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Outbreak of Infection with Multidrug-Resistant *Klebsiella pneumoniae* Carrying bla_{IMP-8} in a University Medical Center in Taiwan

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***Klebsiella pneumoniae* strains with the transferable carbapenem-hydrolyzing metallo-β-lactamases, which include IMP- and VIM-type enzymes, remain extremely rare. To investigate whether IMP- or VIM-producing *K. pneumoniae* isolates had spread at a university medical center in Taiwan, a total of 3,458 clinical isolates of *K. pneumoniae* consecutively collected in 1999 and 2000 were tested by the agar diffusion method, colony hybridization, PCR, and nucleotide sequencing. A total of 40 isolates (1.2%), or 17 nonrepetitive isolates, from 16 patients were found to carry bla_{IMP-8}, a metallo-β-lactamase gene recently identified from a *K. pneumoniae* strain in Taiwan. Carriage of bla_{VIM} or other bla_{IMP} genes was detected in none of the remaining isolates. Of the 17 nonrepetitive bla_{IMP-8}-positive isolates, 15 isolates (88.2%) appeared susceptible to imipenem (MICs, ≤4 μg/ml) and meropenem (MICs, ≤1 μg/ml), indicating the difficulty in detecting bla_{IMP-8} in *K. pneumoniae* by routine susceptibility tests; 14 isolates (82.4%) produced SHV-12 as well; and 14 isolates (82.4%) were also resistant to fluoroquinolones. The organisms caused wound infections in eight patients and bloodstream infections in three patients. They were not directly associated with the death of nine patients. Before the recovery of the bla_{IMP-8}-positive isolates, all 16 patients had undergone various surgical procedures, and 15 patients had been admitted to the surgical intensive care unit, suggesting a nosocomial outbreak. Two major patterns were observed by pulsed-field gel electrophoresis for 14 of the 17 nonrepetitive isolates, indicating that the clonal spread was mainly responsible for the outbreak.**

The emergence of acquired metallo-β-lactamases (MBLs) in gram-negative bacilli is becoming a therapeutic challenge because the enzymes usually possess a broad hydrolysis profile that includes carbapenems and extended-spectrum β-lactams (13). Two major groups of MBLs have been described: IMP- and VIM-type enzymes (13). IMP-1 was the first identified acquired MBL (17) and has spread among *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and other nonfastidious gram-negative nonfermenters in Japan (8, 9, 22, 23). In the past three years, a number of acquired MBLs were identified in Europe (12, 19, 21) and the Far East (7, 10, 27, 28, 30). IMP-2 was identified from a clinical isolate of *Acinetobacter baumannii* in Italy (21). VIM-1 was identified from a clinical isolate of *P. aeruginosa* in Italy (12), and outbreaks of the VIM-1-producing *P. aeruginosa* isolates have been recognized in Greece (26) and Italy (4). VIM-2 was first identified from a clinical isolate of *P. aeruginosa* in France (19) and has been found in *P. aeruginosa* in Japan (N. Shibata, Y. Arakawa, H. Kurokawa, Y. Doi, and K. Shibayama, Abstr. 101st Gen. Meet. Am. Soc. Microbiol., abstr. C-524, p. 273, 2001) and in *Pseudomonas* and *Acinetobacter* spp. in Korea recently (K. Lee, J. B. Lim, J. Yum, D. Yong, J. R. Choi, Y. Chong, J. M. Kim, and D. M. Livermore, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2003, p. 123, 2000). All acquired MBL genes found so far were inserted in integrons (1, 7, 10, 11, 12, 19, 21, 28, 30).

Both IMP- and VIM-type MBLs have been detected in Taiwan (27, 28). Both IMP-1 and VIM-2 were found in *Pseudomonas putida* and *Pseudomonas stutzeri* (27), and a variant of the VIM-2 enzyme, VIM-3, was found in *P. aeruginosa* (27). A variant of the IMP-2 enzyme, IMP-8, was identified from a clinical isolate of *Klebsiella pneumoniae*, which produced the SHV-12-type extended-spectrum β-lactamase (ESBL) and TEM-1 as well (28). Reports of MBL-producing *K. pneumoniae* isolates remain rare. In Japan, only one IMP-1-producing *K. pneumoniae* isolate was detected in two surveys of gram-negative bacilli (8, 23). Outside Japan, there was only one confirmed report of an IMP-1-producing *K. pneumoniae* isolate collected from Singapore (T. H. Koh, G. S. Babini, N. Woodford, L.-H. Sng, L. M. C. Hall, and D. M. Livermore, Letter, Lancet **353:**2162, 1999). Since *K. pneumoniae* is notorious as a host of resistance plasmids and is one of the major causes of nosocomial infections (5), the present study was carried out in order to investigate the prevalence of *K. pneumoniae* producing IMP- or VIM-type MBLs in a university medical center in Taiwan. A nosocomial outbreak of *K. pneumoniae* carrying bla_{IMP-8} in the intensive care units (ICUs) was recognized, and thus a retrospective analysis of the cases from which the IMP-8-producing isolates were recovered was also conducted.

