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ORIGINAL ARTICLE

Detection of carbapenemase genes and other resistance mechanisms in carbapenem-resistant/cephalosporin-susceptible *Pseudomonas aeruginosa*

[1]Abdelrahman Elsawy*, [2]Khalid Al-Quthami, [2]Ammar Abbas, [2]Hind Khan, [2]Wjanat Hariri, [2]Naelah Hanawi, [2]Shada Almowaled, [2]Renan Ghazawi, [2]Shirin Kaki, [2]Salwa Andejani, [2]Rakan Felemban, [2]Razaz Brashi, [2]Eyad Oqap, [3]Ashraf E. Sorour

[1]Medical Microbiology Department, AlAzhar Faculty of medicine, Cairo, Egypt
[2]Medical Microbiology Laboratory Department, Alnoor Specialist Hospital, Holy Makkah, Saudi Arabia
[3]Medical Microbiology and Immunology Department, Faculty of medicine – Cairo University, Cairo, Egypt

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Corresponding Author:
Abdelrahman Elsawy
Medical Microbiology Department, AlAzhar Faculty of medicine, Cairo, Egypt
Tel.: 00966-561663844
elsawyeg@gmail.com

ABSTRACT

Background: *Carbapenem- resistant P. aeruginosa isolates are increasingly observed. Carbapenem resistance in P. aeruginosa is modulated by acquired carbapenemases in association with non-carbapenemases mechanisms. An uncommon phenotype of carbapenem resistant but cephalosporine susceptible (Carb- R/ Ceph- S) Ps. aeruginosa clinical isolates have been reported.* ***Objective:*** *We aimed to assess mechanisms of carbapenem resistant in this characteristic uncommon phenotype.* ***Methodology:*** *A total of 168 carbapenem resistant clinical isolates of Ps. aeruginosa were recovered form admitted cases in a Tertiary Care Hospital during the period from November 2021 to October 2022. All Carb- R/ Ceph- S Pseudomonas aeruginosa isolates were subjected to the following to detect carbapenems resistant mechanism (s): Genotypic discovery of carbapemenases production, phenotypic detection both of AmpC overproduction and efflux pumps overproduction.* ***Results:*** *48 isolates (28.6%) were cephalosporine susceptible (Carb-R/ Ceph-S). Genotypic discovery of carbapenemases encoding genes by multiplex PCR and phenotypic detection of AmpC overproduction and efflux pumps overproduction were done to identify the possible mechanisms of carbapenem resistant in the studied phenotypes. None of 48 Carb- R/ Ceph-S P. aeruginosa isolates were carrying carbapenemases encoding genes, 60.4% (29/48) had efflux pumps overproduction and 4.2% (2/48) had AmpC overproduction. The highest rate of antimicrobial resistance was to Tigecycline and Colistin and the smallest rate of antimicrobial resistance was to Piperacillin/ Tazobactam, and Tobramycin and Amikacin.* ***Conclusions:*** *None of Carb-R/ Ceph-S P. aeruginosa harboring carbapenemases encoding genes, whereas; efflux pumps overproduction and AmpC overproduction were detected in 60.4% and 4.2% respectively.*

INRTODUCTION

Pseudomonas (Ps) aeruginosa is one of the most important and ubiquitous nosocomial pathogens especially in Intensive Care Units (ICUs)[1,2]. *Ps. aeruginosa* has a remarkable capability to retain resistance to utmost antimicrobial agents[3,4]. These mechanisms affect the development of multidrug resistant (MDR) *Ps. aeruginosa* isolates, and lead to complications in treatment[5, 6]. In addition, MDR isolates of *Ps. aeruginosa* are responsible for outbreaks in rehabilitated cases [7]. As carbapenems are more stable against hydrolysis by the most serine- β- lactamases [8]; carbapenems are extensively used as first- line medicines to treat nosocomial infections and are effective against multidrug- resistant *Ps. aeruginosa* and other bacterial infections producing the cephalosporinase AmpC or extended spectrum β-lactamases[9]. Nonetheless, carbapenem- resistant *Ps. aeruginosa* (CRPA) isolates are frequently observed, presumably due to the global clinical use of carbapenems[10-12]. Carbapenem resistance in *Ps. aeruginosa* is modulated by acquired carbapenemases in association with non-carbapenemases natural mechanisms similar as down- regulation or loss of OprD porin, efflux pumps hyperexpression, chromosomal AmpC- lactamase product and target differences [13, 14]. Thus, more delicate opinions for empirically treating cases infected with CRPA should be considered.

