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Short Communication

Carbapenem-resistant-only *Pseudomonas aeruginosa* infection in patients formerly infected by carbapenem-susceptible strains

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ABSTRACT

Pseudomonas aeruginosa isolates that were initially carbapenem-susceptible and later became selective carbapenem-resistant following antimicrobial therapy were identified from individual cases during the same hospitalisation. Cross-resistance to other β-lactams was not found and their susceptibilities remained identical in consecutive isolates. Real-time quantitative reverse transcription PCR was performed to investigate the role of OprD, an outer membrane protein regulating the entry of carbapenems, in the appearance of carbapenem-resistant-only *P. aeruginosa* (CROPA). Fifteen paired isolates of carbapenem-susceptible *P. aeruginosa* (CS-PA) and CROPA were identified. All of the cases had carbapenem exposure history within 1 month before the appearance of CROPA (mean 10 days). Reduced OprD expression was found in 93% (14/15) of the isolates, suggesting that *oprD* inactivation was the major contributor to selective carbapenem resistance. Of the 14 cases with CROPA due to *oprD* mutation, 71% (10/14) were persistent infection, as genotype analysis revealed that their paired strains were isogenic; 29% (4/14) represented re-infections as they were heterogenic, suggesting that *oprD*-deficient CROPA existed in the hospital and that carbapenem selective pressure promoted its spread to patients. We conclude that CROPA may occur soon after the use of carbapenems to treat CS-PA infections and that *oprD* mutation is the major mechanism of resistance in CROPA. Restriction of empirical use of carbapenems by antibiotic stewardship is important to halt the occurrence of CROPA.

1. Introduction

Pseudomonas aeruginosa is one of the major nosocomial pathogens. Infections by *P. aeruginosa* are often difficult to treat because of their limited susceptibility to commonly used antimicrobial agents [1]. There are a limited number of antimicrobial agents with reliable activity against *P. aeruginosa*, including antipseudomonal penicillins, cephalosporins, fluoroquinolones and carbapenems. Carbapenems are often the most consistently effective agents for the treatment of serious *P. aeruginosa* infections [2]. However, resistance to carbapenems has risen steadily among *P. aeruginosa* and is often associated with resistance to other antibiotics [3].

Carbapenem resistance in *P. aeruginosa* typically occurs through the loss of OprD [4], an outer membrane protein regulating the entry of carbapenems. Loss of OprD is the major determinant of non-metallo-β-lactamase-mediated resistance to carbapenems [5]. In addition to inactivation of OprD, overexpression of the intrinsic efflux system (MexAB–OprM) or production of carbapenem-hydrolysing β-lactamases (AmpC) may also be related to the occurrence of carbapenem resistance [6].

Carbapenem resistance often occurs following prolonged treatment of *P. aeruginosa*-infected patients, and cross-resistance to other β-lactams has been observed [3]. However, rapid onset of selective carbapenem resistance during treatment occurred in 15 patients in our hospital; in all cases the isolates were initially carbapenem-susceptible (CS). Susceptibilities to all other tested β-lactams remained identical in the carbapenem-resistant (CR) and CS pairs.

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Table 1
Comparison of demographic and clinical characteristics between cases with persistent infection and cases with re-infection of carbapenem-resistant-only *Pseudomonas aeruginosa* (CROPA).

