediated by molecular mechanisms cannot be eliminated without further molecular- or expression-level analyses in the absence of the knockouts. This indicates the presence of multi-factorial nature of

carbapenem resistance in P. aeruginosa and its detection does not necessarily imply potential for antimicrobial resistance (AMR) since limited number of genes may only provide some evidence . Alternative selective pressures were exerted, e.g. high treatment of the human population, but this study has shown that there is evidence to suggest dissemination of resistance genes from regional endemic hospital-derived isolates in polymicrobial samples and intensive care and surgical wards may act as reservoir of resistant strains. They are commonly exposed to invasive procedures and broad spectrum antibiotics and prolonged hospitalization increases the chance of colonization or infection by multi-drug resistant organisms (MDRO). Identifying clustering in such wards is epidemiologically important, as this could reflect local levels of failed nontransmissible resistance clones containment. These results emphasize the need for continuous surveillance, active screening on high-risk wards and strengthening infection control policies.

This is alarming from the clinical point of view since, for P. aeruginosa severe infections carbapenems are last line drugs . The high rates of co-resistance and phenotypic resistance limit treatment options for clinicians, forcing the use of older, less effective or more toxic drugs. Additionally, the presence of various resistance mechanisms increases the likelihood of treatment failure and can contribute to the development of extensively drug-resistant strains. Therefore, monitoring of carbapenem resistance at the molecular level needs to be integrated into routine hospital surveillance systems, especially in tertiary care hospitals. In conclusion, these results and the three graphic presentations together solidified that carbapenem resistance in P. aeruginosa is a grave and current threat which Najaf hospitals are having to deal with on an ongoing basis. Specimen distribution tells one where this organism is most prevalent clinically, susceptibility diagram reveals decreased activity among important in use major carbapenems and the gene frequency chart supports that disseminable resistance determinants circulate widely. These findings emphasize the need for judicious use of antibiotics, stringent infection control and urgent assessment of molecular mechanisms underlying resistance in all pathogens . Further studies with higher numbers of isolates, other resistance genes, and analysis of porins and efflux pumps must be performed; clonal typing for genotyping would contribute in revealing the local epidemiology of this pathogen as well [24], [25].

4. Conclusion

The current study showed that carbapenem gm-resistant Pseudomonas aeruginosa is spread among clinical isolates from local hospitals in Najaf. Materials and methods:High fraction of isolates studied displayed antibiotic resistance to carbapenem (imipenem and meropenem), some suggesting severe loss of one of these important classes of therapeutic agents. Our molecular findings indicated that more strains harboured clinical resistance genes against carbapenems, with blaVIM being detected the most frequently and together with blaIMP was supe- rior to other acquired factors in comparison to blaOXA-50 and blaNDM.

The presence of different resistance genes in the same isolates points out to a genetically divergent and polygenic background of carbapenem resistance in these studied strains. The detection of carbapenem-resistant isolates that do not carry the tested genes, further suggests that mechanisms such as porin loss, efflux pump overexpression or AmpC hyperproduction might also result in detected resistance pattern .

The results validate P. aeruginosa as a relevant reservoir for carbapenem resistance in hospital settings that poses significant challenges to infection control and ultimately threatens patient safety. Conclusion The study emphasizes the necessity of the continued periodical antimicrobial susceptibility testing, molecular surveillance studies for resistance genes and strict infection control policies along proper antibiotic use in Najaf hospitals.

This study collectively provides local data regarding the genetic background of carbapenem resistance in P. aeruginosa, which may provide a scientific rationale for

future epidemiological studies related to this issue as well as hospital antibiotic policy formulation.

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