Pan-drug-resistant *Pseudomonas aeruginosa* causing nosocomial infection at a university hospital in Taiwan

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ABSTRACT

This study evaluated the clinical and microbiological characteristics of 16 patients who were colonised or infected with 26 isolates of pan-drug-resistant *Pseudomonas aeruginosa* (PDRPA; intermediately-resistant or resistant to all cephalosporins, piperacillin–tazobactam, aztreonam, carbapenems, ciprofloxacin and aminoglycosides) in a university hospital during 1999–2002. All the isolates had colistin MICs ≤4 mg/L, 19 (73%) isolates had $bla_{\text{VIM-3}}$, and 25 (96%) isolates had class I integrons (*int*I). Time-kill studies for two PDRPA blood isolates demonstrated synergism for cefepime–amikacin after 24 h. Pulsed-field gel electrophoresis analysis of the isolates revealed a polyclonal nature (12 pulsotypes), although clonal dissemination of PDRPA isolates among these patients was also present.

Keywords Colistin, pan-drug resistance, *Pseudomonas aeruginosa*, resistance, synergism, time-kill studies

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Pseudomonas aeruginosa is a major cause of nosocomial infection, particularly among immunocompromised patients or patients treated in intensive care units (ICUs) [1–5]. Anti-pseudomonal β-lactams, fluoroquinolones, aminoglycosides and carbapenems are used for treating *P. aeruginosa* infections [1–5], and intensive use of these antimicrobial agents has facilitated the rapid emergence of resistance in this species [1,6–8]. Imipenem-resistant and pan-drug-resistant isolates of *P. aeruginosa* have been reported as the cause of nosocomial outbreaks of infection in ICUs and burn units in the past decade [8–10].

During the period January 1999 to December 2002, 26 pan-drug-resistant *P. aeruginosa* (PDRPA) isolates, defined as isolates intermediately-resistant or resistant to all antimicrobial agents available for clinical use (cephalosporins, piperacillin–tazobactam, aztreonam, carbapenems, ciprofloxacin and aminoglycosides) according to routine disk-diffusion susceptibility results [11], were recovered from 16 patients at National Taiwan University Hospital, a 2000-bed hospital located in northern Taiwan (Table 1). There were two patients who were infected or colonised with PDRPA in 1999, four in 2000, five in 2001, and five in 2002.

All 16 patients had various underlying diseases. Ten of the 16 patients acquired PDRPA infection in an ICU. Three of the patients had catheter-related bacteraemia, six had an indwelling device associated with urinary tract infection, four had ventilator-associated pneumonia, one had a wound infection and multiple episodes of catheter-related bacteraemia, and one had nosocomial pneumonia and urinary tract infection. Each patient had received a carbapenem (imipenem or meropenem) (six patients) or an extended-spectrum cephalosporin (cefepime or ceftazidime) (ten patients), either alone or in combination with ciprofloxacin or an aminoglycoside, before isolation of PDRPA.

The interval between admission and the first isolation of PDRPA for the 16 patients ranged from 2 to 290 days, with a mean of 74 days. Three patients (patients 1, 11 and 15) had more than one PDRPA isolate recovered from various clinical specimens during their period of admission (three isolates from each individual patient were identical; see below). Three patients (patients 2, 3 and 4) died before PDRPA was isolated. The remaining 13 patients received cefepime or meropenem, plus an aminoglycoside or ciprofloxacin. Seven (43.8%) patients (patients 2–6, 13 and 14) died within 30 days of isolation of PDRPA.

MICs of ten antimicrobial agents for the 26 isolates were determined using the agar dilution

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Table 1. Clinical and microbiological characteristics of 16 patients with pan-drug-resistant *Pseudomonas aeruginosa* (n = 26) infections