MATERIALS AND METHODS

Bacterial strains, clinical isolates and patients. A total of 3,458 clinical isolates of *K. pneumoniae* were consecutively collected at the National Cheng Kung University Medical Center, a 900-bed university hospital in southern Taiwan, from January 1999 to December 2000. Of these isolates, 1,622 and 1,836 isolates were collected in 1999 and 2000, respectively. All these isolates were identified by using the conventional techniques (5) and/or the API 20E system (bioMérieux, Marcy l'Etoile, France). MBLs have been shown to confer resistance to ceftazi-

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dime and cephamycins; however, susceptibilities to carbapenems for MBL producers covered a wide range (4, 7, 8, 10, 12, 13, 17, 19, 21–23, 27, 28, 30). Thus, only the isolates that met the following criteria on the basis of the results of the disk diffusion method were selected for further experiments: reduced susceptibilities to imipenem (inhibition zone diameter, <16 mm) or meropenem (inhibition zone diameter, <16 mm), or resistance to both ceftazidime (inhibition zone diameter, ≤14 mm) and cefoxitin (inhibition zone diameter, ≤14 mm). The medical records of the patients with MBL-producing isolates were reviewed. The bacterial strains used as controls for colony hybridization and PCR included bla_{VIM-1}-containing *P. aeruginosa* VR-143/97 (12), bla_{VIM-2}-carrying *P. putida* NTU-91/99 (27), bla_{VIM-3}-containing *P. aeruginosa* NTU-26/99 (27), bla_{IMP-1}-carrying *P. putida* NTU-92/99 (27), bla_{IMP-2}-containing *A. baumannii* AC-54/97 (21), and bla_{IMP-8}-carrying *K. pneumoniae* KPO787 (28).

Colony blot hybridization. Colony blot hybridization was performed as described elsewhere (6, 27). The DNA probes generated by PCR amplification of the entire bla_{VIM-1}, bla_{VIM-2}, bla_{IMP-1}, and bla_{IMP-2} were prepared as described previously (27) and were labeled with [α-^{32}P]dCTP (Amersham Pharmacia Biotech, Hong Kong, China) by the random priming technique with a commercial kit (Gibco BRL, Life Technologies, Gaithersburg, Md.). Since there are only two nucleotide differences between bla_{VIM-2} and bla_{VIM-3} (27) and four nucleotide differences between bla_{IMP-2} and bla_{IMP-8} (28), the VIM-3- and IMP-8-producing control strains can also be hybridized with the bla_{VIM-2}- and bla_{IMP-2}-specific probes, respectively.

PCR amplification and DNA sequencing. Plasmids from clinical isolates were prepared by a rapid alkaline lysis procedure (24). PCR assays were performed to amplify the entire sequences of the bla_{IMP-1}-, bla_{IMP-2}-, bla_{VIM-1}-, bla_{VIM-2}-, bla_{SHV}-, and bla_{TEM}-related genes as described previously (27, 28). The amplicons were purified with PCR cleanup kits (Roche Molecular Biochemicals, Mannheim, Germany) and were sequenced on an ABI PRISM 310 sequencer analyzer (Applied Biosystems, Foster City, Calif.). The PCR and sequencing primers used were described elsewhere (27, 29). bla_{IMP-8} and bla_{VIM-3} from the control strains were successfully amplified with the primers for bla_{IMP-2} and bla_{VIM-2}, respectively.

Transfer of resistance. Conjugation experiments were performed as described previously (20, 29) with streptomycin- and rifampin-resistant *Escherichia coli* C600 as the recipient (2). Tryptic soy agar plates supplemented with 500 μg of streptomycin (Sigma Chemical Company, St. Louis, Mo.) per ml or 64 μg of rifampin (Sigma) per ml and 10 μg of ceftazidime (Glaxo Group Research Ltd., Greenford, United Kingdom) per ml were used to select transconjugants. Plasmids from *E. coli* transconjugants were digested with *Eco*RI (Roche Molecular Biochemicals). Digested DNA samples were analyzed by electrophoresis on 0.8% agarose gels. The gels were stained with ethidium bromide (Sigma), and plasmid bands were visualized under UV light. The plasmid sizes of transconjugants were estimated by adding up restriction fragments.

