

## Emergence of *Klebsiella pneumoniae* Isolates Producing Inducible DHA-1 $\beta$ -Lactamase in a University Hospital in Taiwan

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Ten nonrepetitive clinical isolates of *Klebsiella pneumoniae* exhibiting an unusual inducible  $\beta$ -lactam resistance phenotype were identified between January 1999 and September 2001 in a university hospital in Taiwan. In the presence of 2  $\mu$ g of clavulanic acid, the isolates showed a one to four twofold concentration increase in the MICs of ceftazidime, cefotaxime, and aztreonam but remained susceptible to cefepime (MICs,  $\leq 0.5$   $\mu$ g/ml) and imipenem (MICs,  $\leq 0.5$   $\mu$ g/ml). PCR, sequence analysis, and isoelectric focusing revealed production by these isolates of TEM-1, SHV-11, and DHA-1, a plasmid-encoded inducible AmpC  $\beta$ -lactamase originally found in a *Salmonella enterica* serovar Enteritidis strain. Transfer of the resistance by conjugation experiments was not successful, but Southern hybridization showed that *bla*<sub>DHA-1</sub> was located on 70-kb plasmids, suggesting that the *bla*<sub>DHA-1</sub>-containing plasmids in the *K. pneumoniae* isolates were non-self-transmissible. Five isolates were recovered from patients in two surgery wards and two intensive care units. Acquisition of the DHA-1 producers could be traced back to previous hospitalizations 1 to 5 months earlier for the other five patients. Six and seven patterns among the isolates were demonstrated by plasmid analysis and ribotyping, respectively, indicating that the spread of the DHA-1 producers was due to both horizontal transfer of *bla*<sub>DHA-1</sub> and dissemination of endemic clones.

Chromosome-mediated AmpC  $\beta$ -lactamases have been described in a wide variety of gram-negative bacilli, such as *Pseudomonas aeruginosa* and *Enterobacter* spp. (8, 15, 16, 25). In most genera of the family *Enterobacteriaceae*, AmpC is inducible and, when overexpressed, can confer resistance to both oxyimino- and 7- $\alpha$ -methoxy-cephalosporins and monobactams (8, 15, 25). Many plasmid-mediated AmpC enzymes, such as CMY-type  $\beta$ -lactamases, have been found in bacterial species that naturally lack a chromosomal AmpC  $\beta$ -lactamase, such as *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Salmonella* spp. (2, 4–6, 10–14, 21, 28, 30). It is believed that such  $\beta$ -lactamases arose through the transfer of chromosomal AmpC genes onto plasmids (21).

Unlike chromosome-mediated AmpC, plasmid-encoded AmpC enzymes are almost always expressed constitutively (4–6, 11–14, 21, 30). Plasmid-mediated inducible  $\beta$ -lactamases are extremely rare. DHA-1 from a clinical isolate of *Salmonella enterica* serovar Enteritidis from Saudi Arabia is the first identified plasmid-encoded inducible cephalosporinase (2). The counterpart of *bla*<sub>DHA-1</sub> was the chromosomal AmpC gene of *Morganella morganii* (3, 22). The inducibility of DHA-1 is due to the presence of a regulator *ampR* gene, which is also related to that of *M. morganii*, upstream of *bla*<sub>DHA-1</sub> on the same plasmid (2, 22, 29). A DHA-1-related  $\beta$ -lactamase, named DHA-2, was identified more recently from a *K. pneumoniae* isolate in France (10). The enzyme also confers an inducible  $\beta$ -lactam resistance phenotype.

Recently, the standard confirmatory test for the detection of extended-spectrum  $\beta$ -lactamases (19) revealed an unusual ceftazidime and cefotaxime resistance phenotype in clinical

isolates of *K. pneumoniae* in a university hospital in Taiwan. Thus, a retrospective analysis was carried out to characterize these isolates and their various clinical and epidemiological features. We found inducible expression of DHA-1 by these isolates. To our knowledge, this is the first report of the appearance of DHA-1 in the Far East and is also the first report of fairly widespread of DHA-1-producing *K. pneumoniae* within a health care institution.

