Bacteremia Due to Extended-Spectrum- β -Lactamase-Producing *Aeromonas* spp. at a Medical Center in Southern Taiwan[▽]

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Although extended-spectrum- β -lactamase (ESBL)-producing aeromonads have been increasingly reported in recent years, most of them were isolates from case reports or environmental isolates. To investigate the prevalence of ESBL producers among *Aeromonas* blood isolates and the genes encoding ESBLs, consecutive nonduplicate *Aeromonas* blood isolates collected at a medical center in southern Taiwan from March 2004 to December 2008 were studied. The ESBL phenotypes were examined by clavulanate combination disk test and the cefepime-clavulanate ESBL Etest. The presence of ESBL-encoding genes, including *bla*_{TEM}, *bla*_{PER}, *bla*_{CTX-M}, and *bla*_{SHV} genes, was evaluated by PCR and sequence analysis. The results showed that 4 (2.6%) of 156 *Aeromonas* blood isolates, 1 *Aeromonas hydrophila* isolate and 3 *Aeromonas caviae* isolates, expressed an ESBL-producing phenotype. The ESBL gene in two *A. caviae* isolates was *bla*_{PER-3}, which was located in both chromosomes and plasmids, as demonstrated by Southern hybridization. Of four patients with ESBL-producing *Aeromonas* bacteremia, two presented with catheter-related phlebitis and the other two with primary bacteremia. Three patients had been treated with initial noncarbapenem β -lactams for 5 to 10 days, and all survived. In conclusion, ESBL producers exist among *Aeromonas* blood isolates, and clinical suspicion of ESBL production should be raised in treating infections due to cefotaxime-resistant *Aeromonas* isolates.

Aeromonads, oxidase-producing Gram-negative rods, are aquatic microorganisms and have been implicated in a variety of human diseases (11). Three well-known principal classes of β -lactamases recognized in aeromonads are class C cephalosporinases, class D penicillinases, and class B metallo- β -lactamases (MBL) (11), whereas production of extended-spectrum β -lactamases (ESBLs) has received little attention. ESBLs belong to the class A β -lactamases according to Ambler's classification (1a). They confer resistance to all penicillins, older and newer cephalosporins, and monobactams but not to cephamycins or carbapenems, and they are inactivated by β -lactamase inhibitors such as clavulanate. ESBL-producing aeromonads have been increasingly reported in recent years. The earliest report of a clinical case, in 2003, described a fecal *A. caviae* strain harboring ESBL *bla*_{TEM-24} from an aged patient with intestinal ischemia (16). Later on, ESBL-producing environmental isolates were reported, including isolates carrying *bla*_{PER-1} from sludge in Switzerland (24), isolates carrying *bla*_{PER-1}, *bla*_{PER-6}, *bla*_{SHV-12}, *bla*_{VEB-1a}, *bla*_{TLA-2}, or *bla*_{GES-7} from the Seine River (8, 15), and *Aeromonas hydrophila* isolates from an urban river in China (15). However, no study focused on the prevalence and clinical manifestations of infections caused by ESBL-producing aeromonads. The aim of this

study was to investigate the prevalence of ESBL producers among *Aeromonas* blood isolates, to investigate the genes encoding ESBLs, and to describe the clinical features of infected patients. A literature review in search of clinical cases was also conducted, with the hope of better understanding the current status of ESBLs among clinical *Aeromonas* isolates.

