J Microbiol Immunol Infect
2004;37:343-349

Clinical experiences of bacteremia caused by metallo-β-lactamase-producing Gram-negative organisms

Nan-Yao Lee[1], Jing-Jou Yan[2,3], Hsin-Chun Lee[1,3], Kung-Hung Liu[1], Shao-Tsung Huang[1], Wen-Chien Ko[1,3]

Division of Infectious Diseases, Departments of [1]Internal Medicine and [2]Pathology, National Cheng Kung University Hospital, Tainan; and [3]Department of Medicine, Medical College, National Cheng Kung University, Tainan, Taiwan, ROC

Received: March 23, 2004 Revised: April 26, 2004 Accepted: May 17, 2004

The emergence of acquired metallo-β-lactamase (MBL) in Gram-negative bacilli is regarded as a therapeutic challenge since such enzymes are capable of hydrolyzing all β-lactams in vitro except the monobactams. The clinical characteristics and outcome of 8 episodes of Gram-negative bacteremia caused by MBL-producing isolates from January 1997 through December 2000 (*Klebsiella pneumoniae*, 6 isolates; *Pseudomonas stutzeri*, 4; *Pseudomonas aeruginosa*, 1; and *Pseudomonas putida*, 1) were analyzed. The median age of the patients was 61 years (range, 2-95 years). Most patients (n = 6, 75%) had more than 1 comorbid illness or condition and 6 patients acquired bacteremia in the intensive care unit. The median time from admission to the first positive culture was 34.5 days (range, 1-99 days). Pneumonia was the most common site of infection. Five patients (62.5%) received a carbapenem to treat bacteremia. The median time to defervescence was 6 days (range, 2-12 days). No bacteriologic failure was noted during or after antimicrobial therapy. The overall mortality rate from bacteremia caused by Gram-negative, MBL-producing organisms was nil at 14 or 28 days.

Key words: Beta-lactamases, bacteremia, carbapenems, Gram-negative bacteria

Gram-negative bacilli (GNB) with the potential to develop β-lactam resistance during therapy are common causes of hospital-acquired bacteremia, and there is a concern that their ability to become resistant during treatment may lead to treatment failure [1,2]. However, β-lactamases remain the most important contributing factor. Metallo-β-lactamases (MBL) belong to class B of the Ambler molecular classification [3] and to group 3 of the Bush-Jacoby-Medeiros functional classification [4], and are emerging resistance determinants in several bacterial species of clinical relevance, including members of the family *Enterobacteriaceae*, *Pseudomonas* spp. and other non-fastidious Gram-negative non-fermenters [5,6]. Acquired MBL expression in GNB is becoming a therapeutic challenge since these enzymes are capable of hydrolyzing all β-lactams except the monobactams [1,7].

During the past decade, a number of acquired MBLs have been identified in Gram-negative pathogens and were categorized into 2 major types: IMP and VIM [1,3,8-13]. IMP-1 was initially confined to Japanese isolates and was thought to be a major threat when it appeared on a transposable element in 1991 [14]. Its dissemination throughout Japanese hospitals among a variety of *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates alerted the infectious disease community to the possibility that this class of enzyme might spread rapidly to confer resistance worldwide to most antimicrobial agents that contain a β-lactam ring [15,16]. Furthermore, a second family of acquired MBLs, the VIM types, has been detected in several countries, including as Italy [17], Greece [18], France [10], and Taiwan [16], indicating the problem will be a global issue. However, the appropriate antibiotic therapy for treating infections due to MBL-producing GNB bacteremia remains unknown [19]. The clinical impact of infection with MBL-producing strains remains undefined. Thus, the objectives of this study were to analyze prevalence, clinical responses and outcome of treatment of bacteremia caused by MBL production.

Materials and Methods

Bacterial strains

In total, 8 episodes of bacteremia caused by MBL-producing isolates were documented from a total of 623

Corresponding author: Wen-Chien Ko, Department of Internal Medicine, National Cheng Kung University Hospital, No. 138, Sheng Li Road, 704, Tainan, Taiwan, ROC.
E-mail: winston@mail.ncku.edu.tw

consecutive Gram-negative bacteremia episodes from January 1997 to December 2000 at National Cheng Kung University Medical Center, a university-affiliated medical center with approximately 900 beds. Isolates from 623 episodes included members of the family *Enterobacteriaceae, Pseudomonas* spp. and other non-fastidious Gram-negative non-fermenters. These MBL-producing isolates included 6 *Klebsiella pneumoniae*, 4 *Pseudomonas stutzeri*, 1 *P. aeruginosa* and 1 *Pseudomonas putida*. All of these isolates were identified using conventional techniques [20,21] and/or the API 20E system (bioMerieux, Marcy l'Etoile, France). Methods for screening MBL producers, polymerase chain reaction amplification, DNA sequencing and hybridization assays have been described elsewhere [12,22,23].