Analytical IEF. Crude preparations of β-lactamases were obtained by sonication (3) and were subjected to analytical isoelectric focusing (IEF) as described previously (14, 27, 29). β-Lactamase activity was detected by overlaying the gels with 0.5 mM nitrocefin (Oxoid, Basingstoke, United Kingdom) in 50 mM HEPES (pH 7.5) supplemented with 2 mM $ZnCl_2$ (21, 27).

Susceptibility tests. The MICs of five β-lactam agents were determined by the agar dilution method, and the susceptibilities to eight non-β-lactam antibiotics were determined by the disk diffusion method. Both tests were performed and interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS) (15, 16). The antimicrobial agents used for the agar dilution tests and their sources are as follows: aztreonam, Bristol-Myers Squibb, New Brunswick, N.J.; cefoxitin, Sigma Chemical Company; ceftazidime, Glaxo Group Research Ltd.; cefotaxime, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.; imipenem, Merck Sharp & Dohme, West Point, Pa.; and meropenem, Sumitomo Pharmaceuticals Ltd., Osaka, Japan. Antimicrobial disks were all obtained from Becton Dickinson Microbiology Systems, Cockeysville, Md., including amikacin, chloramphenicol, ciprofloxacin, gentamicin, ofloxacin, pefloxacin, tobramycin, and trimethoprim-sulfamethoxazole.

PFGE analysis. Genomic DNAs prepared by the procedure of Piggot et al. (18) were digested overnight with 10 U of *Xba*I (New England Biolabs, Beverly, Mass.) as recommended by Tenover et al. (25) and were subjected to pulsed-field gel electrophoresis (PFGE) with the Pulsaphor plus system (Amersham Pharmacia Biotech) as described previously (29). DNA fragments were separated in 1% agarose gels in 0.5× Tris-borate-EDTA buffer at 180 V for 30 h, with pulse times ranging from 5 to 35 s. The results were interpreted according to the criteria of Tenover et al. (25)

RESULTS

Screening of isolates. On the basis of the NCCLS criteria, only five of the 3,458 isolates showed reduced susceptibilities to imipenem (inhibition zone diameter, <16 mm) or meropenem (inhibition zone diameter, <16 mm). The five isolates also demonstrated resistance to ceftazidime (inhibition zone diameter, ≤14 mm) and cefoxitin (inhibition zone diameter, ≤14 mm). One hundred and thirty-five isolates exhibited resistance to both ceftazidime and cefoxitin but were susceptible to imipenem and meropenem. These 140 isolates were selected for further experiments.

IMP-8 producers and clinical features. The bla_{IMP-2}-specific probe yielded a strong hybridization signal with 40 of the 140 isolates in colony hybridization experiments. Of the 40 isolates, 36 were found to carry bla_{IMP-8}, bla_{SHV-12}, and bla_{TEM-1} by PCR and nucleotide sequencing, and four were found to harbor bla_{IMP-8}, bla_{SHV-11}, and bla_{TEM-1}. Nine and 31 bla_{IMP-8}-positive isolates were collected in 1999 and 2000, respectively. The bla_{VIM} and bla_{IMP-1} genes were not detected in the remaining 100 isolates in both colony hybridization experiments and PCR assays.

The 40 bla_{IMP-8}-positive isolates were recovered from 16 patients. Twenty of these isolates were recovered from wound specimens, 12 were from sputa, five were from blood samples, two were from tips of central venous tips, and one was from a urine sample (Table 1). Of these 40 samples, 29 were submitted from the surgical ICU, 6 were from the medical ICU, and 4 were from the surgical wards. Prior to the isolation of IMP-8 producers, all 16 patients had undergone various surgical procedures, and all patients except patient 14 had been admitted to the surgical ICU. The underlying diseases and causes of admission of the 16 patients are summarized in Table 1. Before isolation of the IMP-8 producers, five patients had received extended-spectrum β-lactams and one of them had also received meropenem. bla_{IMP-8}-positive isolates were associated with wound infections in eight patients and caused bloodstream infections in three patients. After isolation of the IMP-8 producers from blood samples, patients 7 and 8 received imipenem and patient 14 received meropenem. Nine patients died during the ICU stay due to multiorgan failure not directly related to infections with IMP-8 producers.

Analytical IEF. On IEF gels, all 40 IMP-8-producing isolates had three major bands with pIs of 5.4, 7.6, and 8.2. The pI 7.6 band probably represented the chromosomal SHV β-lactamase of *K. pneumoniae*, the pI 5.4 band might represent the TEM-1 β-lactamase, and the pI 8.2 band might represent either IMP-8 alone or a mixture of IMP-8 and SHV-12 (29).