MATERIALS AND METHODS

Bacterial isolates. *Aeromonas* blood isolates identified in the clinical microbiology laboratory of National Cheng Kung University Hospital (NCKUH), a university-affiliated medical center in southern Taiwan, from March 2004 to December 2008 were collected and stored at -70°C until use. For each patient, if multiple isolates of the same species with identical antimicrobial susceptibility profiles were obtained, only the first was taken into account. The genus *Aeromonas* was identified by the positive oxidase test, fermentation of D-glucose, motility, the absence of growth in 6.5% sodium chloride, and resistance to the vibriostatic agent O/129 (150 μg), and the identification was confirmed by the API 20E system (BioMérieux, Marcy-l'Etoile, France). Identification of *Aeromonas* species was based on the sequence analysis of the partial *rpoB* gene by PCR with the primers Pasrpb-L (5'-GCAGTGAAAGARTTCTTTGGTTC-3') and Rpb-R (5' GTTCATGTTNGNACCCAT 3') under the conditions previously described (12). The sequences of amplified DNA products were compared with reference sequences available at the GenBank database using a BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Antimicrobial susceptibility tests. The ESBL phenotype was examined by tests proposed for the detection of ESBLs in *Enterobacteriaceae* by the Clinical and Laboratory Standards Institute (CLSI) (3). *Aeromonas* isolates that demonstrated a diameter of inhibition zone of ceftazidime (30 μg) of ≤ 22 mm or of cefotaxime (30 μg) of ≤ 27 mm by the disk diffusion method—i.e., reduced susceptibility to expanded-spectrum cephalosporins—were examined by a phenotypic confirmatory test, i.e. the ceftazidime-clavulanate and cefotaxime-clavulanate combination disk test (CDT), and the cefepime-clavulanate ESBL Etest (AB Biodisk, Solna, Sweden). The presence of ESBL was determined by a

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TABLE 1. PCR primers used to detect genes encoding ESBLs, MBL (*bla*_{CphA}), and AmpC-β-lactamase (*bla*_{MOX}) among four ESBL-producing *Aeromonas* blood isolates

β-Lactamase(s) targeted	Primer name	Primer sequence (5'–3')	Reference or source
<i>bla</i> _{TEM}	TEM-2A(F) TEM-1B(R)	CCCCTATTTGTTTATTTTCT GACAGTTACCAATGCTTAAT	34
<i>bla</i> _{SHV}	SHV-3A(F) SHV-2B(R)	CCGGGTATTCTTATTGTC TAGCGTTGCCAGTGCTCGAT	19
<i>bla</i> _{PER}	PER-A(F) PER-B(R)	ATGAATGTCATTATAAAAGC TTAATTTGGGCTTAGGGCAGAA	18
<i>bla</i> _{CTX-M} group 1 (<i>bla</i> _{CTX-M-1} , <i>bla</i> _{CTX-M-3} , <i>bla</i> _{CTX-M-151})	MultiCTXMGp1(F) MultiCTXMGp1-2(R)	TTAGGAARTGTGCCGCTGYA CGATATCGTTGGTGGTRCCAT	5
<i>bla</i> _{CTX-M} group 2 (<i>bla</i> _{CTX-M-2})	MultiCTXMGp2(F) MultiCTXMGp1-2(R)	CGTTAACGGCAGCATGAC CGATATCGTTGGTGGTRCCAT	5
<i>bla</i> _{CTX-M} group 9 (<i>bla</i> _{CTX-M-9} , <i>bla</i> _{CTX-M-14})	MultiCTXMGp9(F) MultiCTXMGp9(R)	TCAAGCCTGCCGATCTGGT TGATTCTCGCCGCTGAAG	5
<i>bla</i> _{CTX-M-13}	CTXM-13U(F) CTXM-13L(R)	GGTAAAAAATCACTGCGTC TTGGTGACGATTTTAGCCGC	28
<i>bla</i> _{CphA}	CPHA(F) CPHA(R)	GCTTAGAGCTCCTAAGGAGCAAGATGAAAGGTTGG GCATAGGTACCTTATGACTGGGGTGCGGCCTTG	17
<i>bla</i> _{MOX}	MOX(F) MOX(R)	CAACGACAATCCATCCTGTG CCTATGCTGGGGTTGGAGTA	This study