Given the significance of carbapenems for the treatment of infections caused by *Ps. aeruginosa*, it is essential to clarify the mechanisms involved in unusual and/ or inadequately known phenotypes. Knowledge of these mechanisms alert for an adaption to the precise

pressure wielded by antimicrobial and drug resistance development, therefore affecting the treatment of infections caused by these pathogens [15]. Clinical isolates of *Ps. aeruginosa* that displayed an uncommon phenotype of antibiotic resistance to carbapenems, but susceptibility to broad- spectrum cephalosporins (Carb- R/ Ceph- S) have been reported by many studies [16-18]. In these cases, cephalosporins could be used as an alternative medication to treat these cases.

Aim of the Study

The aim of this study was to assess the mechanisms of carbapenem resistant in this characteristic uncommon phenotype; (Carb- R/ Ceph- S).

METHODOLOGY

This descriptive study conducted in a Tertiary Care Hospital (Al-Noor specialist Hospital – Makkah – Kingdom of Saudi Arabia) through one year starting from November 2021 to October 2022. The sampling was a part of *Ps. aeruginosa* of routine hospital laboratory procedure and an informed concurrence was attained from cases. The study was approved by the Laboratory Ethical Committee. Demographic and clinical information, as gender, age and duration of hospitalization were collected.

Bacterial isolates:

A total of 473 *Ps. aeruginosa* isolates were recovered from different clinical samples including; blood, urine, sputum, wound swabs, tissues and broncho-alveolar lavage. Blood samples were processed using BD BACTEC FX Blood Culturing System (Becton Dickinson, USA) and positive samples were sub-cultured on MacConkey agar and unselective blood agar media. All other samples were dressed on MacConkey agar, chocolate agar and unselective blood agar media. Bacterial isolates were identified through VITEK 2 COMPACT system (bioMérieux, USA) using identification GN ID card.

Antimicrobial susceptibility testing

All *Ps. aeruginosa* isolates were tested for their susceptibility to different antimicrobial agents through VITEK 2 COMPACT system (bioMérieux, USA) using AST- GN card. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Carbapenem resistance (Carb- R) was defined when *Ps. aeruginosa* isolates were resistant to meropenem and/or imipenem and cephalosporins susceptibility (Ceph- S) was defined when the isolates were sensitive to ceftazidime and cefepime based on CLSI guidelines [19].

All CRPA isolates were subjected to multiplex PCR assay to detect genes encoding carbapemenases enzymes product.

All Carb- R/ Ceph- S *Ps. aeruginosa* isolates were subjected to the following tests to detect carbapenems resistant mechanism (s): Genotypic discovery of carbapemenases production, phenotypic detection of AmpC overproduction and phenotypic detection of efflux pumps overproduction.

Phenotypic detection of AmpC overproduction

AmpC overproduction was detected by detecting the ceftazidime MIC of the isolate using agar plate supplemented with cloxacillin (250 µg/ ml), as cloxacillin inhibits AmpC β- lactamase effects. At least a twofold decreased concentration of ceftazidime MIC in the presence of cloxacillin compared to MIC of ceftazidime without cloxacillin was considered as an AmpC overproduction [20].

Phenotypic detection of efflux pumps overexpression

The inhibitory effect of phenylalanine- arginine beta- naphthylamide(PaβN; as an efflux pump asset) at a concentration of 40 µg/ ml on the MIC of imipenem and meropenem was detected according to former studies. At least twofolds dropped MIC in the presence of PAβN compared to MIC value without inhibitor was considered as an overexpression of efflux pumps [21].

Genotypic detection of carbapemenases production

Presence of carbapemenases encoding genes; blaKPC, blaNDM, blaVIM, blaOXA- 48, and blaIMP; was tested qualitatively in all Carb- R *Ps. aeruginosa* isolates through multiplex PCR assay using GeneXpert system and Cepheid Xpert Carba- R cartilage(Cepheid, Sunnyvale, CA, USA). .

Quality control reference strains:

Escherichia coli ATCC 25922 and *Ps. aeruginosa* ATCC 27853 were used as quality control reference strains for standard microbiology testing throughout this study [20].

Statistical analysis

The results were analyzed using the SPSS software version 22 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 473 non-duplicate *Ps. aeruginosa* isolates were recovered from different clinical specimens from patients admitted to a Tertiary Care Hospital (Al-Noor specialist Hospital – Makkah – Kingdom of Saudi Arabia)during 2021 to October 2022.