				Isolate			MIC (mg/L)				
Patient	Age/sex	Underlying conditions	Location[b]	Designation	Source	Date (day/month/year) of isolation (days)[c]	Imipenem	Meropenem	bla_{VIM-3}	*int*1	PFGE pulsotype
1[a]	76/M	Previous CVA, ARF	3C2	A	Sputum	8/4/1999 (18)	32	32	–	+	I
2	78/F	Colon cancer, CAD, ARF	4C1	B	Sputum	28/5/1999 (16)	16	16	–	+	II
3	69/M	Lung cancer, previous CVA	14A	C	Blood	27/12/1999 (46)	32	> 32	–	–	III
4	17/M	AML, post-BMT, neutropenia (post-C/T)	4C2	D	Blood	29/3/2000 (50)	16	32	–	+	IV
5	12/F	SLE	4C2	E	Genital discharge	24/4/2000 (120)	> 32	> 32	+	+	V
6	57/F	DM, CRI	13D	F	Sputum	14/11/2000 (90)	> 32	> 32	+	+	VI
7	70/F	Liver cirrhosis, MPGN	13D	G	Urine	19/12/2000 (49)	> 32	> 32	+	+	VI
8	76/M	CVA, BPH with cystofix indwelling	4W1	H	Urine	16/7/2001 (3)	16	8	+	+	VII
9	62/F	VHD with CHF	14B	I	Sputum	1/8/2001 (88)	> 32	> 32	+	+	VI
10	70/M	DM, vocal cord paralysis, tracheostomy	3C1	J	Sputum	29/10/2001 (2)	16	16	–	+	VIII
11[a]	20/M	Flame burn (95% of TBSA)	3A1	K	Blood	12/11/2001 (120)	8	32	+	+	IX
12	75/M	COPD, BPH	15A	L	Wound	31/12/2001 (33)	16	16	+	+	VIII
13	25/M	Flame burn, ARF (93% of TBSA)	12C	M	Urine	16/3/2002 (140)	> 32	> 32	+	+	X
14	9/F	Flame burn (93% of TBSA)	3A1	N	Blood	5/6/2002 (70)	> 32	> 32	+	+	XI
15[a]	66/M	DM, previous TB, cryptococcal meningitis	3C1	O	Sputum	2/9/2002 (47)	> 32	> 32	+	+	XI
16	35/M	Pemphigus vulgaris, steroid use, previous CVA	3A1	P	Urine	14/10/2002 (290)	> 32	> 32	+	+	XII

PFGE, pulsed-field gel electrophoresis; CVA, cerebrovascular accident; ARF, acute renal failure; CAD, coronary artery disease; AML, acute myeloblastic leukaemia; BMT, bone marrow transplantation; C/T, chemotherapy; SLE, systemic lupus erythematosus; DM, diabetes mellitus; TBSA, total body surface area; CRI, chronic renal insufficiency; MPGN, membranoproliferative glomerulonephritis; BPH, benign prostatic hypertrophy; COPD, chronic obstructive pulmonary disease; VHD, vulvular heart disease.
[a]Multiple isolates belonging to the same pulsotype were obtained from this patient.
[b]Intensive care units (3A1, 3C1, 3C2, 4C1, and 4C2) and general wards (4W1, 12C, 13B, 13D, 14A, 14B and 15A).
[c]Indicates intervals (days) between admission and isolation of PDRPA.

method [12]. These isolates were intermediately-resistant or resistant (50%) to amikacin, and resistant to aztreonam (27%), cefepime (12%), ceftazidime (8%), ciprofloxacin (4%), levofloxacin (4%), imipenem (4%) and meropenem (4%), but not to piperacillin–tazobactam. The MIC range of colistin was 0.5–4 mg/L, with an MIC_{50} of 1 mg/L and an MIC_{90} of 4 mg/L. All PDRPA isolates were negative for the bla_{IMP-1}, bla_{IMP-2}, bla_{VIM-1}, bla_{VIM-2} or bla_{VIM-4} genes [13,14]. Nineteen (73%) of the 26 PDRPA isolates harboured bla_{VIM-3}, and 25 (96%) had class I integrons (*int*I) (Table 1) [15,16]. The bla_{VIM-3} gene, shown previously by sequencing to be contained within *int*I, was not transferable by conjugation.

Time-kill curves were determined for two PDRPA isolates recovered from two patients with bloodstream infections as described previously [17]. Antimicrobial agents were tested alone and in four combinations: ceftazidime, aztreonam, cefepime plus amikacin, and ceftazidime plus ciprofloxacin. All combinations, except the ceftazidime

plus ciprofloxacin combination, resulted in synergism after 24 h with 0.5× the MICs for the two PDRPA isolates. The combination regimens also resulted in a higher rate of killing of these two isolates over the first 2–4 h, when compared with the most active single agent (Fig. 1). At 0.25× the MICs, synergism after 24 h was observed only with the cefepime plus amikacin combination.