≥5-mm increase in zone diameters for ceftazidime/clavulanate, cefotaxime/clavulanate, or cefepime/clavulanate compared with those for ceftazidime, cefotaxime, or cefepime alone by CDT. Either a cefepime MIC reduction by ≥3 2-fold dilutions with clavulanate or a rounded phantom inhibition zone below the cefepime gradients with no ellipse visible around the cefepime end was also indicative of the presence of ESBL activity (30). The MICs of doxycycline, imipenem, ertapenem, piperacillin-tazobactam, cefotaxime, ceftazidime, and levofloxacin for ESBL-producing isolates were determined by Etest, and the results were interpreted following CLSI recommendations for *A. hydrophila* complex (4). Genetic relatedness of ESBL producers of the same species was examined by arbitrarily primed PCR with primers ERIC-1R (5'-ATGTAAGCTCCTGGGGATTAC-3') and ERIC-2R (5'-AGTAAGTACTGGGGTGAGC G-3') (25).

Detection of ESBL genes by PCR. For all phenotypically confirmed ESBL-producing *Aeromonas* isolates, PCR amplification, cloning, and DNA sequence analyses were conducted to determine the ESBL genotypes, including *bla*_{TEM}, *bla*_{PER}, *bla*_{CTX-M}, and *bla*_{SHV} genes, as well as the presence of the MBL *bla*_{CphA} gene and the AmpC β-lactamase *bla*_{MOX} gene by using previously described PCR primers and conditions (5, 17–19, 28, 34) (Table 1). The sequences of amplified DNA products were compared with the GenBank database to identify the types of β-lactamase genes.

Localization of the *bla*_{PER-3} gene. The location of the *bla*_{PER-3} gene was analyzed by using the S1 nuclease technique as described previously (22). Southern hybridization was performed with a digoxigenin (DIG)-labeled *bla*_{PER-3} gene specific probe, obtained by PCR amplification with the primers PER-A(F) and PER-R1 (5'-CTCGTCTCCCTGATACGCTTTC-3') using a DIG system (DIG DNA labeling and detection kit; Roche Diagnostics, Germany) according to the manufacturer's instructions (29).

Patients and literature review. Medical records of patients with ESBL-producing *Aeromonas* bacteremia were reviewed to collect the clinical data. The severity of acute illness at the onset of *Aeromonas* bacteremia was assessed within 1 day after admission by the Pittsburgh bacteremia score, a previously validated scoring system that was based on mental status, body temperature, blood pressure, requirement for mechanical ventilation, and recent cardiac arrest (2). Critical illness was defined as a Pittsburgh bacteremia score of ≥4 points. An English-language literature review was also conducted to find clinical cases of patients with ESBL-producing *Aeromonas* infections by querying the PubMed

database between April 1993 and April 2011 with the keywords “*Aeromonas*” and “extended-spectrum beta-lactamase”.

RESULTS

Isolates with the ESBL phenotype. During the study period, 156 consecutive nonduplicate *Aeromonas* blood isolates were collected. Fifty-five (35%) *Aeromonas* blood isolates with reduced susceptibility to expanded-spectrum cephalosporins were examined for the ESBL phenotype by the CDT and ESBL Etest. By CDT with ceftazidime, cefotaxime, and cefepime with and without clavulanate, a ≥5-mm increase in zone diameter was demonstrated in isolates of *A. hydrophila* A2-970201 (from patient A), *Aeromonas caviae* A2-970915 (patient B), *A. caviae* A2-971106 (patient C), and *A. caviae* A2-960104 (patient D). By ESBL Etest, the same four isolates demonstrated a MIC reduction by ≥3 2-fold dilutions with clavulanate. Overall, four isolates (2.6%) of 156 blood isolates, one *A. hydrophila* isolate and three *A. caviae* isolates, expressed ESBL phenotypes. Arbitrarily primed PCR of three *A. caviae* isolates showed three distinct gel patterns, suggestive of genetically unrelated strains. By Etest, all four ESBL producers were susceptible to imipenem, ertapenem, and levofloxacin and resistant to cefotaxime and ceftazidime. Two isolates were susceptible to cefepime, and three were susceptible to piperacillin-tazobactam. A profile of the antimicrobial susceptibility of these isolates is shown in Table 2.