Susceptibility tests

The minimum inhibitory concentrations (MICs) of antimicrobial agents were determined by the agar dilution method, and susceptibilities to antibiotics were determined by the disk diffusion method, as previously described [12,23]. Both tests were performed and interpreted according to the recommendations of the National Committee for Clinical Laboratory Standards [24-26]. The antimicrobial agents used for agar dilution tests were as follows: aztreonam (Bristol-Myers Squibb, New Brunswick, NJ, USA), ceftazidime (Glaxo Group Research Ltd., Greenford, UK), cefotaxime (Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ, USA), imipenem (Merck Sharp & Dohme, West Point, PA, USA), meropenem (Sumitomo Pharmaceuticals Ltd., Osaka, Japan), piperacillin and tazobactam (Lederle Laboratories, Pearl River, NY). Antimicrobial disks were all obtained from Becton Dickinson Microbiology Systems (Cockeysville, MD, USA), including amikacin, chloramphenicol, ciprofloxacin, gentamicin, ofloxacin, pefloxacin, tobramycin, and trimethoprim-sulfamethoxazole.

Study design and review of medical records

This was a retrospective observational study. The clinical information collected for patients with bacteremia caused by MBL-producing isolates included demographic data, underlying disease, prior hospitalization history, prior antimicrobial therapy, use of a mechanical ventilator within the previous month, presence of an indwelling catheter, associated focal infection, clinical parameters relevant to the disease severity, microbiological data, laboratory findings, and radiological imaging. Data on antimicrobial agents used to treat bacteremia caused by MBL-producing isolates, and clinical and bacteriological response were recorded.

Definitions

Bacteremia was defined by the isolation of MBL-producing pathogens from at least 1 blood culture set from a specimen drawn before death. An episode of bacteremia was considered to be clinically significant when signs or symptoms of systemic infection were observed concurrently with bacteremia, or any clinical evidence of local infection was present.

Fever was defined as a body temperature of ≥38°C. Time to defervescence was defined as the period between the day of onset of fever and the first day of 3 consecutive days with a sustained body temperature lower than 37°C. Nosocomial bacteremia was defined as bacteremia occurring more than 48 hours after admission. Polymicrobial bacteremia was defined as the isolation of more than 1 organism in a single blood culture. Severity of acute illness was assessed at the time of positive blood culture by using the Pitt bacteremia score, a previously validated scoring system based on mental status, vital signs, requirement for mechanical ventilation, and recent cardiac arrest [27]. Critical condition was defined as a Pitt bacteremia score of more than 4 points. The source of infection was regarded to be pneumonia, urinary tract infection, meningitis, incisional wound infection, other soft tissue infection, intra-abdominal infection, or primary bloodstream infection, according to the definitions of the Centers for Disease Control and Prevention [28]. Prior antibiotic therapy was defined as receipt of a systemic antimicrobial agent for at least 72 hours within the preceding 4 weeks prior to the occurrence of the bacteremic episode.

The definitions of complicating morbiditive bloodstream infections were as follows: renal insufficiency was defined as a serum creatinine level of more than 2.0 mg/dL or a requirement for dialysis; septic shock hypotension was defined as arterial blood pressure <90 mm Hg or 40 mm Hg less than the patient's normal blood pressure in a patient unresponsive to fluid resuscitation, along with organ dysfunction; and acute respiratory distress syndrome (ARDS), an oxygenation abnormality, was defined as an arterial partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (F_1O_2) ≤200, in conjunction with acute bilateral pulmonary infiltrates and no evidence for heart failure or volume overload.

Clinical response of an infection to treatment was classified as cure (resolution of all symptoms and signs

related to the infection), improvement (resolution or reduction of the majority of symptoms and signs of infection without worsening or new symptoms and signs related to the infection), failure (no resolution, or worsening or new symptoms or signs related to the infection), or indeterminate (inability to assess due to lack of follow-up information or confounding due to the patient's underlying medical condition). Clinical response was evaluated at 14 days after treatment or at the end of treatment.

Bacteriological response to treatment was classified as eradication (absence of MBL-producing pathogens in cultures performed during and after completion of therapy), presumed eradication (absence of MBL-producing pathogens in cultures during therapy in a patient who had clinical improvement but did not receive follow-up or whose site of infection completely healed during therapy and did not receive repeated culture despite clinical improvement), persistence (persistent isolation of MBL-producing pathogens in cultures, persistent symptoms in patients with infections not accessible to follow-up culture or appearance signs of infection requiring new antimicrobial therapy), or indeterminate (inability to assess because of lack of follow-up information).

