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Original Article

Antimicrobial therapy and control of multidrug-resistant *Pseudomonas aeruginosa* bacteremia in a teaching hospital in Taiwan

Ching-Hsiang Leung[1], Nai-Yu Wang[2], Chang-Pan Liu[3,4], Li-Chuan Weng[2], Feng-Chih Hsieh[2], Chun-Ming Lee[3,4,5]

[1]Division of Endocrinology and Metabolism, Department of Medicine, [2]Microbiology Section, Department of Medical Research, and [3]Division of Infectious Diseases, Department of Medicine, Mackay Memorial Hospital; [4]Mackay Medicine Nursing and Management College; and [5]Taipei Medical University, Taipei, Taiwan

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Background and Purpose: The emergence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* is a challenging clinical problem. This study investigated the source of an outbreak of MDR *P. aeruginosa* infections and the role of combination therapy in its management.

Methods: MDR *P. aeruginosa* isolates were collected at the MacKay Memorial Hospital, Taipei, Taiwan, and antibiotic synergy was investigated based on antibiotic susceptibility tests using a combination of antibiotics. Isolates of patients with MDR *P. aeruginosa* bacteremia were selected for genetic analysis by pulsed-field gel electrophoresis.

Results: A combination of ceftazidime, amikacin, and sulbactam had significant synergistic effects against bloodstream MDR *P. aeruginosa* isolates and was more beneficial clinically compared with other antibiotic combinations. The major source of MDR *P. aeruginosa* infection was located and stringent infection control measures were enforced.

Conclusion: The results of this study suggest that use of triple antimicrobial therapy (ceftazidime, amikacin, and sulbactam) can be a useful alternative treatment for MDR *P. aeruginosa* infection in certain circumstances.

Key words: Anti-infective agents; Bacteremia; Colistin; Drug resistance, multiple; *Pseudomonas aeruginosa*; Sulbactam

Introduction

Pseudomonas aeruginosa has emerged as one of the most important pathogens causing nosocomial Gram-negative bacilli infection and is a major cause of opportunistic infection [1]. *P. aeruginosa* is recognized as a particularly dangerous pathogen owing to its natural resistance to many anti-microbial agents and its capacity to acquire new resistance mechanisms under pressure from antibiotics [2].

Multiple mechanisms of antimicrobial resistance have been described for *P. aeruginosa*. The organism contains an inducible beta (β)-lactamase, making it intrinsically resistant to many β-lactam antibiotics that induce this enzyme and are then hydrolyzed by it [3]. *P. aeruginosa* encodes several efflux pumps that pump β-lactam antibiotics and other classes of antimicrobial agents out of the bacterial cell [4]. In addition, the membrane of the bacterium is impermeable to many antibiotics, and thus also contributes to drug resistance [5,6]. The interplay of these mechanisms results in multiple antimicrobial resistance of *P. aeruginosa*. Various antibiotic combinations have been tried to treat multidrug-resistant (MDR) *P. aeruginosa* infections. However, a single efflux mutation may affect both the β-lactams and the fluoroquinolones, thereby reducing the effectiveness of combinations that include these drugs [2].

At the Mackay Memorial Hospital (MMH), Taipei, Taiwan, there have been severe bloodstream infections

Corresponding author: Dr. Chun-Ming Lee, MD, Division of Infectious Diseases, Department of Medicine, Mackay Memorial Hospital, No. 92, Sec. 2, Chun-San N. Road, Taipei, Taiwan.
E-mail: leecm@ms2.mmh.org.tw

caused by a strain of MDR *P. aeruginosa* that was resistant to all commercially available antimicrobial agents in Taiwan. Although the prevalence of *P. aeruginosa* infection remained relatively stable at the hospital from 2001 to 2004, there was a sharp increase in the number of MDR *P. aeruginosa* isolates in 2004 compared with the preceding 3 years. Additionally, in 2004, many patients admitted to the medical intensive care unit (ICU) developed MDR *P. aeruginosa* bacteremia, and an outbreak was suspected. Colistin was not available for use during this period, and treating MDR *P. aeruginosa* infection with a greatly restricted choice of antibiotics was a challenge.