Detection of ESBL genes. Two *A. caviae* isolates, A2-970915 and A2-971106, carried the *bla*_{PER} gene, which was 100% (927/927 nucleotides) identical to the complete sequence of the *A. caviae* ESBL *bla*_{PER-3} gene (GenBank accession number

TABLE 2. Profiles of antimicrobial susceptibility and the genes encoding ESBLs, MBL, and AmpC β -lactamases in ESBL-producing *Aeromonas* blood isolates

Parameter ^a	Result ^b for:				
	<i>A. hydrophila</i> (A2-970201)	<i>A. caviae</i> (A2-970915)	<i>A. caviae</i> (A2-971106)	<i>A. caviae</i> (A2-960104)	ESBL-producing <i>K. pneumoniae</i> (ATCC 700603) ^c
Disk diffusion, zone diam (mm)					
Cefotaxime/cefotaxime-clavulanate (Δ)	14/19 (5)	7/30 (23)	7/27 (20)	17/23 (6)	22/28 (6)
Ceftazidime/ceftazidime-clavulanate (Δ)	12/25 (13)	7/29 (22)	7/26 (19)	15/23 (8)	14/26 (12)
Cefepime/cefepime-clavulanate (Δ)	22/30 (8)	17/29 (12)	9/24 (15)	18/24 (6)	23/28 (5)
MIC by Etest (μ g/ml)					
Cefepime/cefepime-clavulanate	6/<0.064	>16/<0.064	>16/<0.064	6/0.125	<0.25/0.064
Cefepime	6 (S)	24 (I)	>256 (R)	6 (S)	1
Doxycycline	2	8	12	1.5	16
Imipenem	0.25 (S)	0.19 (S)	0.25 (S)	0.25 (S)	0.19
Ertapenem	0.032 (S)	0.012 (S)	0.047 (S)	0.25 (S)	0.032
Piperacillin-tazobactam	2 (S)	16 (S)	24 (I)	12 (S)	4
Cefotaxime	24 (R)	>256 (R)	>256 (R)	32 (R)	3
Ceftazidime	48 (R)	>256 (R)	>256 (R)	48 (R)	12
Levofloxacin	0.125 (S)	0.75 (S)	2 (S)	0.38 (S)	0.38
Detection of genes with specific primers					
ESBL genes					
<i>bla</i> _{PER}	—	<i>bla</i> _{PER-3}	<i>bla</i> _{PER-3}	—	—
<i>bla</i> _{TEM}	— ^d	—	—	—	—
<i>bla</i> _{SHV} ^c	—	—	—	—	—
<i>bla</i> _{CTX-M} ^e	—	—	—	—	—
MBL gene, <i>bla</i> _{CphA}	+	—	—	—	—
AmpC β -lactamase gene, <i>bla</i> _{MOX}	+	—	+	+	—

^a Δ , increase of zone diameter (mm).^b S, susceptible; I, intermediate; R, resistant.^c *Klebsiella pneumoniae* ATCC 700603 harboring the *bla*_{SHV-18} gene was used as a quality control strain.^d The *bla*_{TEM-116} gene, whose product is not an ESBL, was found.^e Two clinical strains of *K. pneumoniae* harboring the *bla*_{CTX-M-9} gene and the *bla*_{CTX-M-13} gene were used as quality control strains.

AY740681). Three isolates, A2-970201, A2-970915, and A2-971106, possessed the *bla*_{TEM-116} gene, with 99.2% to 100% identity to the *A. hydrophila* *bla*_{TEM-116} gene (GenBank accession no. FJ767900). None of the four ESBL-producing isolates had *bla*_{CTX-M} or *bla*_{SHV} genes. The genes responsible for the ESBL phenotype in isolates A2-970201 and A2-960104 were not identified.