### MATERIALS AND METHODS

**Bacterial isolates and patients.** The standard screening and confirmation methods for the detection of extended-spectrum  $\beta$ -lactamases (19) were routinely performed at the Department of Pathology, National Cheng Kung University Hospital, a 900-bed teaching hospital in southern Taiwan. Between January 1999 and September 2001, 10 nonrepetitive isolates of *K. pneumoniae* from 10 patients demonstrated reduced inhibition zone diameters for both ceftazidime and cefotaxime in combination with clavulanic acid versus those for ceftazidime and cefotaxime when tested alone (see Table 1), suggesting production of  $\beta$ -lactamases induced by clavulanic acid. All these isolates were identified by conventional techniques (9) and/or the API 20E system (bioMérieux, Marcy l'Etoile, France). The medical records of the patients from whom the isolates were recovered were reviewed.

**Antagonism testing.** The disk antagonism method initially used to detect inducibility of chromosomal  $\beta$ -lactamases (16) was performed with a slight modification to test the 10 *K. pneumoniae* isolates. Disks of inducing agents and disks of cephalosporins were placed on the surface of Mueller-Hinton agar plates and separated by 25 mm (see Fig. 1). The cephalosporins used were cefotaxime, ceftazidime, aztreonam, and cefepime. Clavulanic acid (10  $\mu$ g) and cefoxitin (30  $\mu$ g) were used as inducing agents. The plates were examined after overnight incubation at 37°C.

**Susceptibility testing.** MICs were determined by the standard agar dilution method (18). The antimicrobial agents and their sources were as follows: amoxicillin and clavulanic acid, SmithKline Beecham Pharmaceuticals, Surrey, United Kingdom; aztreonam and cefepime, Bristol-Myers Squibb, New Brunswick, N.J.; cefotaxime, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.; cefoxitin, Sigma Chemical Company, St. Louis, Mo.; ceftazidime, Glaxo Group Research Ltd., Greenford, United Kingdom; and imipenem, Merck Sharp & Dohme, West Point, Pa. The susceptibilities to six non- $\beta$ -lactam agents were determined by the standard disk diffusion method (19). Antimicrobial disks were obtained from Becton Dickinson Microbiology Systems, Cockeysville, Md., including amikacin,

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TABLE 1. Susceptibility patterns of DHA-1-producing *K. pneumoniae* isolates<sup>a</sup>

Isolate	Extended-spectrum β-lactamase confirmatory test (inhibition zone, mm)										MIC (μg/ml)										Disk diffusion test									
	CAZ		CAZ + CLA <sup>b</sup>		CTX	CTX + CLA <sup>b</sup>		AMC	PIP	TZP	FOX	CAZ	CAZ + CLA <sup>c</sup>	CTX	CTX + CLA <sup>c</sup>	ATM	ATM + CLA <sup>c</sup>	CFP	CFP + CLA <sup>c</sup>	IPM	IPM + CLA <sup>c</sup>	GN	TOB	AMK	OFX	CIP	SXT			
	CAZ	CAZ + CLA <sup>b</sup>	CTX	CTX + CLA <sup>b</sup>	AMC	PIP	TZP	FOX	CAZ	CAZ + CLA <sup>c</sup>	CTX	CTX + CLA <sup>c</sup>	ATM	ATM + CLA <sup>c</sup>	CFP	CFP + CLA <sup>c</sup>	IPM	IPM + CLA <sup>c</sup>	GN	TOB	AMK	OFX	CIP	SXT						
387	17	12	19	15	>256	>256	16	>256	16	64	4	16	4	8	<0.13	0.13	0.13	S	S	S	S	S	S	S	S	S				
416	21	14	23	17	>256	>256	32	>256	8	32	1	4	2	4	<0.13	0.25	0.25	R	R	R	R	R	R	R	R	R				
1490	13	11	23	15	>256	>256	32	>256	32	128	4	16	8	32	0.5	0.13	0.13	R	R	R	R	R	R	R	R	R				
1596	15	12	21	16	>256	>256	32	>256	64	256	16	64	8	32	0.5	0.25	0.25	R	R	R	R	R	R	R	R	R				
197	16	13	23	16	>256	>256	32	>256	16	64	2	8	8	32	<0.13	0.5	0.5	R	R	R	R	R	R	R	R	R				
274	17	13	22	17	>256	>256	32	>256	16	32	2	4	4	16	<0.13	0.25	0.25	R	R	R	R	R	R	R	R	R				
281	15	9	25	22	>256	>256	16	>256	32	128	4	16	8	32	0.5	0.5	0.5	R	R	R	R	R	R	R	R	R				
325	22	14	24	17	>256	>256	8	>256	4	64	1	8	1	8	<0.13	0.25	0.25	R	R	R	R	R	R	R	R	R				
397	20	12	24	20	>256	>256	8	>256	8	32	1	8	1	8	<0.13	0.25	0.25	R	R	R	R	R	R	R	R	R				
1067	21	11	26	23	>256	>256	8	>256	8	64	2	16	2	16	<0.13	0.5	0.5	S	S	S	S	S	S	S	S	S				