The primary aim of this study was to find alternative ways to treat MDR *P. aeruginosa* infection in the absence of colistin using a combination of antibiotics based on antibiotic susceptibility tests. The second aim was to investigate the cause of the outbreak so that steps could be taken to control the spread of MDR *P. aeruginosa* infection.

Methods

Patients and bacterial isolates

MMH is a 2000-bed teaching medical centre in Taiwan, with 42 beds in the medical ICU. All MDR *P. aeruginosa* isolates cultured in the hospital's clinical microbiology laboratory from January 2001 to December 2004 were collected. Only 1 isolate per patient was used for the study. Medical records of patients with 1 or more blood cultures positive for MDR *P. aeruginosa* during this period were reviewed. Antimicrobial susceptibility tests were performed on all the isolates. The bloodstream isolates of patients with MDR *P. aeruginosa* bacteremia were analyzed using pulsed-field gel electrophoresis (PFGE) to determine their relationship.

Definitions

An MDR *P. aeruginosa* isolate was defined as one that was resistant to ceftazidime, aztreonam, cefepime, ticarcillin-clavulanate, piperacillin-tazobactam, imipenem-cilastatin, meropenem, amikacin, tobramycin, gentamicin, and ciprofloxacin on disk diffusion susceptibility testing, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [7]. The infection was considered to be nosocomial if a blood culture that yielded positive findings was obtained more than 48 h after hospitalization.

Mortality was attributed to bacteremia if the patient was being treated for bacteremia when death occurred, unless pathological and clinical data clearly indicated otherwise.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the disk diffusion method on Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, MD, USA). The minimal inhibitory concentration (MIC) values were determined by the agar dilution method, according to CLSI guidelines [7]. The antimicrobial agents tested included amikacin, ceftazidime, cefepime, ciprofloxacin, meropenem, sulbactam, and piperacillin-tazobactam. Colistin was later included in the susceptibility testing when it became available in Taiwan in 2006. Meropenem was selected from the carbapenems for susceptibility testing. Fresh overnight cultures of the isolates on blood agar plates were resuspended in sterile normal saline to a 0.5 McFarland standard and then spotted with a multipoint inoculator on Mueller-Hinton agar containing serial 2-fold dilutions of amikacin, ceftazidime, cefepime, meropenem, ciprofloxacin, colistin, or piperacillin. The MICs for ceftazidime, cefepime, meropenem, ciprofloxacin, colistinmethate sodium, and piperacillin were also determined in the presence of a fixed concentration of amikacin (20 μg/mL), tazobactam (4 μg/mL), or sulbactam (8 μg/mL) [8]. Visible growth was recorded after incubation at 35°C for 18 to 20 h.

Pulsed-field gel electrophoresis of genomic DNA

Genomic DNA of the blood isolates was prepared in 0.5% SeaPlaque GTG agarose (FMC BioProducts, Rockland, ME, USA) as previously described [9], and incubated at 37°C for 6 h with 10 units of SpeI (BioLabs, Beverly, MA, USA) in enzyme buffer. The digested DNA fragments were separated in 1% SeaKem GTG agarose gel (FMC BioProducts) on a CHEF-DRII apparatus (Bio-Rad Laboratories, Hercules, CA, USA). Electrophoresis was performed with 0.5X Tris-borate-ethylenediaminetetraacetic acid buffer at 6 V/cm at 14°C. DNA fragments were then visualized by fluorography with ethidium bromide. A lambda DNA ladder (Amersham Pharmacia Biotech, Piscataway, NJ, USA) was used as the molecular size marker. Isolates were considered closely related if they showed differences of less than 3 bands [10].