Other genes encoding β -lactamases, including the *bla*_{CphA} gene in *A. hydrophila* A2-970201 and the *bla*_{MOX-6}-like gene (96% to 99% identical to *A. caviae* *bla*_{MOX-6}; GenBank accession no. GQ152601) in *A. hydrophila* A2-970201, *A. caviae* A2-971106, and *A. caviae* A2-960104, were found (Table 2).

Localization of the *bla*_{PER-3} gene. The result of Southern hybridization for determining the localization of *bla*_{PER-3} gene in two *A. caviae* isolates demonstrated that the *bla*_{PER-3} gene was localized on both chromosomes and plasmids of these two isolates (Fig. 1).

Patients and literature review. Clinical details of four patients with ESBL-producing *Aeromonas* bacteremia are shown in Table 3. They developed *Aeromonas* bacteremia at 5 to 19 days after admission, and three (patients B, C, and D) did not receive antibiotics in the preceding 1 month. Two patients presented with catheter-related phlebitis, and two patients with cancers of the digestive tract presented with primary bacteremia; the Pittsburgh bacteremia scores of all four patients were less than 4. Three patients (B, C, and D) had been empirically treated with penicillin derivatives or cephalosporin for 5 to 10

days, and all four survived for at least 2 weeks after the onset of bacteremia.

A literature review found four clinical cases, including a pediatric patient with *A. hydrophila* sepsis and pneumonia (26), an aged patient with necrotizing fasciitis caused by an *A. hydrophila* isolate harboring *bla*_{TEM-24} (7), an aged patient with intestinal ischemia with a fecal isolate of *A. caviae* harboring *bla*_{TEM-24} (16), and an aged patient with pneumonia caused by an *A. caviae* isolate harboring *bla*_{CTX-M-3} (35) (Table 3). Among three published cases with known clinical courses, the clinical conditions of two patients with pneumonia and one patient with necrotizing fasciitis deteriorated with initial non-carbapenem antimicrobial therapy.

DISCUSSION

To date, though there is no standard method for detection of ESBLs among aeromonads, most studies have adopted clavulanate-based synergy tests (7, 26), such as those recommended for phenotypic confirmation of ESBL-producing *Enterobacteriaceae* by CLSI (3). However, using expanded-spectrum cephalosporins as ESBL substrates, antagonism by clavulanate on ESBL may be masked by the coexistence of AmpC β -lactamases. *Aeromonas hydrophila* and *A. caviae* isolates have been reported to possess chromosomal AmpC β -lactamase genes (11), as noted in our three isolates. Therefore, the synergy test with cefepime, a novel cephalosporin not hydrolyzed by AmpC

FIG. 1. The localization of the *bla*_{PER-3} gene was determined by genomic mapping with S1 nuclease digestion by pulsed-field gel electrophoresis (A) and hybridizations with probes for the *bla*_{PER-3} gene (B). The genomic DNA of *A. caviae* A2-970915 and *A. caviae* A2-971106 was undigested (lanes 1 and 5) or was digested with S1 nuclease (lanes 2 and 4). Lane 3, Lambda ladder PFG marker (New England BioLabs). ←, linearized chromosomes; ★, plasmids.

β-lactamases, was also applied in this study. The results of different methods for detection of the ESBL phenotype—i.e., CDT using ceftazidime, cefotaxime, and cefepime with and without clavulanate and the cefepime-clavulanate ESBL Etest—were concordant, with all detecting the same four ESBL producers. However, the limited number of ESBL isolates in this study makes it uncertain that the performance of a ceftazidime- or cefotaxime-based combination disk method would be identical to that of the cefepime-based synergy test. Although more investigations are warranted, the cefepime-based synergy test may be helpful in screening the ESBL phenotype among aeromonads carrying AmpC β-lactamases.