Genotypes (pulsotypes) of the 26 isolates were determined by pulsed-field gel electrophoresis using restriction enzyme *Spe*I [17]. In total, 12 pulsotypes of PDRPA were identified among these 16 patients. Three patients (patients 6, 7 and 9) yielded isolates of PDRPA that belonged to the same pulsotype (VI); two of these patients had been admitted to the same ward in 2000, while one was admitted to a different ward in 2001. Two patients (patients 14 and 15) who yielded isolates that belonged to pulsotype XI were admitted to different ICUs on the same floor of the hospital, but 3 months apart. The same pulsotypes were identified from each of the three patients who yielded more than one PDRPA isolate during their hospital stays, with an interval that ranged from 8 days (patient 1) to 80 days (patient 11).

These findings highlight three important points. First, although some dissemination of two of the 12 clones was observed, the high genotypic heterogeneity among the PDRPA isolates is indicative of the polyclonal nature of emergence of these resistant bacteria. Second, since the spread of metallo-β-lactamase-producing *P. aeruginosa* isolates has been reported worldwide [13,14,16,18], the fact that most PDRPA isolates in the present study contained $bla_{\text{VIM-3}}$ supports previous findings [13], and also suggests that horizontal transmission of a $bla_{\text{VIM-3}}$-containing integron may have occurred between these PDRPA isolates [16]. Further study on these PDRPA isolates to elucidate the mode of transfer of $bla_{\text{VIM-3}}$, as well as other mechanisms conferring multiple drug resistance, are ongoing. Third, the time-kill analyses indicated that the cefepime plus amikacin combination seemed to be the most favourable of the tested options for treating infections caused by PDRPA. However, the in-vitro data should be validated by assessing the clinical efficacy of various combinations of antimicrobial agents before specific recommendations can be made to modify existing treatment guidelines for infections caused by PDRPA [19]. In the present study, all PDRPA isolates had colistin MICs of ≤4 mg/L, which indicates that this agent may be an alternative choice for the treatment of PDRPA infections [20], although this agent is currently not available in Taiwanese hospitals.

In summary, the emergence of PDRPA may be a harbinger of the so-called post-antibiotic era. A stringent antibiotic control policy should be exercised as part of efforts to control the emergence and spread of these multiresistant organisms, and strict compliance with infection control measures is essential to reduce the likelihood of nosocomial spread of infection.

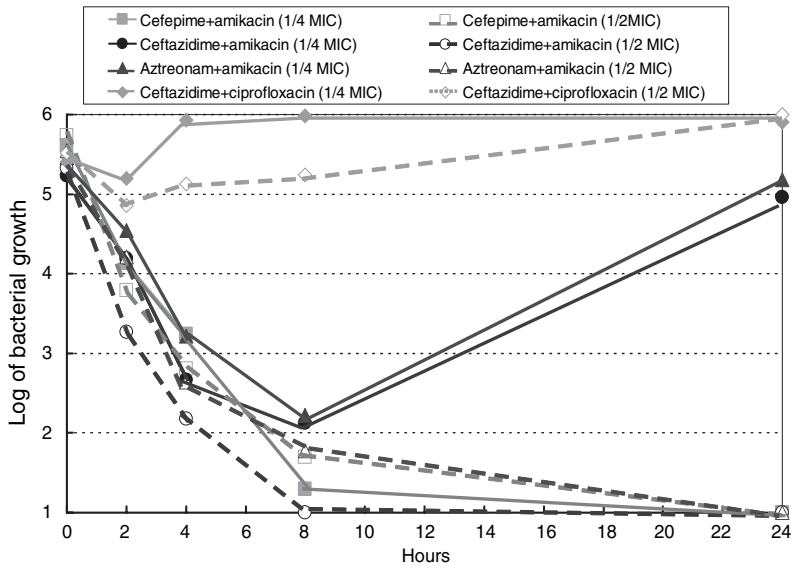

Fig. 1. Representative killing curves at 0.5× and 0.25× the MICs for one of the pan-drug-resistant *Pseudomonas aeruginosa* (PDRPA) blood isolates (isolate K1; pulsotype IX-1).

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RESEARCH NOTE

The relationship between serotypes and PFGE genotypes in isolates of *Streptococcus pneumoniae* from Hungary

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ABSTRACT

The relatedness of 112 penicillin-non-susceptible isolates of *Streptococcus pneumoniae* from Hungary was determined by pulsed-field gel electrophor-

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