PFGE. Except for two isolates from patient 13, the repetitive IMP-8-producing isolates recovered from the same patient had identical PFGE patterns. Thus, there were 17 nonrepetitive isolates identified in the outbreak. The PFGE results are summarized in Table 2 and are shown in Fig. 1. Five PFGE patterns were identified. Of the 14 isolates producing both IMP-8 and SHV-12, 11 isolates had pattern A. Isolates 3599/00, 00d401, and 99w853 had distinct patterns B, D, and E, respectively. Although isolate 00m869 exhibited a resistance phenotype different from those of isolate 3396/00 and 00t801, all three isolates had pattern C.

TABLE 1. Origins of bla_{IMP-8}-containing *K. pneumoniae* isolates and clinical characteristics of 16 patients with these isolates

Patient no.	Age (yr)/sex[a]	Disease[b]	Isolate	Collection date (day/mo/yr)	Ward[c]	Source[d]	Type of infection	Previous therapy[e]	Outcome
1	79/M	CAD, DM	99w853	23/7/99	SICU	Urine	Colonization	None	Death
2	70/M	Laryngeal cancer	99a300	1/9/99	SICU	Sputum	Colonization	CAZ	Death
			99b203	11/9/99	MICU	Sputum	Colonization		
3	73/M	PPU, COPD	99c025	20/9/99	SICU	Sputum	Colonization	None	Recovered
4	35/F	Placenta percreta complicated by perforation of urinary bladder	99c196	22/9/99	SICU	Wound	Wound infection	None	Recovered
			99e790	23/10/99	Surgical ward	Wound	Wound infection		
5	65/M	Traumatic ICH	99c515	27/9/99	SICU	Sputum	Colonization	None	Recovered
			99c893	30/9/99	SICU	Wound	Wound infection		
6	79/M	PPU, DM	00e843	25/10/99	SICU	Wound	Wound infection	CTX, ATM	Death
7	47/M	Traumatic ICH, DM, liver cirrhosis	3396/00	1/1/00	SICU	Blood	Bactermia	None	Death
			3397/00	1/1/00	SICU	Blood	Bacteremia		
8	69/F	Traumatic ICH, multiple fractures	00k621	3/1/00	SICU	Wound	Wound infection	None	Recovered
			00k622	3/1/00	SICU	Wound	Wound infection		
			3599/00	4/1/00	SICU	Blood	Bacteremia		
			3606/00	4/1/00	SICU	Blood	Bacteremia		
			00k908	5/1/00	SICU	Wound	Wound infection		
			001060	7/1/00	SICU	Wound	Wound infection		
			001330	10/1/00	SICU	Wound	Wound infection		
			001545	12/1/00	SICU	Wound	Wound infection		
			001650	13/1/00	SICU	Wound	Wound infection		
9	68/M	Traumatic multiple fractures	00m393	26/1/00	Surgical ward	Sputum	Colonization	None	Death
			00p177	24/2/00	SICU	Sputum	Colonization		
			00p251	25/2/00	SICU	Sputum	Colonization		
			00q795	13/3/00	SICU	Wound	Wound infection		
			00r091	17/3/00	SICU	Sputum	Colonization		
			00r134	17/3/00	SICU	Sputum	Colonization		
			00r149	17/3/00	SICU	Wound	Wound infection		
10	73/F	Urinary tract cancer	00m749	26/1/00	SICU	Sputum	Colonization	None	Recovered
11	69/F	PPU, liver cirrhosis	00m869	27/1/00	SICU	Wound	Wound infection	CTX, ATM	Death
			00n070	31/1/00	SICU	Wound	Wound infection		
			00o044	11/2/00	SICU	Wound	Wound infection		
			00o227	24/2/00	SICU	Wound	Wound infection		
12	71/M	Lung cancer	00t801	17/4/00	SICU	Wound	Wound infection	None	Death
13	73/M	CAD, DM	00d401	2/8/00	MICU	Sputum	Colonization	CAZ	Death
			00f195	22/8/00	MICU	Sputum	Colonization		
14	95/F	Parkinsonism, COPD	00h908	20/9/00	MICU	CVC tip	Colonization	CAZ, ATM	Death
			2907/00	23/9/00	MICU	Blood	Catheter-associated bacteremia	MEM	
15	76/F	Cholangitis, cholelithiasis	00j910	12/10/00	Surgical ward	Wound	Wound infection	None	Recovered
			00j911	12/10/00	Surgical ward	Wound	Wound infection		
16	76/F	CAD, congestive heart failure	00m578	13/11/00	MICU	CVC tip	Colonization	None	Recovered

[a] M, male; F, female.
[b] CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; ICH, intracranial hemorrhage; PPU, perforated peptic ulcer.
[c] MICU, medical intensive care unit; SICU, surgical intensive care unit.
[d] CVC, central venous catheter.
[e] Only broad-spectrum β-lactams are listed. ATM, aztreonam; CAZ, ceftazidime; CTX, cefotaxime; MEM, meropenem.