Results

Patients and bacterial isolates

13,306 *P. aeruginosa* isolates were identified as follows: 3503 isolates were identified in 2001, 3437 isolates in

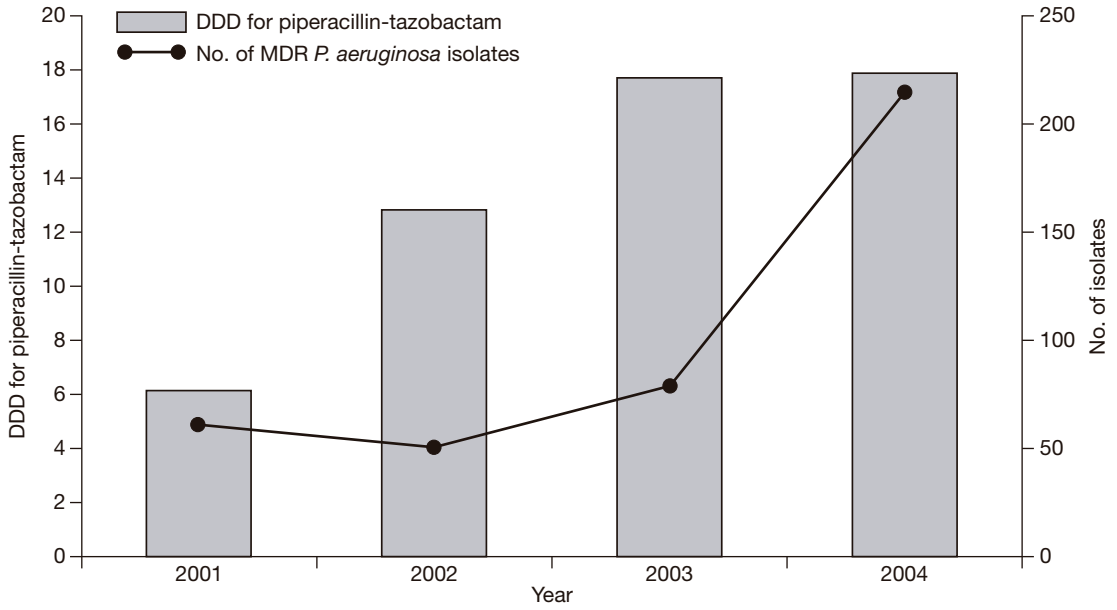

Fig. 1. Defined daily dose (DDD) for piperacillin-tazobactam and frequency of multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolates per year from 2001 to 2004.

2002, 2976 isolates in 2003, and 3390 isolates in 2004. The numbers of MDR *P. aeruginosa* isolates and the defined daily dose (DDD) for piperacillin-tazobactam from 2001 to 2004 are shown in Fig. 1. The numbers of MDR *P. aeruginosa* isolates during the years concerned were 61 (1.74%) in 2001, 51 (1.48%) in 2002, 79 (2.65%) in 2003, and 214 (6.31%) in 2004, for a total of 405. Of these, in 2004, there were 10 bloodstream isolates of MDR *P. aeruginosa*. Annual piperacillin-tazobactam use at the hospital increased progressively from 2001 to 2004. DDD for piperacillin-tazobactam was 6.11 in 2001, 12.84 in 2002, 17.69 in 2003, and 17.85 in 2004. In contrast, use of other antibiotics remained relatively stable during the same period. Nine major PFGE patterns among the 214 MDR *P. aeruginosa* isolates (type A to I) are shown in Fig. 2.

The mean ± standard deviation age of the 10 patients with MDR *P. aeruginosa* bacteremia was 76.4 ± 11.6 years (range, 55 to 88 years). Demographic and clinical characteristics of the patients with MDR *P. aeruginosa* bacteremia, antibiotics administered prior to and after the culture results were available, and patient outcomes are shown in Table 1. All 10 MDR *P. aeruginosa* blood isolates were from patients who had stayed in the medical ICU for a mean period of 17.8 days, had been mechanically ventilated, and had nasogastric feeding tubes and indwelling central venous and urinary catheters. Enhanced barrier nursing and strict isolation and segregation of infected patients in the medical ICU were insufficient to prevent the spread of MDR *P. aeruginosa* bacteremia. An outbreak of nosocomial infection was thus suspected, and investigation included examination of other types of medical facilities and the hospital environment, as