Among four ESBL producers, two *A. caviae* isolates and one *A. hydrophila* isolate harbored the *bla*_{TEM-116} gene. Studies demonstrated that none of the environmental *Aeromonas* isolates (1) or clinical *Klebsiella pneumoniae* isolates carrying the *bla*_{TEM-116} gene expressed ESBL phenotypes (14), and therefore the *bla*_{TEM-116} gene is not thought to be associated with ESBL activity. The *bla*_{PER-3} gene was first identified in an *A. caviae* isolate in France and was found to be located within the class 1 integron In39 (31). Its product is the ESBL PER-3, and it is considered to be responsible for the ESBL activity in two *A. caviae* isolates in the present study. We further demonstrated that the *bla*_{PER-3} gene was located in both chromosome and plasmids of the two isolates. So far, this is the second report of *bla*_{PER-3} ESBL among aeromonads in the literature. The original acquisition of the *bla*_{PER-3} gene in *A. caviae* is

TABLE 3. Clinical features of patients with ESBL-producing *Aeromonas* infections in the present study and the literature

Patient	Species	Age/gender ^b	Coexisting condition	Specimen for culture	Clinical diagnosis	Acquisition of infections	Pittsburgh bacteremia score	Treatment ^c	Outcome at 2 weeks
Present study									
A (A2-970201)	<i>A. hydrophila</i>	70/F	Post-lumbar spine surgery for degenerative disease	Blood	Bacteremia, hand phlebitis	Hospital, probably phlebitis	1	NA, due to hospital transfer	Survived
B (A2-970915)	<i>A. caviae</i>	55/M	Tongue cancer with lung metastasis	Blood	Primary bacteremia	Hospital, unknown route of entry	1	TZP (d1-5), IPM (d6-10)	Survived
C (A2-971106)	<i>A. caviae</i>	52/M	Esophageal cancer	Blood	Primary bacteremia (polymicrobial ^d)	Hospital, unknown route of entry	1	AMC (d1-3), CTX (d4-7), ETP (d8-14)	Survived
D (A2-960104)	<i>A. caviae</i>	65/F	Aplastic anemia	Blood	Bacteremia, hand phlebitis	Hospital, probably phlebitis	1	FEP (d1-3), CAZ (d4-10), LVX (d4-17)	Survived
Reported in the literature									
2003 (16)	<i>A. caviae</i>	76/M	NA ^d	Feces	Intestinal ischemia	NA	NA	NA	NA
2004 (7)	<i>A. hydrophila</i>	87/F	Rheumatoid polyarthritis	Wound	Necrotizing fasciitis	NA	NA	Disease progressed with AMC, CRO, MET	NA
2005 (26)	<i>A. hydrophila</i>	3/M		Blood	Bacteremia, pneumonia	Community, ingestion of nonpotable water	NA	Disease progressed with CTX, OXA, VAN, SAM, and KLA, improved with IPM	Survived
2010 (35)	<i>A. caviae</i>	68/M	Lung cancer	Sputum	Pneumonia	Community	NA	CPZ, CIP (d1-5), IMP (d5-8)	Died

^a Abbreviations of antibiotics: IPM, imipenem; AMC, amoxicillin/clavulanate; CTX, cefotaxime; ETP, ertapenem; CAZ, ceftazidime; LVX, levofloxacin; TZP, piperacillin-tazobactam; CIP, ciprofloxacin; CPZ, cefoperazone; MET, metronidazole; FEP, cefepime; CRO, ceftriaxone; OXA, oxacillin; VAN, vancomycin; KLA, clarithromycin. NA, not available. d, days. D1 was defined as the day of bacteremia onset. "D1-5" represented the period from the day of bacteremia onset to the fifth day after bacteremia onset.

^b Age in years; F, female; M, male.

^c Co-pathogens isolated from blood: *Escherichia coli*, *Acinetobacter baumannii*, *Enterococcus faecium*, *Streptococcus anginosus*, coagulase-negative staphylococci.