Susceptibility tests. The susceptibilities of the nonrepetitive bla_{IMP-8}-positive isolates to various β-lactams are summarized in Table 2. All 40 isolates were resistant to ceftazidime, cefotaxime, and cefoxitin. Thirty-six isolates, or 14 nonrepetitive isolates, which all carried bla_{IMP-8}, bla_{SHV-12}, and bla_{TEM1}, were also resistant to aztreonam (MICs, ≥64 μg/ml), while only four isolates, or three nonrepetitive isolates, which harbored bla_{IMP-8}, bla_{SHV-11}, and bla_{TEM-1} were susceptible to this agent (MICs, 0.06 to 0.25 μg/ml). Only five isolates, or two nonrepetitive isolates, exhibited resistance to imipenem (MICs, >256 μg/ml) and reduced susceptibilities to meropenem (MICs, 8 to 16 μg/ml). All the other isolates appeared susceptible to carbapenems. The agar diffusion tests revealed that all 40 isolates studied were also resistant to non-β-lactam antibiotics (Table 2).

Conjugation experiments. The 17 nonrepetitive bla_{IMP-8}-positive isolates were subjected to conjugation experiments. The bla_{IMP-8}-containing plasmids were successfully transferred from 13 of 17 isolates to *E. coli* C600. PCR and nucleotide sequencing showed that 11 transconjugants carried bla_{IMP-8}, bla_{SHV-12}, and bla_{TEM1} and that two transconjugants carried bla_{IMP-8} and bla_{TEM-1}. Expression of bla_{IMP-8} by the transconjugants was confirmed by IEF analysis. Resistance to chloramphenicol, trimethoprim-sulfamethoxazole and aminoglycosides was also transferred to *E. coli* from all *K. pneumoniae* isolates except isolate 00m869, in which chloramphenicol resistance was not transferable (Table 2). The sizes of the plasmids transferred to *E. coli* were all >100 kb.

DISCUSSION

The present study indicates that MBLs in *K. pneumoniae* remained uncommon in the university medical center. Of the 3,458 *K. pneumoniae* isolates, only 40 isolates (1.2%) from 16 patients were found to carry bla_{IMP-8}. Neither the other bla_{IMP} genes nor the bla_{VIM} genes were detected in the remaining

TABLE 2. Antimicrobial susceptibilities and PFGE patterns of 17 nonrepetitive *K. pneumoniae* isolates and their transconjugants