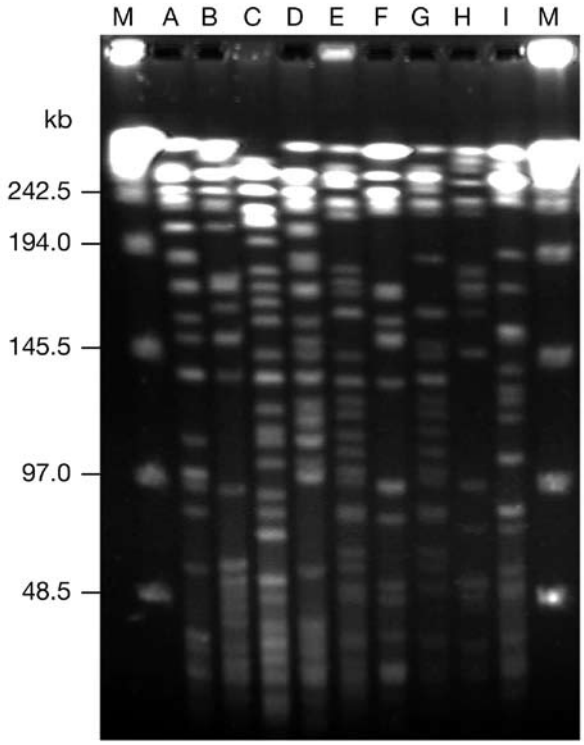

Fig. 2. Pulsed-field gel electrophoresis (PFGE) outcomes for 214 multidrug-resistant *Pseudomonas aeruginosa* isolates after digestion of chromosomal DNA with SpeI. Lane M, molecular size marker; lanes A to I, PFGE types A to I.

Table 1. Clinical data and antibiotic treatment of 10 patients with multidrug-resistant *Pseudomonas aeruginosa* bacteremia

Patient no.	Age (years)/ gender	APACHE II score/outcome	Comorbidity	Antipseudomonal antibiotics administered	
				Before culture result	After culture result
1	55/F	24/died[a]	DM, pneumonia, GU	Vancomycin + meropenem	Ceftazidime
2	76/F	15/died[a]	AMI, CHF, CRF receiving HD	Piperacillin-tazobactam + amikacin	
3	57/F	20/died[a]	Lymphoma with brain metastasis	Teicoplanin + piperacillin-tazobactam	
4	75/M	16/died[a]	DM, COPD, confined to bed after CVA	Teicoplanin + ciprofloxacin	Imipenem-cilastin + sulbactam
5	87/M	25/died[a]	COPD, AF, CRF receiving HD	Cefepime	Meropenem + sulbactam
6	84/M	19/died[a]	Esophageal cancer, CRF receiving HD	Imipenem-cilastin	
7	78/M	24/died[a]	COPD, confined to bed after CVA	Meropenem	
8	82/F	27/survived	DM, COPD, confined to bed after CVA	Amoxicillin-clavulanate + ciprofloxacin	Ceftazidime + sulbactam + amikacin
9	82/M	35/survived	DM, COPD	Piperacillin-tazobactam + amikacin	Ceftazidime + sulbactam + amikacin
10	88/F	18/survived[b]	DM, CRF receiving HD	Teicoplanin + piperacillin-tazobactam + amikacin	Ceftazidime + sulbactam + amikacin

Abbreviations: APACHE = Acute Physiology and Chronic Health Evaluation; F = female; M = male; DM = diabetes mellitus; GU = gastric ulcer; AMI = acute myocardial infarction; CHF = congestive heart failure; CRF = chronic renal failure; HD = hemodialysis; COPD = chronic obstructive pulmonary disease; CVA = cerebrovascular accident; AF = atrial fibrillation

[a]Patients 1 to 7 died of septic shock.

