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

Chi-Jung Wu,1,2,5 Yin-Ching Chuang,6,7 Mei-Feng Lee,6 Chin-Chi Lee,1,2,4 Hsin-Chun Lee,2,4 Nan-Yao Lee,2,4 Chia-Ming Chang,2,4 Po-Lin Chen,1,2,4 Yu-Tzu Lin,5 Jing-Jou Yan,3 and Wen-Chien Ko2,4,*

Departments of Graduate Institute of Clinical Medicine,1 Internal Medicine,2 and Pathology,3 and Center for Infection Control,4 National Cheng Kung University Medical College and Hospital, Tainan, Taiwan; National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Taipei, Taiwan5; Department of Medical Research, Chi Mei Medical Center, Tainan, Taiwan; and Department of Internal Medicine, Chi Mei Medical Center, Liou Ying, Tainan, Taiwan7

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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 cephamycin-clavulanate ESBL Etest. The presence of ESBL-encoding genes, including blaTEM, blaPER, blaCTX-M, and blaSHV 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 blaPER-3, which was located in both chromosomes and plasmids, as demonstrated by Southern hybridization. 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 β metallo-β-lactamas (MBL) (11), whereas production of extended-spectrum β-lactamases (ESBL) has received little attention. ESBLs belong to the class B 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 blaTEM-24 from an aged patient with intestinal ischemia (16). Later on, ESBL-producing environmental isolates were reported, including isolates carrying blaper-1 from sludge in Switzerland (24), isolates carrying blaper-1, blaper-6, blashv-12, blaverb-1a, blaLA-2, or blages-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 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 Pasrpob-L (5′-GCAGTTAAAGAGTTCTTGGTT-3′) and Rpsrb-R (5′-GTTGCGATGTTGACCACT-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
amplified DNA products were compared with the GenBank database to identify PCR primers and conditions (5, 17–19, 28, 34) (Table 1). The sequences of \( \text{bla}_{/H9252} \) gene and the AmpC \( \text{Aeromonas}_{/H11032} \_G-3 \) (25) were examined by arbitrarily primed PCR with primers ERIC-1R (5\( /H11032 \)-CTCGTCTCCCTGATACGCTTTC-3\( /H11032 \)), ERIC-2R (5\( /H11032 \)-CTCGTCTCCCTGATACGCTTTC-3\( /H11032 \)), and PER-R1 (5\( /H11032 \)-CTCGTCTCCCTGATACGCTTTC-3\( /H11032 \)) using a DIG system (DIG DNA labeling and detection kit; Roche Diagnostics, Germany) according to the manufacturer’s instructions (29).

Localization of the \( \text{bla}_{\text{PER}-3} \) gene. The location of the \( \text{bla}_{\text{PER}-3} \) gene was analyzed by using the S1 nuclease technique as described previously (22). Southern hybridization was performed with a digoxigenin (DIG)-labeled \( \text{bla}_{\text{PER}-3} \) gene specific probe, obtained by PCR amplification with the primers PER-A(F) and PER-B(r) (5\( /H11032 \)-CTCGTCTCCCTGATACGCTTTC-3\( /H11032 \)) and PCR (5\( /H11032 \)-CTCGTCTCCCTGATACGCTTTC-3\( /H11032 \)) 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 \( \text{Aeromonas} \) bacteremia were reviewed to collect the clinical data. The severity of acute illness at the onset of \( \text{Aeromonas} \) 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 \( \text{Aeromonas} \) bacteremia by querying the PubMed database between April 1993 and April 2011 with the keywords “\text{Aeromonas}” and “extended-spectrum beta-lactamase”.