<sup>a</sup> AMC, amoxicillin-clavulanic acid; AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CFP, cefepime; CTX, cefotaxime; FOX, cefoxitin; GN, gentamicin; IPM, imipenem; OFX, ofloxacin; PIP, piperacillin; SXT, trimethoprim-sulfamethoxazole; TOB, tobramycin; TZP, piperacillin-tazobactam; S, susceptible; I, intermediate susceptible; R, resistant.

<sup>b</sup> Clavulanic acid at a concentration of 10 μg/ml.

<sup>c</sup> Clavulanic acid at a fixed concentration of 2 μg/ml.

ciprofloxacin, gentamicin, ofloxacin, tobramycin, and trimethoprim-sulfamethoxazole.

**IEF.** Crude preparations of β-lactamases were obtained from the isolates by sonication (7) and subjected to analytical isoelectric focusing (IEF) as described previously (17, 30). Cells induced by 16 μg of cefoxitin per ml were incubated for 3 h before harvesting (2). β-Lactamase activity was detected by overlaying the gels with 0.5 mM nitrocefin in 0.1 M phosphate buffer, pH 7.0.

**PCR and DNA sequencing.** Plasmids from the isolates were extracted by a rapid alkaline lysis procedure (27) and used as templates in PCRs. The entire *bla*<sub>DHA-1</sub> gene was amplified with the oligonucleotide primers DHA-1A (5'-CTGATGAAAAAATCGTTATC-3') and DHA-1B (5'-ATCCAGTGCACATAAATA-3'), corresponding to nucleotides -3 to 17 and 1138 to 1119, respectively, of the DHA-1 structural gene (2). The PCR conditions were as follows: 3 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C; and finally 7 min at 72°C. The entire sequences of *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>-related genes were amplified with the primer pairs as described previously (30). The amplicons were purified with a commercial kit (Roche Molecular Biochemicals, Mannheim, Germany) and sequenced on an ABI Prism 310 sequencer analyzer (Applied Biosystems, Foster City, Calif.).

**Transfer of resistance.** Conjugation experiments were performed as described previously (24, 30) with streptomycin-resistant *Escherichia coli* C600 as the recipient (1). Tryptic soy agar plates supplemented with 500 μg of streptomycin (Sigma) per ml and 64 μg of cefoxitin per ml were used to select the transconjugants. *K. pneumoniae* strain W142 harboring *bla*<sub>CMY-8</sub> was used as the control (30).

**Plasmid analysis and Southern hybridization.** Plasmids from the isolates were analyzed by electrophoresis on a 0.8% agarose gel. *E. coli* strain NCTC 50192 (National Collection of Type Cultures, London, United Kingdom), which contained four plasmids of 7, 36.3, 63.8, and 148.5 kb, was used as a source of molecular size markers. The gel was stained with ethidium bromide (Sigma), visualized under UV light, and subjected to Southern hybridization according to the original protocol (26). The *bla*<sub>DHA-1</sub>-specific probe was a PCR-generated amplicon labeled with [ $\alpha$ -<sup>32</sup>P]dCTP (Amersham Pharmacia Biotech) by the random priming technique with a commercial kit (Gibco-BRL Life Technologies, Gaithersburg, Md.).

**Ribotyping.** The chromosomal DNA of the isolates was extracted and purified as described previously (23). The genomic DNA was restricted with *Eco*RI or *Bst*EII (Roche Molecular Biochemicals) (20). The digests of chromosomal DNA were electrophoresed at 35 V for 18 h in a 0.8% agarose gel, transferred to a nylon membrane (Amersham Pharmacia Biotech), and then hybridized with a [ $\alpha$ -<sup>32</sup>P]dCTP-labeled cDNA copy of *E. coli* rRNA (Roche Molecular Biochemicals) obtained by reverse transcription with avian myeloblastosis virus reverse transcriptase (Gibco-BRL) as described previously (23).