[b]Died 1 month later due to *Chryseobacterium meningosepticum* bacteremia.

well as obtaining cultures from medical equipment, medical personnel, fomites, and the surrounding environment for environmental pathogens. One isolate was obtained from a piece of equipment in the medical ICU, and was selected for comparison with 10 blood isolates by PFGE. In August 2004, continued investigation revealed that the isolate obtained from a brush used to clean all trap bottles in the medical ICU and all 10 bloodstream isolates had an identical PFGE profile, type C (Fig. 3). At that time, it was the practice to use a single brush to clean all trap bottles (which collected the secretions from the airways of patients in the medical ICU), and this was frequently reused.

Once the infection source and transmission route were identified, infection control measures were promptly implemented. The practice of reusing brushes to clean trap bottles in the medical ICU was immediately halted and disposable brushes were used instead. Decontamination of the medical ICU environment was performed using 500 parts per million sodium chlorite. Other measures taken to prevent and control the spread of nosocomial MDR *P. aeruginosa* infection included staff education, strict isolation of infected patients,

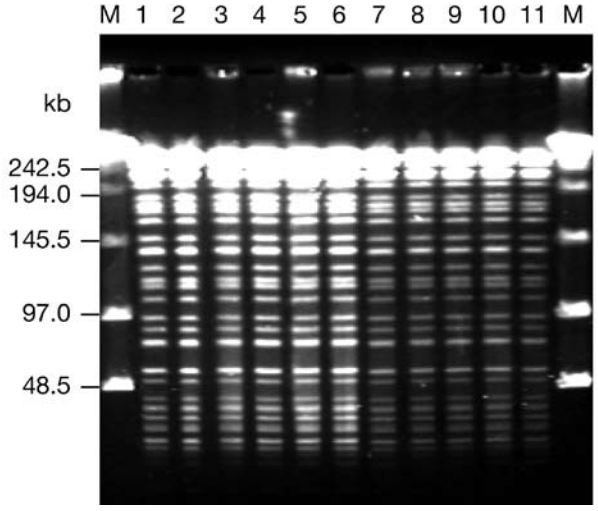

Fig. 3. Pulsed-field gel electrophoresis of 10 multidrug-resistant *Pseudomonas aeruginosa* bloodstream isolates and 1 isolate from the reused brush. Lane M, molecular size marker; lanes 1 to 10, isolate numbers 1 to 10; lane 11, isolate from reused brush.

greater attention to asepsis, proper hand-washing technique by staff and contacts, and increased vigilance, especially for patients at high risk. Extra precautions were

taken during insertion of central venous catheters to limit infection. These included proper hand-washing technique; full barrier precautions using cap, mask, sterile gloves, gown, and drapes; decontamination of skin around the central catheter insertion site; close supervision by a senior doctor; and having a nurse in attendance. Additional infection control policies included removal of central venous catheters as soon as they were not indicated or replacement within 7 days. After 6 months, the infection rate due to central venous catheter infection was reduced from 7.7% to 3.3%. The outbreak of MDR *P. aeruginosa* infection was brought under control in the subsequent follow-up period.

Antimicrobial susceptibility testing and MIC determination

Using disk diffusion, all 214 MDR *P. aeruginosa* isolates (including the 10 bloodstream isolates) were resistant to ceftazidime, cefepime, meropenem, ciprofloxacin and piperacillin (data not shown). The in vitro susceptibilities for the 10 bloodstream isolates of MDR *P. aeruginosa*, as determined by agar dilution, are shown in Table 2. An 8-fold reduction in MIC was observed for all isolates with the combination of ceftazidime, amikacin and sulbactam, and all were within the susceptible range of 8 μg/mL ceftazidime or lower. No synergistic effect was observed for the other antibiotic combinations. For colistin, the in vitro susceptibility was 86.9% (186/214) for MDR *P. aeruginosa* isolates and 90% (9/10) for the bloodstream isolates, but this agent was not available for clinical use in Taiwan when this study was conducted.

