

Molecular Detection of *oprD* resistant Gene among Carbapenem Resistant *Pseudomonas aeruginosa* Clinical Isolates, Khartoum State-Sudan

Mihad Mansour Elzaki¹, Mohamed Hussein Arbab², Nagla Mohamed Ahmed^{1*}

1. Faculty of Medical Laboratory Sciences, Al-Neelain University, Khartoum, Sudan

2. Faculty of Medical Laboratory Sciences, Omdurman Ahlia University, Khartoum, Sudan

Abstract

Background

Carbapenems are the most important therapeutic options that effect against serious infections caused by multidrug resistant *Pseudomonas aeruginosa* isolates; however recently carbapenems resistant isolates of *P. aeruginosa* are increasing worldwide. It is often due to the impermeability that arises via the loss of the OprD porin, the overproduction of an active efflux pump system, and the production of carbapenem-hydrolyzing β -lactamase

Objectives: This study aimed to detect *oprD* gene among carbapenem resistance *P. aeruginosa* clinical isolates using conventional PCR technique during the period from September 2018 to August 2021.

Methods: Hundred carbapenem resistance *Pseudomonas aeruginosa* clinical isolates were collected from different specimens from Soba Hospital, Fedail Hospital and Military Hospital for this study in Khartoum State, Sudan. All isolates were re-identified using conventional bacteriological techniques and their susceptibility to carbapenem was tested using Kirby-Bauer method for confirmation. All isolates were investigated for the presence of *oprD* gene using polymerase chain reaction (PCR).

Results: Of the 100 clinical isolates, the *oprD* gene expressed in 10 (10%) isolates and it was not detected among the remainder (90%), the higher percentage was found among the isolates from wound swabs 5 (50%), whereas it was not detected among the isolates from high vaginal swabs, nibble discharge and sputum samples (P value = 0.02).

Conclusions: The *oprD* gene was not detected among the vast majority of isolates such findings can supports the idea that the basic mechanism of carbapenem resistance could be via the loss of *oprD* porine gene.

Key words: Carbapenem resistance, *Pseudomonas aeruginosa*, OprD porin, Molecular technique, Portal of entry.

Corresponding author: Nagla Mohamed Ahmed, nag.mas@hotmail.com

Introduction

Pseudomonas aeruginosa is a motile, non-fermenting, gram negative opportunistic bacterium which it responsible for many different infections in patients with compromised host defenses (e.g., cancer, burn, HIV, and cystic fibrosis) [1]. Intensive care unit (ICU) hospitalized patients constitute one of the risk

groups that are more susceptible to acquire pseudomonas infections as they may develop ventilator-associated pneumonia (VAP) and sepsis [2–4]. Moreover, increasing emergence of multidrug-resistant *P. aeruginosa* (MDR PA) isolates during therapy is critical problem which is reported in 27%– 72% of patients with initially susceptible *P. aeruginosa*, leading to increased

health care costs and prolonged hospitalization [5]. Furthermore virulent *P.aeruginosa* is frequently life-threatening and often challenging to treat because it expresses a combination of bacteria-associated factors (intrinsic and acquired antimicrobial resistance, expression of different virulence factors [5].

It has become increasingly clear that resistance development in *P. aeruginosa* is multi- factorial, with mutations in genes encoding porins, efflux pumps, penicillin-binding proteins, and chromosomal β -lactamase, all contributing to resistance to β -lactam, carbapenems, aminoglycosides and fluoroquinolones [6].

Carbapenem antibiotics are recognized as one of the most potent agents with activity against *Pseudomonas aeruginosa* and remain important for the therapy of serious infections due to these pathogens. The mortality associated with carbapenem resistance *P.aeruginosa* infections is high (20% to 50%), making optimal and timely treatment is essential [7].

Recently, however, the strains with resistance to carbapenems have been increasing among clinical isolates of *P. aeruginosa* resistance to carbapenems in *Pseudomonas aeruginosa* is often due to impermeability that arises via the loss of OprD porin, the over production of an active efflux pump system, and the production of carbapenem-hydrolyzing β -lactamase [8].