Antimicrobial susceptibility tests revealed that 305 isolates (64.5%) were carbapenem susceptible and 168 isolates (35.5%) were carbapenem resistant (Carb-R). While 120 isolates out of 168 (71.4%) Carb.-R isolates were also cephalosporin resistant (Ceph-R); 48 isolates out of 168 (28.6%) were cephalosporin susceptible (Ceph-S).

Demographic study of the 48 Carb-R / Ceph-S *P. aeruginosa* isolates showed that 39 isolates out of the 48 (81.25%) were recovered from male patients and 9 isolates (18.75%) were recovered from female patients. Patients' age varied from 18 years to 88 years (the mean of age was 49±3). Out of 48 Carb-R/Ceph-S *P. aeruginosa* isolates; 21 isolates (43. 8%) were isolated

from sputum specimens, 15 isolates (31.2%) were isolated from wound swabs, 3 isolates (6.2%) were isolated from urine specimens, 7 isolates (14.6%) were isolated from blood specimens and 2 isolates (4.2%) were isolated from tissue specimens.

The rate of antimicrobial resistance of 48 Carb-R/Ceph-S *Ps. aeruginosa* isolates was highest to Tigecycline (45 isolates out of 48 "93.8%") followed by Colistin (40 isolates out of 48 "83.3%")' Levofloxacin (24 isolates out of 48 "50%"), Aztreonam (22 isolates out of 48 "45.8%"), Ciprofloxacin (12 isolates out of 48 "25%"), Ticarcillin / clavulanic acid (11 isolates out of 48 "22.9%"), Gentamicin (8 isolates out of 48 "16.7%"), Piperacillin / Tazobactam (4 isolates out of 48 "8.3%") and was lowest to both Tobramycin and Amikacin (2 isolates out of 48 "4.2%").

Carbapenemases encoding genes were detected by Gene-Xpert multiplex PCR assay in in76 isolates (63.4%) Carb-R / Ceph-R *P. aeruginosa* isolates; 23 isolates (13.7%) had *bla* NDM gene and 53 isolates (31.5%) had *bla* OXA-48 gene (table 1). Whereas; Carbapenemases encoding genes were not detected in all Carb-R/Ceph-S *Ps. aeruginosa* isolates (0%).

Table 1: Carbapenemases encoding genes in Carbapenem resistant *Ps. aeruginosa* isolates

Carbapenemases genes	Carbapenem resistant *Ps. aeruginosa* isolates Total n. 168 (100%)		Total Number of genotypic positive isolates
	Cephalosporine susceptible n.= 48 (28.6%)	Cephalosporine resistant n.= 120 (71.4%)	
bla KPC	0.0 (0.0%)	0.0 (0.0%)	0.0 (0.0%)
bla VIM	0.0 (0.0%)	0.0 (0.0%)	0.0 (0.0%)
bla NDM	0.0 (0.0%)	23 (19.2%)	23 (13.7%)
bla IPM	0.0 (0.0%)	0.0 (0.0%)	0.0 (0.0%)
bla OXA-48	0.0 (0.0%)	53 (44.2%)	53 (31.5%)
Total number	0.0 (0.0%)	76 (63.4%)	76 (45.2%)

Overproduction of efflux pumps was detected phenotypically in 29 isolates out of 48 Carb-R / Ceph-S *Ps. aeruginosa* isolates (60.4%); 23 isolates of them (47.9%) were phenotypically positive for both imipenem and meropenem, 2 isolates (4.2%) were phenotypically positive for imipenem only and 4 isolates (8.3%) were phenotypically positive for meropenem only (table 2).

AmpC overproduction was detected phenotypically in only 2 isolates out of 48 Carb-R / Ceph-S *P. aeruginosa* isolates (4.2%). These 2 isolates had a combined antimicrobial resistant mechanism as they were also phenotypically positive regarding efflux pumps overproduction for both imipenem and meropenem (table 2).

Table 2: Phenotypic detection of Efflux pumps overproduction and AmpC overproduction in Carb. R. / Ceph. S. *Ps. aeruginosa* isolates

Phenotyping	Carb. R. / Ceph. S. *Ps. aeruginosa* isolates n. = 48		
	Efflux pumps overproduction	AmpC overproduction	Combined AmpC & Efflux pumps overproduction
Phenotypic positive	29 (60.4%)	0.0 (0.0%)	0.0 (0.0%)
Positive for both imipenem and meropenem	23 (47.9%)	0.0 (0.0%)	0.0 (0.0%)
Positive for imipenem only	2 (4.2%)	2 (4.2%)	2 (4.2%)
Positive for meropenem only	4 (8.3%)	0.0 (0.0%)	0.0 (0.0%)
Phenotypic negative	19 (39.6%)	46 (95.8%)	46 (95.8%)
Total number	**48 (100%)**		

DISCUSSION

Multidrug resistant *Ps. aeruginosa* strains are common causes of nosocomial infections worldwide[22], especially in patients with impaired immune systems [23].