Only those patients who received antimicrobials for at least 72 h were included in the analysis of response to antimicrobial therapy. Patients were observed for at least 14 days after the onset of bacteremia to assess clinical outcome, and possible complications of infection.

Results

During the study period, a total of 623 bacteremic isolates were identified, among which 8 episodes were found to be caused by MBL-producing isolates (6 isolates of *Klebsiella pneumoniae*, 1 *P. aeruginosa*, 1 *P. putida*, and 4 *P. stutzeri*). The average annual incidences of isolating MBL-producing organisms among cases of nosocomial bacteremia caused by *K. pneumoniae* and *Pseudomonas* spp., were 48.4 and 14.0 per 1000 person-years, respectively. Of these, 7 episodes (88%) were nosocomially acquired, and only 1 was considered to be community acquired. The demographic and clinical characteristics of these 8 patients are shown in Table 1.

Patient characteristics

Fifty percent of patients were male. Their median age was 61 years (range, 2-95 years). Seven patients (87.5%) had more than 1 underlying comorbid illness or condition. The median time from admission to the first positive culture was 34.5 days (range, 1-99 days). Six patients (75%) acquired bacteremia in the intensive care unit. Polymicrobial bacteremia was noted in 3 episodes (37.5%). Seven patients (87.5%) were critically ill at the time when bloodstream infections with MBL-producing organisms developed. Six patients (75%) had received antimicrobial therapy before the onset of bacteremia. Two patients (25%) received endotracheal intubation, 5 patients (62.5%) and 4 patients (50%) underwent placement of a central venous or arterial access device within 48 hours before bacteremia developed. Pneumonia was the most common type of infection, developing in 6 patients (Table 1).

Susceptibility tests

The susceptibilities of MBL-producing isolates to various β-lactams are summarized in Table 2. All isolates were resistant to ceftazidime and cefotaxime. In addition to the MBL enzyme, 2 of 3 isolates of *K. pneumoniae* expressed an extended-spectrum β-lactamase (ESBL). Only 1 of 5 *Pseudomonas* spp. isolates was resistant to aztreonam (MIC ≥64 μg/mL). Of the 8 MBL-producing isolates, only 3 exhibited resistance to imipenem and meropenem (MIC ≥16 μg/mL), and all were *Pseudomonas* spp.

Antimicrobial therapy and outcome

Table 1 shows the prior β-lactam exposure and antimicrobial therapy of the patients. Five patients (62.5%) received a carbapenem and 6 patients (75%) received combination therapy, including 3 patients who had polymicrobial bacteremia. The median time to defervescence was 6 days (range, 2-12 days). No bacteriologic failure was noted in series examination during or after antimicrobial therapy. Four cases evolved into septic shock, 2 required emergency dialysis for acute renal failure, and 1 developed ARDS. The overall mortality rate related of patients with MBL-producing bacteremia was 0% at 14 days and at 28 days. The length of hospital stay after bacteremia onset was 44 ± 43.5 days. Four patients were discharged alive, resulting in an in-hospital mortality rate of 50%.

Discussion

Because resistance mediated by MBLs is not overcome by conventional β-lactamase inhibitors [5], MBLs are included among the resistance determinants of

Table 1. Clinical characteristics, type of infection, treatment and outcome of patients with bacteremia caused by metallo-β-lactamase-producing organisms

Patient no./ age (years)/ gender	Underlying condition	Critical condition	Organism[a]	Type of infection	Prior β-lactam exposure	Antimicrobial therapy	Bacteriologic outcome	Clinical outcome (2-week)
1/48/M	Diabetes mellitus/liver cirrhosis	Yes	*Klebsiella pneumoniae*[b]	Pneumonia	TIM, OX	TZP + GN, IPM	Cure	Survival
2/69/F	Traumatic accident with intracerebral hemorrhage	Yes	*Klebsiella pneumoniae*[b]	Catheter-related infection	CFZ	IPM, TZP	Cure	Survival
3/95/F	COPD/heart failure/uremia	Yes	*Klebsiella pneumoniae*[b]/ *Acinetobacter baumannii*	Pneumonia	CAZ	MEM	Cure	Survival
4/64/M	COPD	Yes	*Pseudomonas aeruginosa*[b]/ *Stenotrophomonas maltophilia*	Pneumonia	ATM	TIM + AMK	Cure	Survival
5/8/M	Solid malignancy	No	*Pseudomonas putida*[b]	Catheter-related infection	Nil	PIP + AMK	Cure	Survival
6/80/F	Liver cirrhosis/ COPD/ hypothyroidism	Yes	*Pseudomonas stutzeri*[b]/ *Stenotrophomonas maltophilia*	Pneumonia	MOX	IPM + SXT, MOX + AZC	Cure	Survival
7/58/F	Lymphoma/ systemic lupus erythematosus	Yes	*Pseudomonas stutzeri*[b]	Pneumonia	CTX, IPM	CIP + SXT, IMP + SXT	Cure	Survival
8/2/M	No	Yes	*Pseudomonas stutzeri*[b]	Pneumonia	Nil	PCN, CTX + MET	Cure	Survival