The *oprD*-coding gene was mapped between 71 and 75 min on the *P. aeruginosa* PAO1 chromosome, and the OprD protein was shown to be coded by 1332-bp nucleotides [9,10]. Outer membrane protein OprD is considered the

preferred portal of entry for carbapenems and similar drugs such as imipenem and meropenem also enter the cell via OprD . Any loss of OprD expression from the outer membrane significantly decreases the susceptibility of *P. aeruginosa* to carbapenems and has been shown to play a major role in the acquired resistance to imipenem and, to a lesser extent, meropenem [11]. Terzi *et al.* (2015) suggested that loss of OprD might be the first antibiotic resistance mechanism induced by selective pressure of carbapenem [11]. So, this study aimed to detect the presence of *oprD* gene encoding carbapenem resistant *pseudomonas aeruginosa* by using molecular technique.

Materials and methods

Study Design and setting

A descriptive cross sectional study was conducted in different Khartoum State hospitals, (Omdurman Teaching Hospital, Fedail Hospital and Soba Teaching Hospital) during the period from November 2018 to November 2019. Hundred (100) Carbapenem (Meropenem and Imipenem) resistant *Pseudomonas aeruginosa* clinical isolates were obtained from different specimens including: wounds, urine, sputum, ear swabs, high vaginal swabs and nibble discharge were included in this study.

Phenotypic methods

The fresh cultured colonies of the clinical isolates were sub-cultured on nutrient agar and MacConkey agar media to ensure purity and optimum growth. Then they were re-identified using routine culture techniques and their susceptibility to carbapenems were tested using Kirby-Bauer method for confirmation.

DNA extraction:

Bacterial DNA was extracted by using boiling method. All bacterial strains were sub cultured on nutrient agar and the overnight pure bacterial growth were used. Three colonies were emulsified in Eppendorf tubes containing 200µl of distilled water and boiled at 100 °C for 30 minutes then centrifuged at 12000 rpm for 15 minutes. The supernatant containing DNA was transferred to new Eppendorf tubes and stored at - 20°C [12].

PCR amplification procedure

PCR was carried out for all isolates by using specific primer for *oprD* gene F5`GGGGTGTTTTTCGCAGAGTA3`, R5`ATCACAGTAAGAGGGGCC3`, 1780 bp, amplification was performed at final volume 25 µl (5µl Master mix, 5µl from DNA, 2µl primer and 13µl D.W) [13].

The PCR cycling conditions were as follows: 2 min of denaturation at 98 °C and 30 cycles of denaturation at 95°C for 30 sec, annealing at 55°C, for 30 sec, and extension at 72°C for 3 min. PCR Thermocycler machine (Promega, German) was used [13].

PCR products was separated on 1.5% agarose gel by electrophoresis to detect specific amplified product by comparing with standard molecular weight marker 100 base pair (DNA ladder). 5µl of PCR product was loaded into the well. Electrophoresis was carried out at 100 Volts, 60 AMP for 30 minutes. The amplified products of the study samples were visualized by Trans-illuminator Photographed by a digital camera

(German) and transferred to computer data for labeling and storage.

Statistical analysis

Data was analyzed using SPSS 21. Frequencies and percent were obtained for frequency tables, Chi-squared test was used for goodness of fit. The relationships between variables tested were obtained using cross tables and Chi-squared (Fisher exact) test for independence. P-value ≤ 0.05 was considered as significant.

Ethical consideration

The approval and informed consent were obtained from Al-Neelain University Ethical committee and informed consent was taken from the hospitals included in this study.

Results

Hundred (100) carbapenem resistant *P.aeruginosa* clinical isolates were collected from different hospitals in Khartoum State. They had been isolated from different clinical samples as represented in Table (1). Their identification was confirmed using conventional bacteriological techniques and their resistance to carbapenem (imipenem and meropenem) was confirmed using Kirby-Bauer method.