Treatment and outcome

Three of 10 patients died before MDR *P. aeruginosa* bacteremia was detected, and 1 patient died on the day that MDR *P. aeruginosa* was reported (Table 1). Of the 6 remaining patients, 2 patients treated with combination therapy of carbapenem and sulbactam and 1 treated with ceftazidime alone died. The results of MIC determination showed that a combination of ceftazidime, amikacin, and sulbactam reduced the MIC to the range of sensitivity. Therefore, the remaining 3 patients were treated with ceftazidime, amikacin, and sulbactam and all survived the MDR *P. aeruginosa* infection, although 1 patient died from *Chryseobacterium meningosepticum* bacteremia 1 month later.

Discussion

This study clearly showed that a combination of ceftazidime, amikacin, and sulbactam reduced the MICs of ceftazidime to the susceptible range in comparison with those of ceftazidime or amikacin alone, suggesting that this combination could be used against MDR *P. aeruginosa*. This was a major factor in controlling the outbreak of MDR *P. aeruginosa* infection at MMH at a time when colistin was not available for clinical use. Infection with MDR *P. aeruginosa* is a growing problem in many medical facilities, and the occurrence of infections due to strains that are resistant to almost all commercially available antibacterial drugs are not uncommon [11,12]. In MDR *P. aeruginosa* infections, susceptibility testing

Table 2. In vitro susceptibilities of 10 bloodstream isolates of multidrug-resistant *Pseudomonas aeruginosa*, as determined by agar dilution

Antibiotic	Isolate (MIC; μg/mL)									
	1	2	3	4	5	6	7	8	9	10
Amikacin	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Ceftazidime	64	64	64	64	32	64	64	64	64	64
Ceftazidime + amikacin	32	32	32	16	16	32	32	32	32	32
Ceftazidime + sulbactam	16	16	16	16	8[a]	16	16	16	16	16
Ceftazidime + amikacin + sulbactam	8[a]	8[a]	8[a]	8[a]	4[a]	8[a]	8[a]	8[a]	8[a]	8[a]
Meropenem	16	16	16	16	8	16	64	16	16	16
Meropenem + amikacin	16	16	16	16	8	16	64	16	16	16
Meropenem + sulbactam	8	16	16	8	8	8	32	16	16	16
Meropenem + amikacin + sulbactam	8	8	8	8	4[a]	8	64	8	8	16
Colistin	2[a]	4	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]
Colistin + amikacin	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]
Colistin + amikacin + sulbactam	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]

Abbreviation: MIC = minimal inhibitory concentration

[a]Susceptible.

for antimicrobials that are not tested routinely and antibiotic synergy studies should be considered [13]. Combination therapy with multiple antipseudomonal antibiotics should be considered for synergistic effects [11]. Furthermore, combination therapy may exert a selection pressure that allows only subpopulations with reduced virulence to be expressed [11]. The mortality in this series of 10 patients with MDR *P. aeruginosa* bacteremia was high, but all 3 patients who were treated with triple therapy (ceftazidime, amikacin, and sulbactam) survived.

Among the nosocomial pathogens, *P. aeruginosa* is a major cause of morbidity and mortality. Risk factors for mortality include severe sepsis, pneumonia, delay in starting effective antimicrobial therapy, and an increasing Acute Physiology and Chronic Health Evaluation II score [14]. In this series, as the MDR *P. aeruginosa* was resistant to all available antibiotics, timely and effective therapy could not be instituted, resulting in high mortality initially. Colistin, an older antibiotic, was regarded as potentially having considerable nephrotoxicity and neurotoxicity and has not been used for the past 2 decades in most areas of the world [15], including Taiwan. Colistin has been used as a last resort for patients with serious infections and has acceptable efficacy [15-17]. Recent studies of the management of MDR organisms showed a favorable clinical response to colistin ranging from 58% to 74% [15-17]. However, poor results were observed for patients with pneumonia; only 25% had a good outcome [16]. Other factors that correlated with an unfavorable clinical response to colistin were multiple organ failure, acute respiratory syndrome [17], and bacteremia [18].