**RESULTS**

Isolates with the ESBL phenotype. During the study period, 156 consecutive nonduplicate \( \text{Aeromonas} \) blood isolates were collected. Fifty-five (35%) \( \text{Aeromonas} \) blood isolates with reduced susceptibility to expanded-spectrum cephalosporins were examined for the ESBL phenotype by the CDT and ESBL Etest. By CDT with ceftazidine, cefotaxime, and ceftazidime, and with and without clavulanate, a ≥5-mm increase in zone diameter was demonstrated in isolates of \( \text{Aeromonas} \) with ≥5-mm increase in zone diameter was demonstrated in isolates of \( \text{Aeromonas} \) with ESBL activity (30). The MICs of doxycycline, moxifloxacin, 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 reduced susceptibility to expanded-spectrum cephalosporins. Overall, four isolates (2.6%) of 156 blood isolates, one \( \text{A. hydrophila} \) isolate and three \( \text{A. caviae} \) isolates, expressed ESBL phenotypes. Arbitrarily primed PCR of three \( \text{A. caviae} \) 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 cepofixime, 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 \( \text{A. caviae} \) isolates, A2-970915 and A2-971106, carried the \( \text{bla}_{\text{PER}-3} \) gene, which was 100% (927/927 nucleotides) identical to the complete sequence of the \( \text{A. caviae} \) ESBL \( \text{bla}_{\text{PER}-3} \) gene (GenBank accession number
AY740681). Three isolates, A2-970201, A2-970915, and A2-971106, possessed the bla<sub>TEM-116</sub> gene, with 99.2% to 100% identity to the A. hydrophila bla<sub>TEM-116</sub> gene (GenBank accession no. FJ767900). None of the four ESBL-producing isolates had bla<sub>CTX-M</sub> or bla<sub>SHV</sub> 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<sub>cepA</sub> gene in A. hydrophila A2-970201 and the bla<sub>MOX</sub>-like gene (96% to 99% identical to A. caviae bla<sub>MOX</sub>-like gene) 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<sub>PER-3</sub> gene.** The result of Southern hybridization for determining the localization of bla<sub>PER-3</sub> gene in two A. caviae isolates demonstrated that the bla<sub>PER-3</sub> 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 cather-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<sub>TEM-24</sub> (7), an aged patient with intestinal ischemia with a fecal isolate of A. caviae harboring bla<sub>TEM-24</sub> (16), and an aged patient with pneumonia caused by an A. caviae isolate harboring bla<sub>CTX-M-3</sub> (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-carbenapen 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-lactamas-
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 \( \text{bla}_{\text{TEM-116}} \) gene. Studies demonstrated that none of the environmental Aeromonas isolates (1) or clinical Klebsiella pneumoniae isolates carrying the \( \text{bla}_{\text{TEM-116}} \) gene expressed ESBL phenotypes (14), and therefore the \( \text{bla}_{\text{TEM-116}} \) gene is not thought to be associated with ESBL activity. The \( \text{bla}_{\text{PER-3}} \) 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 \( \text{bla}_{\text{PER-3}} \) gene was located in both chromosome and plasmids of the two isolates. So far, this is the second report of \( \text{bla}_{\text{PER-3}} \) ESBL among aeromonads in the literature. The original acquisition of the \( \text{bla}_{\text{PER-3}} \) gene in A. caviae is

![FIG. 1. The localization of the \( \text{bla}_{\text{PER-3}} \) gene was determined by genomic mapping with S1 nuclease digestion by pulsed-field gel electrophoresis (A) and hybridizations with probes for the \( \text{bla}_{\text{PER-3}} \) 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.](https://aac.asm.org/article-pdf/58/12/5816/29793362/5816.pdf)

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

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

**Abbreviations of antibiotics:** IPM, imipenem; AMC, amoxicillin-clavulanate; CTX, cefotaxime; ETP, etepenem; CIP, ciprofloxacin; OXA, oxacillin; KLA, clarithromycin; NA, not available; d, days; D1, day of bacteremia onset. **a** Represented the period from the day of bacteremia onset to the fifth day after bacteremia onset. **b** Co-pathogens isolated from blood: Escherichia coli, Acinetobacter baumannii, Enterococcus faecalis, Stenotrophomonas maltophilia.
undefined, but the bla<sub>PER-3</sub> gene is closely related to the bla<sub>PER-1</sub> 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<sub>PER-3</sub> 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<sub>PER</sub> 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 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 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 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 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 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 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 one patient with necrotizing fasciitis deteriorated with initial noncarbapenem antimicrobial therapy (7, 26, 35).

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<sub>PER-3</sub> genes, which were located in both chromosomales 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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**REFERENCES**

4. CLSI. 2010. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline, 2nd ed. CLSI document M45-A2. Clinical and Laboratory Standards Institute, Wayne, PA.