Based on conventional PCR results (Figure 1), 10 out of 100 (10.0%) carbapenem resistant *P. aeruginosa* clinical isolates were positive for *oprD* gene as in Figure (2)

The frequency of *oprD* gene among the clinical isolates according to type of specimens was represented in Table (2) (P value= 0.02).

Table (1): Distribution of *Pseudomonas aeruginosa* clinical isolates according to type of specimens

Specimens	Frequency (%)
Wound swab	56 (56.0)
Urine	23 (23.0)
High vaginal swab	11 (11.0)
Sputum	6 (6.0)
Ear swab	3 (3.0)
Nibble discharge	1 (1.0)
Total	100 (100.0%)

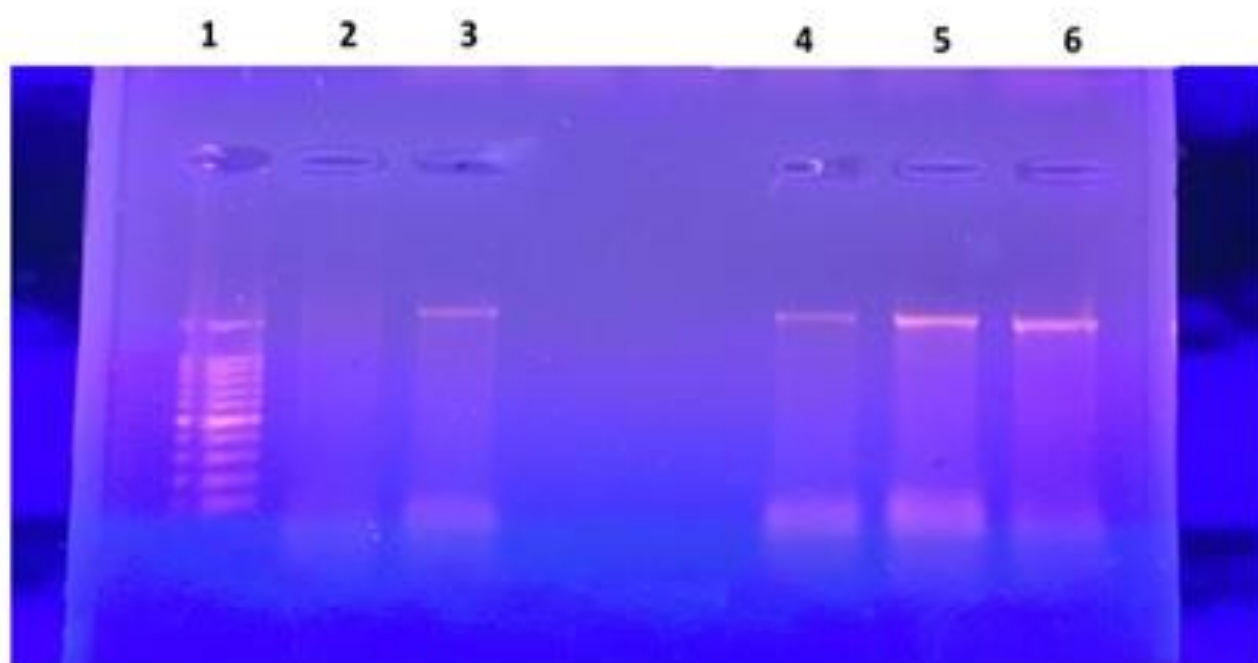


Figure 1 Agarose gel showing the results of amplified *oprD*. Lane: 1 ladder 100 bp, lanes (3, 4, 5, 6) positive *oprD* gene. Length 1780b