^d NA, not available.

undefined, but the *bla*_{PER-3} gene is closely related to the *bla*_{PER-1} gene, with 99% identity (9). Although ESBLs of the PER type were not the most common ESBLs identified, the spread of *Enterobacteriaceae* carrying the PER-1 ESBL gene as a chromosomal insert has been recently reported in Europe (23). Emergence of *bla*_{PER-1} ESBLs was also noted among ESBL-producing *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates in Europe and Asia (6, 32). Further, the horizontal transfer of mobile genetic elements, such as plasmids and integrons, was found to be attributable to an increasing incidence of multidrug resistance among environmental *Aeromonas* isolates (10). Therefore, it is possible that the *bla*_{PER} gene was horizontally transferred by mobile genetic elements between aeromonads and coexistent waterborne bacteria in aquatic environments or between coexistent flora or pathogens in human beings.

As with *Aeromonas* infections described previously (11, 33), the clinical spectrum of patients with ESBL-producing *Aeromonas* infections in the present study and the literature included primary bacteremia, catheter-related infections, necrotizing fasciitis, pneumonia, and gastroenteritis. These infections occurred in both community and nosocomial settings. It is generally believed that patients acquire aeromonads from oral consumption of or direct mucocutaneous contact with contaminated water or seafood (11), whereas the risk factors associated with acquisition of ESBL-producing *Aeromonas* infections are not known due to their rarity. Prior administration of antibiotics is one of the well-known risk factors for infections caused by other ESBL-producing *Enterobacteriaceae* (20). However, most of our patients did not receive prior antibiotics, which made the association of prior exposure of antibiotics with development of ESBL-producing *Aeromonas* infections not evident. Further clinical investigations involving more patients are warranted to identify the risk factors for ESBL-producing *Aeromonas* infections, as well as surveillance of water from both hospitals and communities and suspicious foods to explore the possible sources of infections.

Although clinical studies have shown that survival was better with carbapenem treatment than with a cephalosporin among patients with bacteremia caused by ESBL-producing *K. pneumoniae* or *Enterobacter cloacae* (13, 21), the optimal antimicrobial therapy for ESBL-producing *Aeromonas* infections is not standardized. Among three published cases, the clinical conditions of two patients with pneumonia and one patient with necrotizing fasciitis deteriorated with initial noncarbapenem antimicrobial therapy (7, 26, 35). In contrast, our three patients, not critically ill as defined by their Pittsburgh bacteremia scores, remained stable with empirical noncarbapenem β -lactam agents, and all survived. The causes contributing to the poor outcome of previously published cases were not known. The difference in severity of illness at the time of antibiotic initiation, carriage and expression of toxins of each disease-causing aeromonad, or bacterial loads in clinical diseases might be the possible reasons. Theoretically carbapenems, not hydrolyzed by ESBLs, would work better than penicillins or cephalosporins against ESBL producers. However, antibacterial activity of carbapenems may be hampered by the production of *bla*_{CphA} MBLs in *A. hydrophila*, *A. veronii*, and *A. jandaei* isolates, which is not easily detected by *in vitro* susceptibility tests unless a large inoculum is used (27). It is too early

to conclude the appropriateness of antimicrobial therapy from the clinical experiences of limited cases. Perhaps to avoid the complexity of β -lactamase production in clinical *Aeromonas* isolates, a fluoroquinolone could be the reasonable choice for invasive *Aeromonas* infections.

In conclusion, of 156 *Aeromonas* blood isolates, 4 (2.6%) exhibited the ESBL phenotype, and two *A. caviae* isolates possessed the *bla*_{PER-3} genes, which were located in both chromosomes and plasmids. The complexity of β -lactamase production increases among clinical *Aeromonas* isolates, and clinical use of β -lactam agents for invasive *Aeromonas* infections should be undertaken with caution.

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