## RESULTS

**Inducibility of β-lactamases.** In the standard extended-spectrum β-lactamases confirmatory test, the reduced zone diameters for ceftazidime and cefotaxime in combination with clavulanic acid versus those for ceftazidime and cefotaxime tested alone among the 10 *K. pneumoniae* isolates ranged from 2 to 10 mm (mean, 5.6 mm) and 3 to 8 mm (mean, 5.6 mm), respectively, suggesting production of β-lactamases induced by clavulanic acid (Table 1). Inducibility of the β-lactamases was further recognized by the disk antagonism test, which demonstrated blunting of the cephalosporin disks adjacent to the cefoxitin and clavulanic acid disks (Fig. 1).

**Susceptibility testing.** The results of the susceptibility tests are shown in Table 1. All 10 isolates exhibited high-level resistance to amoxicillin-clavulanic acid and cefoxitin. In the presence of clavulanic acid, a one to four twofold concentration increase in the MICs of ceftazidime, cefotaxime, and aztreonam was noted, while the changes after addition of clavulanic acid in the MICs of cefepime and imipenem were not obvious.

**Identification of β-lactamases.** IEF demonstrated that all 10 isolates displayed three bands of β-lactamase activity with pIs of 5.4, 7.6, and 7.8. The pI 7.6 band probably represented the

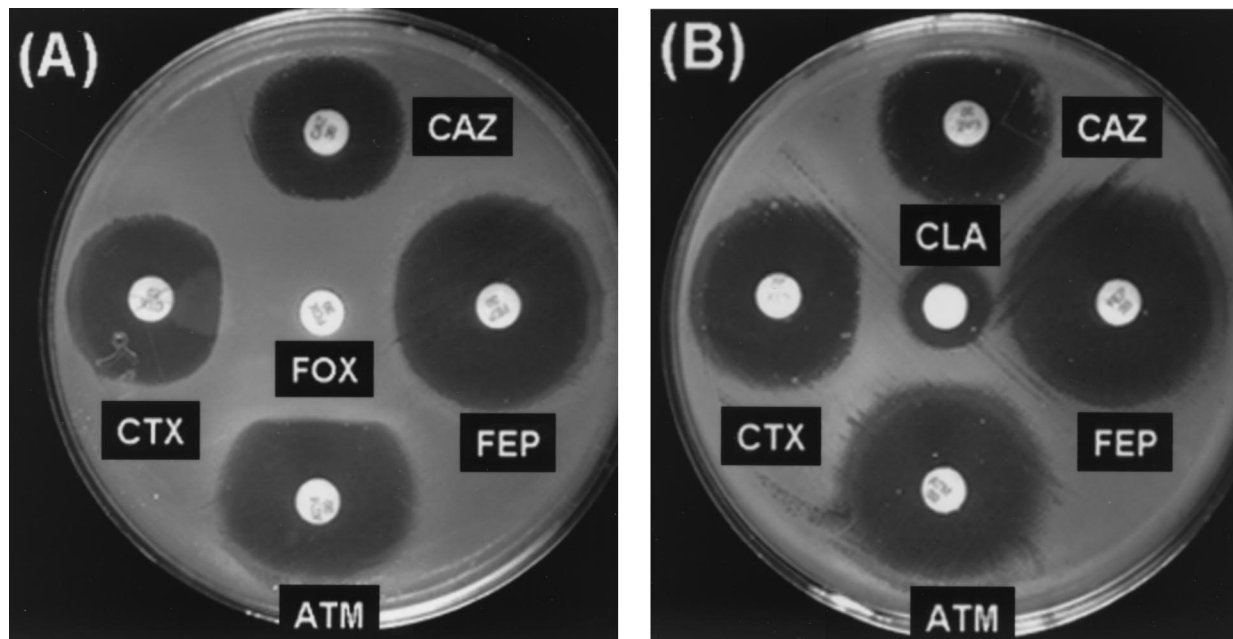


FIG. 1. Double-disk antagonism tests with 30 µg of cefoxitin (A) and 10 µg of clavulanic acid (B). ATM, aztreonam; CAZ, ceftazidime; CLA, clavulanic acid; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin.

chromosomal SHV-1 or SHV-11 type  $\beta$ -lactamase of *K. pneumoniae* (15, 30), the pI 5.4 band might represent the TEM-1  $\beta$ -lactamase (15), and the pI 7.8 band might represent the  $\beta$ -lactamase responsible for the inducible resistance phenotype.