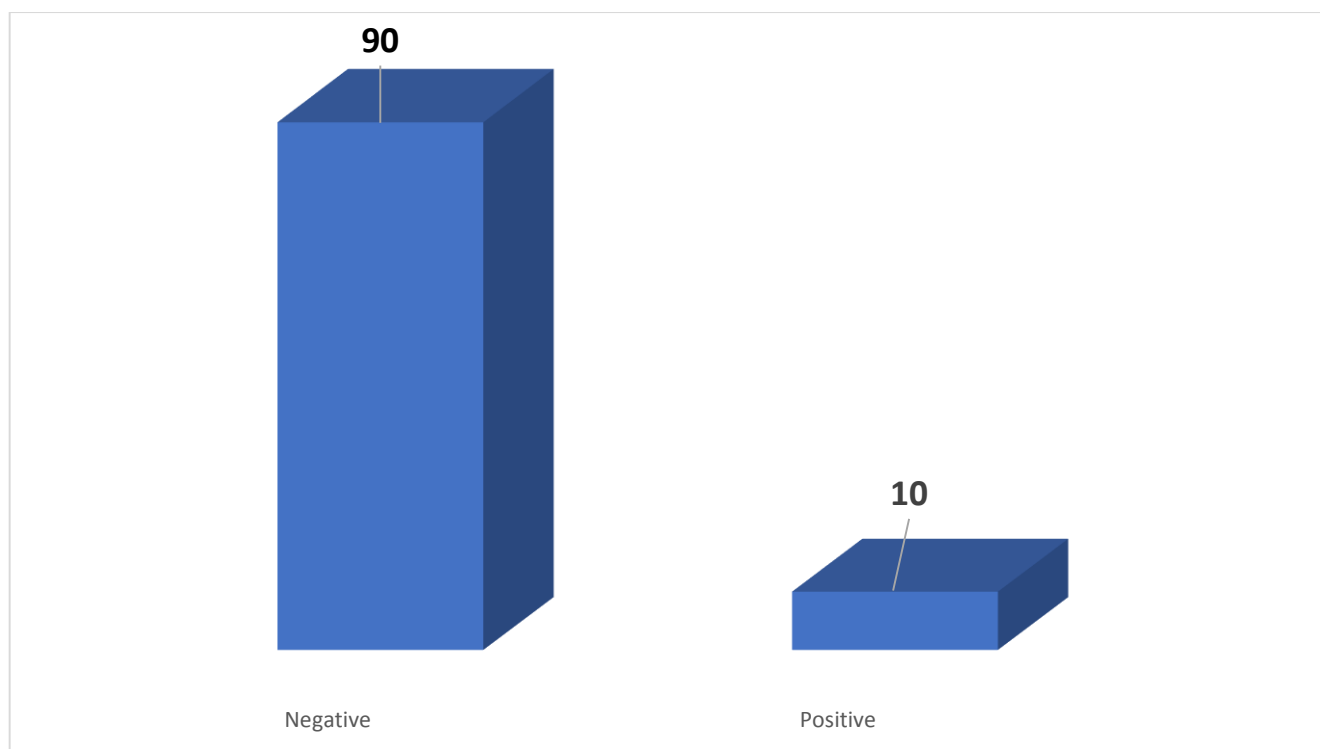


Figure 2: Percentage distribution of *oprD* gene among *P.aeruginosa* clinical isolates

Table 2: Frequency of *oprD* gene among *P. aeruginosa* clinical isolates according to type of specimens

Type of sample	Result	
	Positive (No %)	Negative (No %)
Wound swabs	5 (9.8)	51 (91.1)
Urine	3 (13.0)	20 (86.9)
High vaginal swabs	0 (0.0)	11 (100)
Sputum	0 (0.0)	6 (100)
Ear swabs	2 (66.7)	1 (33.0)
Nibble discharge	0 (0.0)	1 (100)
P value= 0.02		

Discussion

Due to the increase in multi-drug resistant *P. aeruginosa* infections, a greater emphasis has been placed on identifying genetic characteristics underlying bacterial resistance and the clinical implications of these mutations [14].