Genotypes of β-lactamases, isolate and transconjugant	MIC (μg/ml)[a]						Non-β-lactam antibiotics[b] to which resistance is shown	PFGE pattern
	CAZ	CTX	FOX	ATM	IPM	MEM		
Isolate								
IMP-8 + SHV-12 + TEM1								
99a300	>256	64	>256	>256	0.5	0.25	CHL, SXT, GEN, TOB, PEF, OFX, CIP	A
99c025	>256	16	>256	256	1	0.5	CHL, SXT, GEN, TOB, PEF, OFX, CIP	A
99c196	>256	128	>256	>256	1	1	CHL, SXT, GEN, TOB, PEF, OFX, CIP	A
99c893	256	32	>256	>256	1	0.5	CHL, SXT, GEN, TOB, PEF, OFX, CIP	A
00m393	>256	256	>256	>256	0.5	0.25	CHL, SXT, GEN, TOB, PEF, OFX, CIP	A
00m749	>256	64	>256	>256	0.5	0.25	CHL, SXT, GEN, TOB, PEF, OFX, CIP	A
2907/00	>256	32	>256	64	0.5	0.25	CHL, SXT, GEN, TOB, PEF, OFX, CIP	A
00j910	>256	128	>256	>256	1	0.5	CHL, SXT, GEN, TOB, PEF, OFX, CIP	A
00m578	>256	64	>256	>256	4	1	CHL, SXT, GEN, TOB, PEF, OFX, CIP	A
99e843	>256	128	>256	128	1	0.5	CHL, SXT, GEN, TOB	A
00f195	>256	64	>256	64	0.5	0.25	CHL, SXT, GEN, TOB	A
3599/00	>256	128	>256	>256	2	0.5	CHL, SXT, GEN, TOB, PEF, OFX, CIP	B
00m869	>256	128	>256	>256	>256	16	CHL, SXT, GEN, TOB, PEF, OFX, CIP	C
00d401	>256	>256	>256	>256	>256	8	CHL, SXT, GEN, TOB	D
IMP-8 + SHV-11 + TEM1								
99w853	>256	64	>256	0.25	2	0.5	CHL, SXT, GEN, TOB, AMK, PEF, OFX, CIP	E
3396/00	>256	32	>256	0.06	0.25	0.25	CHL, SXT, TOB, AMK, PEF, OFX, CIP	C
00t801	>256	32	>256	0.25	0.5	0.5	CHL, SXT, TOB, AMK, PEF, OFX, CIP	C
Transconjugant								
IMP-8 + SHV-12 + TEM1								
99a300	256	64	>256	16	0.5	0.25	CHL, SXT, GEN, TOB	
99c025	>256	16	>256	32	0.5	0.25	CHL, SXT, GEN, TOB	
99c196	256	32	256	16	1	0.25	CHL, SXT, GEN, TOB	
99c893	128	16	>256	32	0.5	0.25	CHL, SXT, GEN, TOB	
00m393	128	16	128	8	0.25	0.13	CHL, SXT, GEN	
00m749	256	64	256	16	0.25	0.25	CHL, SXT, GEN, TOB	
2907/00	>256	32	256	16	1	0.25	CHL, SXT, GEN, TOB	
00m578	256	16	>256	32	0.5	0.25	CHL, SXT, GEN, TOB	
99e843	256	64	>256	16	0.5	0.25	CHL, SXT, GEN, TOB	
00f195	128	32	128	8	0.5	0.25	CHL, SXT, GEN, TOB	
00d401	128	32	256	16	0.5	0.25	CHL, SXT, GEN, TOB	
IMP-8 + TEM-1								
00m869	256	32	>256	0.13	0.5	0.25	SXT, GEN, TOB	
99w853	256	64	>256	0.06	0.5	0.5	CHL, SXT, GEN, TOB	

[a] CAZ, ceftazidime; CTX, cefotaxime; FOX, cefoxitin; ATM, aztreonam; IPM, imipenem; MEM, meropenem.
[b] CHL, chloramphenicol; SXT, trimethoprim-sulfamethoxazole; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; PEF, pefloxacin; OFX, ofloxacin; CIP, ciprofloxacin.

isolates. Nine IMP-8-producing isolates from six patients were identified among 1,622 isolates collected in 1999 (0.6%), and 31 bla_{IMP-8}-positive isolates from 10 patients were recognized from 1,836 isolates collected in 2000 (1.7%), suggesting an increasing prevalence rate of bla_{IMP-8}-positive *K. pneumoniae* in the university hospital. The increased prevalence rate could be in part due to the nosocomial outbreak.

Correlation between carriage of bla_{IMP-8} and of carbapenem resistance in our isolates was imperfect (Table 2). Only a total of five bla_{IMP-8}-containing isolates, or two nonrepetitive isolates, were resistant to carbapenems. After conjugation, all *E. coli* transconjugants appeared susceptible to carbapenems. Similar findings have also been described in many reports of MBLs (4, 7, 8, 10, 12, 13, 17, 19, 22–23, 27, 28, 30). In addition, it is also noted that the cloned IMP or VIM enzymes confer only low-level resistance to carbapenems in *E. coli* (7, 10, 12, 17, 19, 21, 28, 30). Thus, there have been two speculations about the imperfect correlation: either the MBL genes are not expressed, or substantive resistance might require reduced uptake of the carbapenem as well as the presence of MBLs (13). Koh et al. (T. H. Koh, L. H. Sng, G. S. Babini, N. Woodford, D. M. Livermore, and L. M. C. Hall, Letter, Antimicrob. Agents Chemother. **45:**1939–1940, 2001) described the loss of a major 39-kDa outer membrane protein in an IMP-1-producing *K. pneumoniae* isolate with high-level resistance to carbapenems recently, supporting the speculation that high-level resistance to carbapenems demands impermeability and an IMP enzyme. This model might also apply to our isolates. The fact that most of our bla_{IMP-8}-positive isolates were susceptible to carbapenems indicates the difficulty in detecting *K. pneumoniae* isolates with the IMP enzymes by using routine susceptibility tests. Therefore, determinations of carbapenemase activity or molecular biology techniques are needed for the purposes of epidemiology and, perhaps, patients' treatment.