A 1,141-bp fragment was amplified by PCR with the *bla*<sub>DHA-1</sub>-specific primers for all 10 *K. pneumoniae* isolates. The amino acid sequences of the PCR products deduced from the sequence analysis were identical to the plasmid-mediated cephalosporinase DHA-1 from *S. enterica* serovar Enteritidis (2). The DHA-1 cephalosporinase was consistent with the pI 7.8  $\beta$ -lactamase demonstrated by IEF (2). All the isolates also carried *bla*<sub>TEM-1</sub> and *bla*<sub>SHV-11</sub>, which were identified by PCR with the *bla*<sub>TEM</sub>- and *bla*<sub>SHV</sub>-specific primers and sequence analysis.

**Conjugation experiments and plasmid analysis.** Conjugation experiments failed to demonstrate transfer of inducible cephalosporin resistance from any of the isolates. Cefoxitin resistance was transferred from the control strain to *E. coli* C600 at a frequency of  $10^{-3}$  to  $10^{-4}$  per donor cell. Six different profiles were demonstrated by plasmid analysis among the 10 isolates (Fig. 2). In all isolates analyzed, the presence of a plasmid of approximately 70 kb was detected. Southern hybridization with the *bla*<sub>DHA-1</sub>-specific probe showed that *bla*<sub>DHA-1</sub> was located on the 70-kb plasmid (data not shown).

**Ribotyping.** The genetic relationship among the 10 *K. pneumoniae* isolates was investigated by ribotyping with two different endonucleases. Patterns with at least two discordant bands were considered different (20). The results are listed in Table 2 and partially shown in Fig. 3. Both *EcoRI* and *BstEII* generated seven different patterns. Isolates 1490 and 1596, both of which were collected in early 2000, and isolates 197, 274, and 281, which were all collected in late 2000, had identical ribotypes, suggesting that they derived from two endemic clones.

**Clinical characteristics.** Three isolates were recovered from sputum samples and were considered colonizers. The other seven isolates were associated with three urinary tract infections, two wound infections, one intra-abdominal infection, and one bloodstream infection. The clinical characteristics of the patients infected with or colonized by the DHA-1-producing isolates are summarized in Table 2. Six patients had undergone hemodialysis due to either chronic or acute renal failure before isolation. Five isolates were obtained >48 h after the patients were admitted to the hospital. Three of the five isolates were from the patients in the surgery wards, and two isolates were from the patients in the intensive care units. Although the remaining five isolates were obtained within 48 h after the current admission, all patients with these isolates had been hospitalized in the teaching hospital 1 to 5 months earlier. Notably, four of them had been on hemodialysis either in the university hospital or at community hospitals.

All nine patients for whom complete medical records were available had been exposed to  $\beta$ -lactam agents within 2 weeks before isolation of the DHA-1 producers. Patients 3, 4, 6, and 8 received no specific antimicrobial agents for the DHA-1

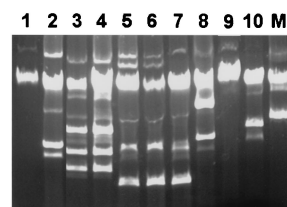


FIG. 2. Plasmid profiles of the 10 *K. pneumoniae* isolates. Lanes 1 to 10, isolates 387, 416, 1490, 1596, 197, 274, 281, 325, 397, and 1067, respectively; lane M, *E. coli* NCTC 50192, which contains four plasmids with molecular sizes of 7, 36.2, 63.8, and 148.5 kb.