In the present study it was found that among the total (n= 100) clinical isolates, the most common isolates were from wound swab specimens this is may be due that *Pseudomonas aeruginosa* is most commonly associated with wound infections [15].

Outer membrane protein OprD is considered the preferred portal of entry for carbapenems and similar drugs such as imipenem and meropenem also enter the cell via OprD [16]. Any loss of OprD expression from the outer membrane significantly decreases the susceptibility of *P. aeruginosa* to carbapenems and has been shown to play a major role in the acquired resistance to imipenem and, to a lesser extent, meropenem [17]. Our study showed that *oprD* expression was negative in the majority of *P. aeruginosa* isolates (90%) and it may be played a significant role in their carbapenem resistance, similar study done by Fournier *et al.* (2013) in French public hospital, they detected loss of *OprD*, as a result of mutations or gene disruptions in 94 out of 109 (86.2%) imipenem-resistant *P. aeruginosa* isolates [18]. Also, other study in Bulgarian hospitals showed that OprD expression was decreased in the vast majority of 29 multi-drug resistant *P. aeruginosa* isolates (97%) and played a significant role in their carbapenem resistance

[19]. Other study done by Sanbongi *et al.*, (2009) in Japan that studied a total of 99 clinical isolates of metallo- β -lactamase-negative *Pseudomonas aeruginosa* for their susceptibilities to carbapenem agents and corresponding *oprD* gene mutations. The OprD sequence of each strain was grouped into two major classes, based on the pattern of alterations. Eighty strains (80.8%) were so-called 'full length type', whose OprD proteins were fully encoded. The remaining 19 strains (19.2%) were so-called 'defective type', which possessed deletions or major alterations that might cause conformational changes in the OprD porin protein. The changes in 'defective type' strains led to 15-, 17- and 23-fold increases in the geometric mean MIC for imipenem, meropenem and biapenem compared with 'full length type' strains, respectively [20].

The present study found that the frequency of *oprD* gene was significantly associated with the type of samples (P value=0.02), the *oprD* gene was not detected among 91.1% of isolates from wound specimens and completely did not express among high vaginal swabs (n=56), sputum (n=6) and nibble discharge (n=1). This indicates that these specimens may harbor isolates with OprD protein deficient which may conferred carbapenem resistance to them.

Conclusion

The present study concluded that the vast majority of isolates did not show the presence of *oprD* gene which it reflects the absence or deletion of OprD protein among these isolates. And this is may be

considered the main mechanism of carbapenem resistant among these clinical isolates.

Conflicts of interest

The authors declare no conflicts of interest.