Monobactams are stable to hydrolysis by MBLs (7, 10, 12, 17, 19, 21, 30); however, 36 of the total of 40 bla_{IMP-8}-containing isolates (90.0%), or 14 of the 17 nonrepetitive isolates (82.4%), were resistant to aztreonam. Production of the SHV-12 ESBL in addition to IMP-8 by these isolates should be responsible for their resistance to aztreonam. Of greater con-

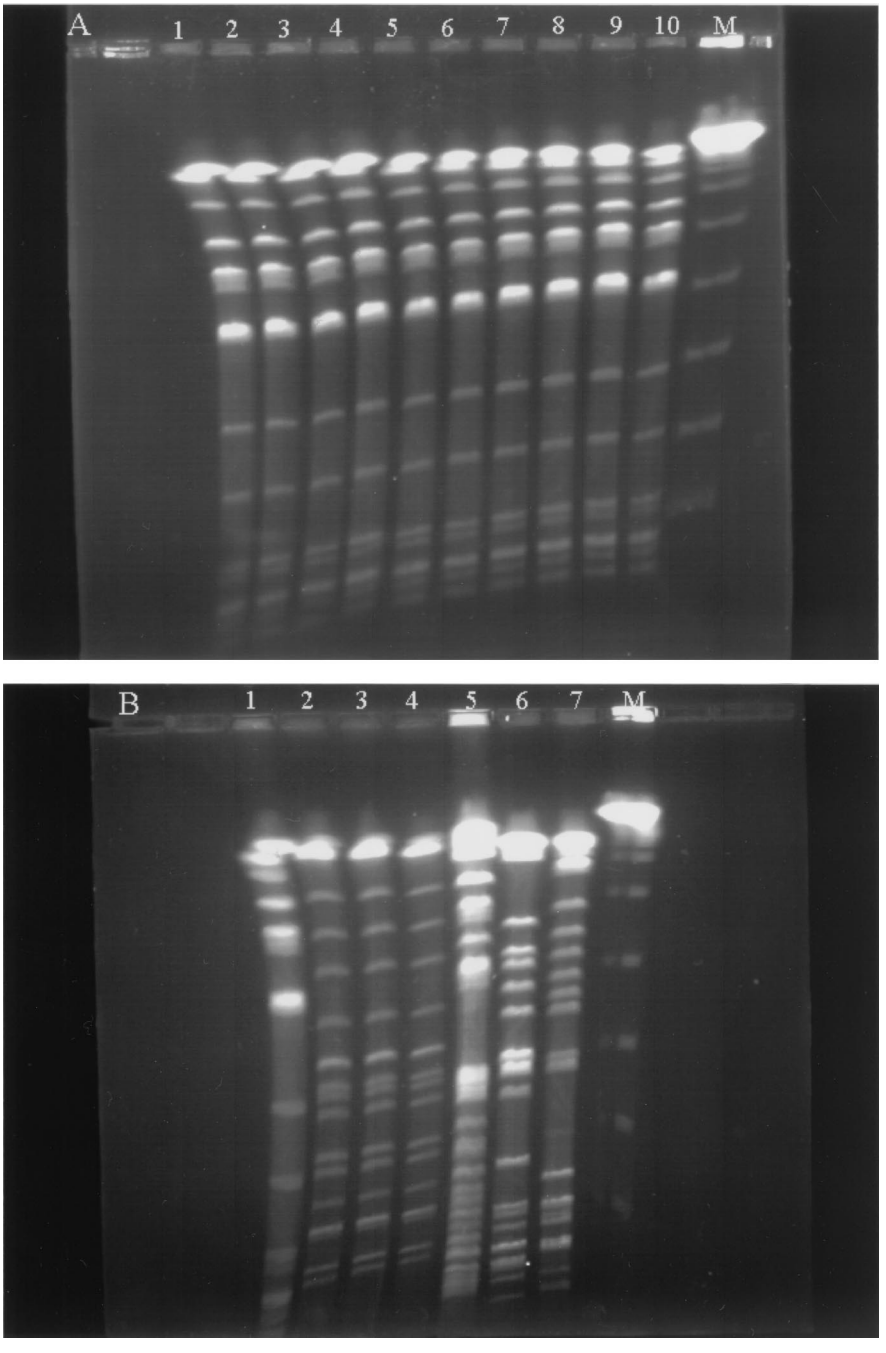

FIG. 1. PFGE of *Xba*I-digested genomic DNAs from 17 nonrepetitive bla_{IMP-8}-positive *K. pneumoniae* isolates. (A) Lane M, bacteriophage lambda DNA concatemers (Gibco BRL), which served as molecular size markers; lanes 1 to 10, pattern A, isolates 99a300, 99c025, 99c196, 99c893, 99e843, 00m393, 00m749, 00f195, 2907/00, and 00j910. (B) Lane M, bacteriophage lambda DNA concatemers; lane 1, pattern A, isolate 00m578; lanes 2 to 4, pattern C, isolates 00m869, 3396/00, and 00t801; lane 5, pattern D, isolate 00d401; lane 6, pattern E, isolate 99w853; lane 7, pattern B, isolate 3599/00. Isolates 00f195 and 00d401 were from the same patient.