TABLE 2. Clinical data, plasmid profiles, and ribotypes of the 10 DHA-1-producing *K. pneumoniae* isolates

Patient no.	Age (yr)/sex	Isolate	Collection date (day/mo/yr)	Source	Type of infection	Ward <sup>a</sup>	Duration between admission and:		Comorbid conditions <sup>b</sup>	Previous medication <sup>c</sup>	Outcome	Plasmid profile	Ribotype ( <i>Eco</i> RI/ <i>Bst</i> EII)
							Isolation (days)	Previous hospitalization (mo)					
1	55/M	387	26/04/99	Wound	Wound	Surgery 7A	14	1	Fractures, head injury	CEF, AMC, CMZ	Recovered	P1	E1/B1
2	74/M	416	06/05/99	Urine	Urinary tract	Reh	1	1	Cirrhosis, spine injury, lung cancer	CEF	Death	P2	E2/B2
3	81/F	1490	22/01/00	Sputum	Colonization	Surgery 7A	23		Fractures, head injury	CFZ, IPM	Death	P5	E5/B5
4	71/M	1596	22/02/00	Sputum	Colonization	CCU	32		CAD, acute renal failure	OXA, TZP, CMZ	Death	P5	E5/B5
5	35/M	197	09/10/00	Ascites	Peritonitis	Surgery 6B	20		Cirrhosis, intra-abdominal bleeding, acute renal failure	CEF, CTX, CRO	Death	P6	E3/B3
6	69/M	274	02/12/00	Wound	Wound	ER	0	1	DM, ESRD	PIP	Recovered	P6	E3/B3
7	77/F	281	06/12/00	Urine	Urinary tract	OPD	0	5	ESRD, stroke, HTN, Parkinsonism	AMC	Death	P6	E3/B3
8	82/F	325	25/12/00	Sputum	Colonization	ER	0	4	ESRD, head injury	Unknown	Death	P3	E4/B4
9	78/F	397	13/04/01	Urine	Urinary tract	MICU	34		Tetanus, pneumonia	PIP, AMC, CEF, CMZ	Recovered	P1	E6/B6
10	74/M	1067	27/08/01	Blood	Bacteremia	ER	1	1	DM, ESRD, stroke	CEF, CTX	Recovered	P4	E7/B7

<sup>a</sup> Reh, rehabilitation; CCU, critical care unit; ER, emergency room; OPD, outpatient department; MICU, medical intensive care unit.

<sup>b</sup> CAD, coronary artery disease; DM, diabetes mellitus; ESRD, end-stage renal disease; HTN, hypertension.

<sup>c</sup> Only β-lactam agents are listed. AMC, amoxicillin-clavulanic acid; CEF, cefazolin; CFZ, cefmetazole; CTX, cefotaxime; CRO, ceftriaxone; IPM, imipenem; OXA, oxacillin; PIP, piperacillin; TZP, piperacillin-tazobactam.

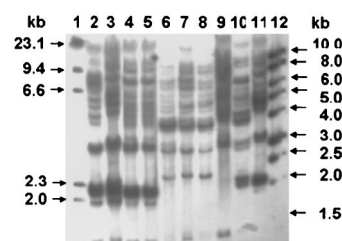


FIG. 3. Ribotypes generated by *Eco*RI. Lane 1, molecular size marker kit II (Roche Molecular Biochemicals); lanes 2 to 11, isolates 387, 416, 1490, 1596, 197, 274, 281, 325, 397, and 1067, respectively; lane 12, 1-kb ladder (Promega Co., Madison, Wis.).

producers, and patient 7 was not treated for the urinary tract infection at the university hospital. Patients 2 and 9 received ciprofloxacin and trimethoprim-sulfamethoxazole, respectively, for 2 weeks, and the *K. pneumoniae* strains were not isolated from urine samples afterward. Patient 1 had received cefotaxime and ciprofloxacin, but his deep soft tissue infection was not eradicated until debridement was performed 1 month after admission. Patient 5 received cefotaxime after isolation and died of intra-abdominal hemorrhages and multiorgan failure due to his underlying diseases 1 week later. Whether the bacterial peritonitis was persistent before his death is not clear. Patient 10 was cured of the bloodstream infection with 2 weeks of meropenem and netilmicin therapy. Five patients died during hospitalization, and patient 7 died during the other hospitalization; however, none of the deaths were directly due to infections caused by the DHA-1 producers.