Carbapenems are the last choice for treatment of many infections caused by drug-resistant bacterial pathogens. Unfortunately, carbapenem-resistant *Ps. aeruginosa* are on the rise. Resistance to carbapenem in *Ps. aeruginosa* may be due to a combination of β-lactamases (especially AmpC) production, carbapenemases production, porin mutations, efflux pump systems overexpression, and/or penicillin-binding protein modifications [24, 25].

Our study demonstrated clinical isolates of *Ps. aeruginosa* exhibiting resistance to carbapenem but remain cephalosporin-susceptible from a tertiary care

hospital clinical setting. These phenotypes have been identified in other countries such as Iran, China and Brazil [16-19]. In our study out of 168 Carb-R *Ps. aeruginosa* isolates that recovered from admitted patients during the period from November 2021 to October 2022, 48 isolates were Carb-R / Ceph-S (28.6%). In another study, these characteristic phenotypes were 19.8% [17].

In our study in addition to carbapenem resistance, 93.8% of isolates were resistant to Tigecycline, 83.3%" were resistant to Colistin unlike other studies that mentioned no resistance to Colistin [7, 26]. The isolates also exhibited resistance to Levofloxacin, Aztreonam and Ciprofloxacin (50%, 45.8% and 25% respectively) but these rates of resistance were different from that mentioned in other studies (87%, 64.3 and 60% respectively) [17, 26]. The lowest rates of resistance were to Gentamicin, Piperacillin / Tazobactam, Tobramycin and Amikacin (16.7%, 8.3%, 4.2 and % 4.2% respectively) and this was also unlike what had been reported in other studies (60.2% 69.6%, 60.2% & 65.2 respectively) [17, 26].

Mechanisms of carbapenem resistance were investigated in this study genotypically by multiplex PCR to detect different types of carbapenemases encoding genes and phenotypically to detect efflux pumps overproduction and AmpC overproduction. None of Carb-R / Ceph-S *Ps. aeruginosa* isolates (0%) were carrying any of carbapenemases encoding genes, but 60.4% of isolates (29 out of 48) had been detected to have efflux pumps overproduction and 4.2% of isolates (2 out of 48) had been detected to have AmpC overproduction. In agreement with these findings *Eloiza et al* in 2017 didn't report in their study any of carbapenemases as a mechanism of carbapenem resistance of the Carb-R / Ceph-S *Ps. aeruginosa* isolates [15]. Other studies previously from Korea and Japan reported the presence of one or more of carbapenemases encoding genes were the mechanism of carbapenem resistance [27, 28]. Meanwhile, AmpC overproduction and efflux pumps overproduction were reported as uni factorial mechanism of carbapenem resistance in Carb-R / Ceph-S *Ps. aeruginosa* in 12.28% and 54.39% respectively [27] other study mentioned that efflux pumps overproduction was the single and only mechanism of carbapenem resistance in 41.7% of Carb-R / Ceph-S *Ps. aeruginosa* [17].

In our study, there were 2 Carb-R / Ceph-S *Ps. aeruginosa* isolates that had a combination of AmpC overproduction and efflux pumps overproduction as mechanisms of carbapenem resistance and these 2 isolates were extensive drug resistant (XDR) isolates as they were resistant to all antimicrobial agent but not ceftazidime and cefepime. On the other hand, in 19 out of 48 isolates there were no detected mechanism (s) for carbapenem resistance, this may be due to other mechanisms, which were not investigated in our study such as mutation of porin proteins.

CONCLUSIONS

Out of 168 carbapenem resistant *Ps. aeruginosa* isolates recovered in our study during a period of one year (November 2021- October 2022), 48 isolates were Carb-R/Ceph-S, none of them harboring carbapenemases encoding genes. Efflux pumps overproduction and AmpC overproduction were detected in 60.4% and 4.2% respectively. The Carb-R / Ceph-S *Ps. aeruginosa* isolates had highest rates of antimicrobial resistance to Tigecycline and Colistin and lowest rate of antimicrobial resistance to Piperacillin / Tazobactam, Tobramycin and Amikacin.

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