References

- Schurek KN, Breidenstein EBM, and Hancock REV, “*Pseudomonas aeruginosa*: a persistent pathogen in cystic fibrosis and hospital-associated infections,” Antibiotic Discovery and Development, pp. 679–715, 2012.
- Williams BJ, Dehnbostel J, and Blackwell TS, “*Pseudomonas aeruginosa*: host defense in lung diseases,” Respirology, vol. 15, no. 7, pp. 1037–1056, 2010. Gellatly SL and Hancock REW, “*Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses,” Pathogens and Disease, vol. 67, no. 3, pp. 159–173, 2013.
- Chastre J and Fagon JY, “Ventilator-associated pneumonia,” American Journal of Respiratory and Critical Care Medicine, vol. 165, no. 7, pp. 867–903, 2002.
- Sonbol, F.I., Khalil, M.A.E.F., Mohamed, A.B. and Ali, S.S., (2015). Correlation between antibiotic resistance and virulence of *Pseudomonas aeruginosa* clinical isolates. Turkish Journal of Medical Sciences, 45(3), pp.568-577.
- Kohler T, Curty LK, Barja F, van Delden C, Pechere J. Swarming of *pseudomonas aeruginosa* is dependent on cell-to-cell signaling and requires flagella and pili. J Bacteriol. 2000 November 1;182(21):5990-6.
- Li H, Estabrook M, Jacoby GA, Nichols WW, Testa R, Bush K. In vitro susceptibility of characterized beta-lactamase-producing strains tested with avibactam combinations. Antimicrob Agents Chemother, 2015; 59(3):1789–1793
- Kirikae T, Mizuguchi Y, Arakawa Y. (2008) Investigation of isolation rates of *Pseudomonas aeruginosa* with and without multidrug resistance in medical facilities and clinical laboratories in Japan. 2008; J Antimicrob Chemother 61: 612–5
- Borgianni L, Prandi S, Salden L, Santella G, Hanson ND, Rossolini GM, et al. Genetic context and biochemical characterization of IMP-18 metallo-beta lactamase identified in a *Pseudomonas aeruginosa* isolate from the United States. Antimicrob. Agents Chemother. 2011; 55:140–145.
- Büscher KH, Cullmann W, Dick W, Opferkuch W. Imipenem resistance in *Pseudomonas aeruginosa* resulting from diminished expression of an outer membrane protein. Antimicrob. Agents Chemother. 1987; 31:703–708.
- Terzi HA, Kulah C, Atasoy AR, Ciftci IH. Investigation of OprD porin protein levels in carbapenem-resistant *Pseudomonas aeruginosa* isolates. Jundishapur journal of microbiology. 2015; 8(12).
- Dahab RA, Alamin AM, Altayb HN. Phenotypic and genotypic detection of carbapenemase enzymes producing gram negative bacilli isolated from patients in

12. Khartoum state. 2017; F1000 Research, 6: 16-56.
13. Sanbongi Y, Shimizu A, Suzuki T, Nagaso H, Ida T, Maebashi K, Gotoh N. Classification of OprD sequence and correlation with antimicrobial activity of carbapenem agents in *Pseudomonas aeruginosa* clinical isolates collected in Japan. Microbiology and immunology. 2009;53(7):361-7
14. Strateva T, Yordanov D. *Pseudomonas aeruginosa* - a phenomenon of bacterial resistance. J Med Microbiol. 2009;58(Pt 9):1133–48.
15. Sakyo S, Tomita H, Tanimoto K, Fujimoto S, Ike Y. Potency of carbapenems for the prevention of carbapenem-resistant mutants of *Pseudomonas aeruginosa*: the high potency of a new carbapenem doripenem. J Antibiot (Tokyo). 2006;59(4):220–8.
16. El Amin N, Giske CG, Jalal S, Keijsers B, Kronvall G, Wretling B. Carbapenem resistance mechanisms in *Pseudomonas aeruginosa*: alterations of porin OprD and efflux proteins do not fully explain resistance patterns observed in clinical isolates. APMIS. 2005;113(3):187–96.
17. Fournier D, Richardot C, Muller E, Robert-Nicoud M, Llanes C, Plesiat P, et al. Complexity of resistance mechanisms to imipenem in intensive care unit strains of *Pseudomonas aeruginosa*. J Antimicrob Chemother. 2013;68(8):1772–80
18. Vatcheva-Dobrevska R, Mulet X, Ivanov I, Zamorano L, Dobrev E, Velinov T, et al. Molecular epidemiology and multidrug resistance mechanisms of *Pseudomonas aeruginosa* isolates from Bulgarian hospitals. Microb Drug Resist. 2013;19(5):355–61
19. Hong DJ, Bae IK, Jang IH, Jeong AH, Kang H, Lee K. Epidemiology and characteristics of metallo- β -lactamase-producing *Pseudomonas aeruginosa*. Infect Chemother. 2015;47(2):81–97
20. López-García A, del Carmen Rocha-Gracia R, Bello-López E, Juárez-Zelocualtecalt C, Sáenz Y, Castañeda-Lucio M, et al. Characterization of antimicrobial resistance mechanisms in carbapenem-resistant *Pseudomonas aeruginosa* carrying IMP variants recovered from a Mexican Hospital. *Infection and drug resistance*, 2018; 11, p.1523.