## DISCUSSION

The plasmid-mediated inducible DHA-1 β-lactamase was first identified in Taiwan in the present study. PCR, sequence analysis, and IEF revealed production of three β-lactamases, TEM-1, SHV-11, and DHA-1, by all 10 *K. pneumoniae* isolates possessing inducible resistance to extended-spectrum β-lactamases. TEM-1 and SHV-11 are restricted-spectrum β-lactamases. Since DHA-1, originally found in an *S. enterica* serovar Enteritidis strain was inducible (2), the enzyme is believed to be responsible for the unusual inducible β-lactam resistance phenotype of our isolates. Transfer of the resistance by conjugation experiments was not successful; however, the *bla*<sub>DHA-1</sub>-specific probe was hybridized to a 70-kb plasmid in all isolates analyzed, suggesting that *bla*<sub>DHA-1</sub> was on non-self-transmissible plasmids. Similar results have been described in reports of DHA-1-producing *S. enterica* serovar Enteritidis and DHA-2-producing *K. pneumoniae* (2, 10). To our knowledge, plasmid-mediated DHA-type β-lactamases have only been reported in isolates from Europe and the Middle East (2, 10, 29). Thus, this is also the first report of the appearance of a DHA-type β-lactamase in the Far East.

In the extended-spectrum β-lactamases confirmatory test, all *K. pneumoniae* isolates revealed decreased inhibition zone diameters for ceftazidime and cefotaxime in combination with clavulanic acid compared with those of these two agents tested alone, indicating that the test could also be used to screen for inducible β-lactamase-producing gram-negative bacilli that naturally lack inducible chromosome-mediated AmpC en-

zymes. All 10 *K. pneumoniae* isolates showed blunting of the cephalosporin disks adjacent to the cefoxitin and clavulanic acid disks in the antagonism test, indicating that the test can also be used to recognize plasmid-mediated  $\beta$ -lactamases.

In the *Enterobacteriaceae*, AmpC-hyperproducing derepressed strains appear frequently in infections caused by organisms naturally producing inducible AmpC enzymes when patients are treated with extended-spectrum  $\beta$ -lactams (16). Therefore, it has been recommended that the inducible-AmpC-producing *Enterobacteriaceae* species should be reported as resistant to all extended-spectrum  $\beta$ -lactams (16). The use of extended-spectrum  $\beta$ -lactams should be restricted accordingly. Studies on determining the therapeutic success or failure of extended-spectrum third-generation cephalosporins in treating infections with plasmid-mediated inducible AmpC producers, such as our DHA-1-producing *K. pneumoniae* isolates, are lacking. Therefore, whether such *K. pneumoniae* strains, like gram-negative organisms naturally producing inducible AmpC enzymes, should also be reported as resistant to all third-generation cephalosporins is not known and deserves further investigation.

The drugs of choice for the treatment of infections with such organisms are also undetermined. Based on MIC data (Table 1) and the confirmatory test for extended-spectrum  $\beta$ -lactamases, a majority of the DHA-1-producing *K. pneumoniae* isolates would not have been reported as resistant to all third-generation cephalosporins. However, after induction by clavulanic acid, these isolates showed reduced susceptibilities to these agents. Moreover, all these isolates remained susceptible to cefepime and imipenem even in the presence of clavulanic acid. Thus, fourth-generation cephalosporins and carbapenems could be better choices for the treatment of infections caused by DHA-1 producers. Alternatively, when the presence of inducible DHA-type enzymes is suspected or detected, physicians should be informed, and the use of strong AmpC-inducing agents, such as clavulanic acid and cephamycins, should be avoided.

Six plasmid patterns and seven ribotypes were found among the 10 DHA-1-producing isolates (Fig. 2 and 3), indicating that the spread of *bla*<sub>DHA-1</sub> was due to both dissemination of endemic clones and horizontal transfer of the resistance gene. Most isolates in the university hospital were obtained from surgery wards and intensive care units. Five isolates were obtained within 48 h after admission; however, all patients from whom the isolates were obtained had been hospitalized in the same university hospital 1 to 5 months before the current admissions. It is not known exactly whether these isolates were from the university medical center or other hospitals. However, since isolates 274 and 281 had a ribotype identical to that of isolate 197, which was obviously from the university hospital, it is very likely that at least patients 6 and 7 had acquired the resistance strain during previous hospitalizations. Six of the 10 patients infected with DHA-1 producers had been on hemodialysis. Since this was a retrospective study, it is not clear whether the nosocomial infections were associated with the hemodialysis systems.

In conclusion, sporadic infections with *K. pneumoniae* possessing an unusual inducible  $\beta$ -lactam resistance phenotype were found in a university hospital in Taiwan. DHA-1 encoded by non-self-transferable plasmids conferred the resistance phe-

notype. The spread of the DHA-1 producers was due to dissemination of endemic clones and horizontal transfer of the resistance gene.

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