Abbreviations: TIM = ticarcillin-clavulanate; OX = oxacillin; TZP = piperacillin-tazobactam; GN = gentamicin; IPM = imipenem/ cilastatin; CFZ = cefazolin; COPD = chronic obstructive pulmonary disease; CAZ = ceftazidime; MEM = meropenem; ATM = aztreonam; AMK = amikacin; PIP = piperacillin; MOX = moxalactam; SXT = trimethoprim-sulfamethoxazole; AZC = azocillin; CTX = cefotaxime; CIP = ciprofloxacin; PCN = penicillin; MET = metronidazole

[a]In all but patients 4, 5, 7 and 8, metallo-β-lactamase-producing isolates were cultured from 2 sets of blood culture bottles.

[b]Gram-negative bacilli producing metallo-β-lactamase.

increasing clinical importance in the strains of non-fastidious Gram-negative non-fermenters and members of the family *Enterobacteriaceae* [1]. Their monitoring has become an important issue in clinical microbiology [5,29]. The emergence of these multi-drug resistant bacteria is a phenomenon of concern to the clinician, because antimicrobial resistance is a major cause of treatment failure in the management of infectious disease [30,31].

The present study indicates that MBLs in Gram-negative bacteremia remain uncommon in Taiwan. Of the 623 nosocomial Gram-negative bacteremic isolates collected during January 1997 to December 2000, only 8 (1.3%) were found to carry MBLs. The potential of IMP- (bla_{IMP}) and VIM- (bla_{VIM}) MBL genes to spread among clinical Gram-negative bacteria has been reported previously [7,15,23,32-34]. Spread of MBLs in Gram-negative bacteria remains a clinical concern, and surveillance of MBL-producing organisms is necessary to prevent their dissemination.

MBL-producing bacteria tend to demonstrate a wide range of resistance to various broad-spectrum β-lactams, including oxymino-cephalosporins, cephamycins, and carbapenems. Consistent with previously reported findings [31,35], all of our MBL-producing isolates were resistant to extended-spectrum β-lactams. However, their susceptibilities to carbapenems and aztreonam were diverse (Table 2). Expression of MBLs could be cryptic or could be suppressed in strains demonstrating low-level carbapenem resistance [36]. Some of the

Table 2. In vitro antimicrobial susceptibilities of the metallo-β-lactamase-producing Gram-negative bacilli bloodstream isolates

Species/ patient no.	β-Lactamase(s)	Minimum inhibitory concentration (μg/mL)							Antimicrobial susceptibility (disk diffusion method)
		PIP	TZP	CTX	CAZ	ATM	IPM	MEM	
Klebsiella pneumoniae									
1	IMP-8, SHV-11, TEM-1	-	-	32	>256	0.06	0.25	0.25	GN(S), AMK(S), ATM(S), TZP(S), IPM(S), PIP(I), AM(R), SAM(R), TIM(R), NN(R), CF(R), FOX(R), CTX(R), CAZ(R), CIP(R), SXT(R), MOX(R), OFX(R)
2	IMP-8, SHV-12, TEM-1	-	-	128	>256	>256	2	0.5	TZP(S), IPM(S), AM(R), SAM(R), TIM(R), GN(R), NN(R), AMK(R), CF(R), FOX(R), CTX(R), CAZ(R), CIP(R), OFX(R), SXT(R), ATM(R), MOX(R), PIP(R)
3	IMP-8, SHV-11, TEM-1	-	-	32	>256	64	0.5	0.25	MEM(S), TZP(I), AM(R), SAM(R), TIM(R), GN(R), AMK(R), CF(R), CAZ(R), CTX(R), CIP(R), SXT(R), MOX(R), PIP(R)
Pseudomonas aeruginosa									
4	VIM-3	256	64	256	>256	8	>32	32	ATM(I), TIM(R), GN(R), NN(R), AMK(R), CAZ(R), CPZ(R), CIP(R), OFX(R), PIP(R), IPM(R)
Pseudomonas putida									
5	IMP-1	64	32	>256	>256	64	16	>32	TIM(R), AMK(R), CAZ(R), CTX(R), CIP(R), PEF(R), SXT(R), MOX(R), TZP(R), PIP(R), IPM(R)
Pseudomonas stutzeri									
6	IMP-1	64	8	>256	>256	16	2	8	PIP(S), IPM(S), AMK(S), CIP(I), CAZ(R), CTX(R), CPZ(R), CIP(R), SXT(R), MOX(R)
7	IMP-1	64	32	>256	64	16	>32	>32	CIP(S), OFX(S), SXT(S),ATM(S), TZP(S), PIP(S), IPM(S), TIM(I), AMK(I), CAZ(R), CTX(R), MOX(R)
8	IMP-1	64	8	>256	>256	1	1	2	GN(S), AMK(S), ATM(S), PIP(S), TZP(S), IPM(S), SAM(R), TIM(R), CAZ(R), CIP(R), SXT(R), MOX(R)