cern is that all the IMP-8-producing isolates were also resistant to most non-β-lactam agents (Table 2). All of them were resistant to at least one kind of aminoglycosides, and 37 of the total of 40 isolates (92.5%), or 14 of the 17 nonrepetitive isolates (82.4%), were resistant to fluoroquinolones (Table 2). Therefore, strict infection control measures should be implemented with the appearance of such multidrug-resistant isolates to prevent their spread.

Five patterns were obtained by PFGE. Eleven and 3 of the 17 nonrepetitive isolates exhibited PFGE patterns A and C, respectively, indicating that the nosocomial outbreak of bla_{IMP-8}-containing *K. pneumoniae* was mainly due to clonal spread. The isolates from patient 14 exhibited the same PFGE and plasmid profiles as those from the surgical ICU, suggesting that the bla_{IMP-8}-positive strain in the medical ICU might have been spread from the surgical ICU. Isolates 00d401 and

00f195, both of which were recovered from patient 13, gave different PFGE patterns, suggesting horizontal transfer of the resistance plasmid between these two isolates. Isolates 00m869, 3396/00, and 00t801 all had PFGE pattern C (Table 2). Isolates 99e843, 00f195, and 00m869 had resistance phenotypes different from those of isolates with the same PFGE patterns. The discrepancy could be due to loss or acquisition of resistance genes in the epidemiologically related isolates under various selective pressures.

In an outbreak caused by IMP-1-producing gram-negative rods at a hospital in Japan (8), 53.8% of the IMP-1-producing *P. aeruginosa* isolates were recovered from patients with malignant diseases, suggesting that malignancy is a risk factor for the acquisition of IMP-1-producing isolates. The present study suggests that surgery is another important risk factor for the acquisition of MBL producers. Each one of our patients had received some kind of surgery, and eight patients (50%) had wound infections associated with the isolation of IMP-8 producers. In contrast, only 3 of the 16 patients (18.8%) had malignant diseases. It is noteworthy that many of our patients with wound infections stayed at the surgical ICU during the same period (Table 2), implying that the $bla_{\text{IMP-8}}$-positive strains could have been spread by the health care staff. Only 1 of the 16 patients (6.3%) had received carbapenems, while 5 patients (31.3%) had been administered extended-spectrum β-lactams prior to the isolation of the $bla_{\text{IMP-8}}$-positive isolates. The data were consistent with those obtained by Hirakata et al. (8) and with the suggestion that the selective pressure from carbapenems was not required for the acquisition of MBL producers. Nine of the 16 patients finally died, but none of them died of the infections caused by $bla_{\text{IMP-8}}$-positive isolates. Serial wound cultures indicated that $bla_{\text{IMP-8}}$-positive isolates could colonize in the wounds for up to approximately 2 months (Table 1). These findings indicate that even though the $bla_{\text{IMP-8}}$-containing isolates were not necessarily responsible for the high mortality of our patients, the morbidity they caused is still a big problem. Three patients with bloodstream infections were administered carbapenems after the recovery of $bla_{\text{IMP-8}}$-positive isolates on the basis of the in vitro susceptibility tests. Two of them eventually died, although they had all survived the infections. Because of the limited numbers of patients, it is not clear whether the favorable response was due to the treatment regimens that included carbapenems. Despite susceptibilities of the $bla_{\text{IMP-8}}$-positive strains to carbapenems, the use of carbapenems for the treatment of infections with MBL-producing strains should be restricted to prevent the possibility of emergence of more potent IMP enzymes under the selective pressure from carbapenems.

In conclusion, the present study indicates the emergence of infections caused by $bla_{\text{IMP-8}}$-positive *K. pneumoniae* in Taiwan. These $bla_{\text{IMP-8}}$-positive isolates were all multidrug resistant and usually produced an ESBL as well. The nosocomial outbreak identified in a university hospital was largely caused by genetically related strains. Although the $bla_{\text{IMP-8}}$-positive isolates were still confined to the ICUs and spread at a low prevalence rate in the university hospital, strict infection control measures against such isolates should be implemented in order to prevent their further dissemination.

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