Characteristic	All ($N = 15$)	Persistent infection ($N = 10$)	Re-infection ($N = 5$)	P-value
Demographics				
Age (years)				
Median (range)	57.00 (0.03–80.00)	50.50 (0.03–69.00)	67.00 (49.00–80.00)	
Mean ± S.D.	51.3 ± 23.9	43.3 ± 24.6	67.2 ± 12.6	0.05
Sex male	11 (73)	7 (70)	4 (80)	0.59
Site of isolates [*n* (%)]				
Blood	6 (40)	5 (50)	1 (20)	0.58
Ascites	4 (27)	3 (30)	1 (20)	1.00
Sputum	1 (7)	0	1 (20)	0.33
Wound discharge	2 (13)	1 (10)	1 (20)	1.00
Pleural effusion	1 (7)	1 (10)	0	1.00
Pericardial effusion	1 (7)	0	1 (20)	0.33
Concomitant diseases [*n* (%)]				
Hepatic dysfunction	7 (47)	3 (30)	4 (80)	0.12
Renal impairment	8 (53)	4 (40)	4 (80)	0.28
Chronic pulmonary disease	7 (47)	5 (50)	2 (40)	1.00
Cardiac disease	6 (40)	4 (40)	2 (40)	1.00
Cerebral vascular disease	2 (13)	1 (10)	1 (20)	1.00
Diabetes mellitus	5 (33)	4 (40)	1 (20)	0.60
Malignancy	8 (53)	4 (40)	4 (80)	0.28
Surgery	12 (80)	9 (90)	3 (60)	0.24
>3 concomitant diseases	9 (60)	5 (50)	4 (80)	0.58
Clinical conditions before appearance of CROPA (days)				
TAR [median (range)][a]	12.00 (1.00–71.00)	12.50 (7.00–71.00)	11.00 (1.00–13.00)	0.27
ICU stay				
Median (range)	16.00 (5.00–60.00)	15.50 (8.00–57.00)	20.00 (5.00–60.00)	0.71
Mean ± S.D.	23.7 ± 18.3	21.7 ± 16.5	27.8 ± 23.1	
Ventilator use [median (range)]	12.00 (0.00–60.00)	16.50 (0.00–60.00)	9.00 (2.00–53.00)	0.36
Antibiotic usage in the month before appearance of CROPA (days)				
Carbapenems	15 (100)	10 (100)	5 (100)	
Timing of introduction				
Pre-TAR	2 (13)	1 (10)	1 (20)	1.00
During TAR	13 (87)	9 (90)	4 (80)	
Duration of exposure				
Median (range)	9.00 (2.00–28.00)	8.50 (2.00–28.00)	10.00 (2.00–12.00)	0.71
Mean ± S.D.	10.2 ± 7.2	11.4 ± 8.2	7.8 ± 4.5	
Glycopeptides	13 (87)	9 (90)	4 (80)	1.00
Duration of exposure				
Median (range)	5.00 (0.00–19.00)	10.00 (0.00–19.00)	3.00 (0.00–10.00)	0.14
Mean ± S.D.	7.1 ± 5.8	8.7 ± 6.1	3.8 ± 3.8	
First-generation cephalosporins	2 (13)	2 (20)	0	0.52
Duration of exposure [median (range)]	0.00 (0.00–2.00)	0.00 (0.00–2.00)	0	0.30
Third-generation cephalosporins	8 (53)	6 (60)	2 (40)	0.61
Duration of exposure [median (range)]	1.00 (0.00–25.00)	2.00 (0.00–15.00)	0.00 (0.00–25.00)	0.70
Piperacillin/tazobactam	3 (20)	1 (10)	2 (40)	0.24
Duration of exposure [median (range)]	0.00 (0.00–8.00)	0.00 (0.00–2.00)	0.00 (0.00–8.00)	0.14
Fluoroquinolones	2 (13)	0	2 (40)	0.10
Duration of exposure [median (range)]	0.00 (0.00–9.00)	0	0.00 (0.00–9.00)	0.04
Aminoglycosides	2 (13)	0	2 (40)	0.10
Duration of exposure [median (range)]	0.00 (0.00–5.00)	0	0.00 (0.00–5.00)	0.04

S.D., standard deviation; TAR, time at risk; ICU, intensive care unit.
[a] TAR was defined as the time interval between detection of the first carbapenem-susceptible *P. aeruginosa* and the detection of CROPA in each individual patient.

This study sought to investigate the mechanism accounting for the occurrence of selective carbapenem resistance in *P. aeruginosa* isolates, with a hypothesis that this resistance results from a loss of OprD expression. Clinical features and risk factors associated with the occurrence of CR-only *P. aeruginosa* (CROPA) in these cases were also analysed.

2. Materials and methods

2.1. Bacterial strains and microbiological investigation

From 2007 to 2011, clinical isolates of *P. aeruginosa* from the microbiology laboratory of Chang Gung Memorial Hospital (Taoyuan, Taiwan) isolated from the same patient during the same hospitalisation that were initially CS and then became CR were collected for investigation. All isolates were identified by standard methods [7] and antimicrobial susceptibilities were determined by the standard disc diffusion method and Etest [8]. Susceptibility and resistance were defined according to the criteria suggested by the Clinical and Laboratory Standards Institute (CLSI) [8]. Susceptibilities to all other tested non-carbapenem antibiotics (amikacin, gentamicin, ceftazidime, aztreonam, ciprofloxacin, cefepime and piperacillin/tazobactam) were identical between CS *P. aeruginosa* (CS-PA) and CROPA pairs.

Inactivating mutations in the *oprD* gene were investigated by real-time quantitative reverse transcription PCR for single paired strains (CS-PA and CROPA) isolated from each case. In each case, two independent PCR products were fully sequenced as described previously [9] and the resulting sequences were compared with that of the reference strain *P. aeruginosa* PAO1. To investigate the existence or integrity of *oprD*, primer set OprD-F (5′-CGCCGACAAGAAGAACTAGC-3′) and OprD-R (5′-GTCGATTACAGGATCGACAG-3′) was employed for PCR analyses.