Abbreviations: PIP = piperacillin; TZP = piperacillin-tazobactam; CTX = cefotaxime; CAZ = ceftazidime; ATM = aztreonam; IPM = imipenem-cilastatin; MEM = meropenem; GN = gentamicin; S = susceptible; AMK = amikacin; I = intermediate; AM = ampicillin; R = resistant, SAM = ampicillin-sulbactam; TIM = ticarcillin-clavulanate; NN = tobramycin; CF = cephalothin; FOX = cefoxitin; CIP = ciprofloxacin; SXT = trimethoprim-sulfamethoxazole; MOX = moxalactam; OFX = ofloxacin; CPZ = cefoperazone; PEF = pefloxacin

strains in this study exhibited a carbapenem-susceptible phenotype, even though they carried the MBL gene. There are 3 potential explanations for this finding. First, a secondary regulatory system, in addition to the structural gene, may exist so that expression of these genes is suppressed. Second, the rate at which carbapenems are hydrolyzed may be low due to a gene dosage effect related to the plasmid copy number [31]. Third, a carbapenem resistance phenotype requires the contribution of resistance mechanisms other than MBL production, such as an efflux pump [37] or the loss of certain outer membrane proteins [38]. Aztreonam is notable for its relatively low MIC level for MBL-producing strains [10,17], and the reduced susceptibilities to aztreonam in some isolates might be due to the coexisting presence of ESBL or other resistance mechanisms.

Due to the differences in resistance phenotype, the treatment of bloodstream infections caused by MBL-producers is controversial. This study and previous antimicrobial susceptibility studies clearly demonstrated that these pathogens not only were resistant to β-lactams but also frequently resistant to other classes of antibiotics [7,16,39]. Although, there are limited data on the treatment of life-threatening infections caused by MBL-producing isolates [31,39,40], the utility of β-lactam agents, including carbapenems, is being threatened by the acquisition of MBL-producing genotypes. However, in our study, 5 patients with bloodstream infections produced by MBL-producing organisms were treated with carbapenems and the other 3 received β-lactam agents based on in vitro susceptibility results (Table 2). All of these patients survived and no microbiological failure was noted during or after antimicrobial therapy.

An animal model of pulmonary infection with a *P. aeruginosa* strain with VIM-type MBL and resistance to imipenem (MIC 128 μg/mL) was established in rats [19]. However, therapy with imipenem at doses equivalent to the highest doses recommended for human infections can significantly reduce bacterial titers in rat lung, as compared with rats without antimicrobial therapy. Such a finding seems to support

the result obtained when using imipenem in treating infections caused by MBL-producing organisms in this study. Two patients in this series with bacteremia caused by non-*aeruginosa Pseudomonas* spp., both of which were resistant to meropenem, were successfully treated with a penicillin derivative in combination with an aminoglycoside. Therefore, there is a potential synergism with the combination regimen for MBL-producing GNB. Further in vitro or animal experiments are urgently needed to delineate appropriate therapy for these multi-drug resistant nosocomial organisms.

Because of the limited number of cases in this study, it is not clear whether the favorable response was due to the use of treatment regimens that included carbapenems. However, the relatively low inoculum size in bloodstream infections and the low MIC values (2 to 8 μg/mL) of carbapenems might have been related to the favorable outcome in our patients. Further clinical studies are needed to reveal the therapeutic role of carbapenems in MBL-producing bacteremia.

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