Several measures were implemented to control the outbreak and prevent the spread of nosocomial MDR *P. aeruginosa* infection at MMH. All patients with MDR *P. aeruginosa* bacteremia had been in the medical ICU. The PFGE pattern of the MDR *P. aeruginosa* strain isolated from the reused brush was indistinguishable from those obtained from all bloodstream isolates, indicating that they were epidemiologically linked. Hence, all patients who were in the medical ICU had a high risk of acquiring MDR *P. aeruginosa* infection from contaminated trap bottles. This suggests a lapse in infection control. The other medical facilities and equipment tested were negative for the specific spreading clone.

Risk factors associated with the development of MDR *P. aeruginosa* have been studied [11,14]. Prior exposure to several antibiotics has been suggested to play an important role in the acquisition of these organisms [11]. Clinical isolates that are resistant to some last-line agents (ceftazidime, amikacin, ciprofloxacin, and carbapenems) have been found in patients in ICUs in Taiwan [19]. MDR *P. aeruginosa* emerges in a stepwise manner after exposure to antipseudomonal antibiotics. Many of these patients had been treated with other antibiotics, including cephalosporins, aminoglycosides, quinolones, and carbapenems prior to infection by MDR *P. aeruginosa*. Harris et al showed that exposure to piperacillin-tazobactam, imipenem, aminoglycosides, vancomycin, and broad-spectrum cephalosporins was associated with the isolation of piperacillin-tazobactam-resistant *P. aeruginosa* [20]. At MMH, there was a progressive increase in piperacillin-tazobactam use from 2001 to 2004, followed by a sharp increase in MDR *P. aeruginosa* infection in 2004 (Fig. 1). While broad-spectrum antibiotics such as carbapenems are potentially helpful for infections caused by *P. aeruginosa*, their increasing use has led to the emergence of carbapenem-resistant *P. aeruginosa* [2,11,12,21]. Another risk factor for the development of MDR *P. aeruginosa* bacteremia was the use of fluoroquinolones and β-lactam antibiotics [11,21]. Hence, a policy of consultation with infectious disease specialists for antimicrobial therapy for patients in the medical care unit was implemented.

At present, there is no ideal strategy for the treatment of MDR *P. aeruginosa* infections. A combination of antibiotics is often used to treat serious *P. aeruginosa* infections [11,22,23]. A large number of clinical and laboratory studies have been performed to look for synergy among various combinations of antibiotics and have produced widely varying results. In a study on *Enterobacter cloacae* bloodstream infection, a combination of cefepime and sulbactam reduced the MICs of most multiresistant isolates compared with those for cefepime alone [24]. In the present study, the effect of sulbactam added to a combination of β-lactam and amikacin to treat MDR *P. aeruginosa* was investigated, and a significant decrease in the MIC of ceftazidime was found. Since this regimen appeared to be effective in vitro, 3 patients were treated with this combination regimen and all survived.

Colistin remains the recommended drug of choice for MDR *P. aeruginosa* infections. Toxicity, particularly nephrotoxicity, is an important concern with colistin [16], although the incidence of toxicity is less frequent and severe than reported previously [25]. Levin et al reported a high incidence of nephrotoxicity

(37%), especially in patients with already compromised renal function [16]. In 2 studies of colistinmethate sodium conducted exclusively in ICUs, the observed nephrotoxicity was 14.0% and 18.6% [17,26]. Patients with MDR *P. aeruginosa* are usually severely ill, with some already having compromised renal function, and the risk of developing side effects from colistin use is an important consideration. At MMH, some patients with MDR *P. aeruginosa* infection who were treated with colistin after it was approved for use in Taiwan developed nephrotoxicity. However, since colistin was only used for a limited number of patients, no statistical conclusions could be reached. Hence, using an effective combination of antibiotics could be another safe method of treating MDR *P. aeruginosa* infection.

This study was limited by the relatively small number of patients, and further large-scale studies could provide more conclusive data.

MDR *P. aeruginosa* infections have been detected in many teaching hospitals in Taiwan and different treatment strategies using various antibiotic combinations have been used. The majority have used a β-lactam and aminoglycoside combination. The results of this study suggest that use of triple antimicrobial therapy, including ceftazidime, amikacin, and sulbactam, could be an alternative way of treating MDR *P. aeruginosa* infection in certain circumstances.

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