Complexity of β-lactamases among clinical
Aeromonas isolates and its clinical implications

Po-Lin Chen a,b, Wen-Chien Ko b,c, Chi-Jung Wu a,b,d,*

a Graduate Institute of Clinical Medicine, National Cheng Kung University, College of Medicine, Tainan, Taiwan
b Department of Internal Medicine, National Cheng Kung University, College of Medicine and Hospital, Tainan, Taiwan
c Center for Infection Control, National Cheng Kung University, College of Medicine and Hospital, Tainan, Taiwan
d National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Tainan, Taiwan

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Introduction

Aeromonas species, an aquatic Gram-negative bacilli, is distributed globally and grows ubiquitously in the natural environment. The role of aeromonads as human pathogens in natural disasters was reinforced by the observation that they ranked as the single most common pathogen identified in tsunami survivors with skin or soft tissue infections in Thailand in 2004.1 Besides skin or soft tissue infections, aeromonads can cause a variety of human diseases in the community or hospital settings, such as gastroenteritis, septicemia, abdominal/peritoneal sepsis, hepatobiliary tract infections, and catheter-related infections.2,3 Both immunocompromised and immunocompetent individuals would acquire infections due to aeromonads, mostly from oral consumption of or direct mucocutaneous contact with...
**β-Lactamases in Aeromonas species**

Contaminated water or foods, A. hydrophila, A. caviae, and A. veronii bv. sobria are the three principal *Aeromonas* species found to be associated with human diseases.

Aeromonads can produce various β-lactamases which confer resistance to a broad spectrum of β-lactams, and therefore in vitro susceptibility testing must be used to guide antimicrobial therapy. Three major classes of chromosomally mediated β-lactamases—Ambler class B, C, and D β-lactamases—have been recognized in *Aeromonas* species. Metallo-β-lactamases (MBLs), AmpC β-lactamases, and penicillinases are the principal class B, C, and D β-lactamases harbored in aeromonads, respectively. Another important class of β-lactamases addressed is class A extended-spectrum β-lactamases (ESBLs), which have been increasingly reported in both clinical and environmental aeromonads. However, conventional in vitro susceptibility tests would sometimes fail to detect these β-lactamases and hence pose a therapeutic challenge. An understanding of the types of β-lactamases harbored in clinically relevant *Aeromonas* species is important, and would be a guide for antimicrobial therapy. In this article, the drug susceptibility profiles of major β-lactamases found in *Aeromonas* species and clinical implications of the complexity of β-lactamases are discussed.

**General susceptibility profiles**

Much of the susceptibility information on aeromonads is based solely upon the most clinically relevant *Aeromonas* species, i.e., *A. hydrophila*, *A. caviae*, and *A. veronii* bv. *sobria*. It is not clear whether those profiles can be extrapolated to other less frequently encountered taxa causing illness. Currently, consensus guidelines for the antimicrobial susceptibility testing of *Aeromonas* spp., including the members of *A. hydrophila* complex, *A. caviae* complex, and *A. veronii* complex, have been published by the Clinical and Laboratory Standards Institute (CLSI), providing information and interpretative criteria for broth microdilution and disk diffusion testing. Of the three major *Aeromonas* species, some species-specific susceptibility variations have been found, as demonstrated by the summary of previous reports (Table 1) in which the methods of susceptibility testing and interpretative criteria varied with studies. Generally, carbapenem resistance was occasionally found in *A. hydrophila* and *A. veronii* isolates, while *A. caviae* isolates were carbapenem-susceptible. *A. hydrophila* and *A. caviae* isolates were cephalothin-resistant and more frequently displayed resistance to cefuroxime, ceftriaxone, or cefotaxime than did *A. veronii* isolates, which were cephalothin-susceptible. Most of the *A. hydrophila*, *A. caviae*, and *A. veronii* isolates displayed resistance to ampicillin and amoxicillin. Of interest, *A. enteropelogenes* (formerly *A. tructi* or *A. trota*) is always susceptible to ampicillin and is the only known *Aeromonas* species that produces only one β-lactamase—molecular class C β-lactamase. In a study by Fosse et al., a series of 417 wild-type *Aeromonas* strains, biochemical identification, and susceptibility testing with 11 β-lactams by the disk-diffusion method revealed five predominant phenotypes: *A. hydrophila* complex/class B, C, and D β-lactamases; *A. caviae* complex/class C and D β-lactamases; *A. veronii* complex/class B and D β-lactamases; *A. schubertii* spp./class D β-lactamase; *A. trota* spp./class C β-lactamase. These observations are in agreement with previous observations and suggest that the distribution of three chromosomally mediated class B, C, and D β-lactamases among aeromonads is species-specific, which could be a useful scheme for taxonomic differentiation and a guide of antimicrobial therapy. Although susceptibility variations between species have been found in selected studies, these results should be considered preliminary at present. For examples, many *A. veronii* bv. *sobria* isolates were hybridized-positive for a class C cephalosporinase gene, cepS. However, *A. veronii* bv. *sobria* 163a, the strain in that CepS cephalosporinase that was originally identified, is actually a strain of *A. hydrophila*, with a 100% identity to the 16S rRNA and rpoB sequences of *A. hydrophila* ATCC7966.

### Table 1: Summary of in vitro drug susceptibilities of clinical isolates of three common *Aeromonas* species from three studies conducted by Janda et al., Wu et al., and Lamy et al.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>A. hydrophila % susceptible</th>
<th>A. caviae % susceptible</th>
<th>A. veronii bv. sobria % susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin or amoxicillin</td>
<td>0/0</td>
<td>13/0.6/7</td>
<td>9/7/3.7</td>
</tr>
<tr>
<td>Ampicillin/sulbactam or Amoxicillin/clavulanate</td>
<td>75/0/15.4</td>
<td>80/0/40</td>
<td>100/7/11.1</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>—/38/88.4</td>
<td>—/59/100</td>
<td>—/82/100</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>—/90/88.4</td>
<td>—/89/100</td>
<td>—/92/100</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>21/5/11.5</td>
<td>13/0/20</td>
<td>82/93/100</td>
</tr>
<tr>
<td>Cefotaxime or ceftriaxone</td>
<td>92/90/—</td>
<td>72/74/—</td>
<td>100/100/—</td>
</tr>
<tr>
<td>Cefepime</td>
<td>—/98/100</td>
<td>—/96/100</td>
<td>—/100/96.3</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>—/98/—</td>
<td>—/89/—</td>
<td>—/100/—</td>
</tr>
<tr>
<td>Imipenem</td>
<td>—/73/84.6</td>
<td>—/96/93.3</td>
<td>—/64/37</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>—/92/100</td>
<td>—/93/100</td>
<td>—/96/100</td>
</tr>
<tr>
<td>Amikacin</td>
<td>—/95/100</td>
<td>—/100/100</td>
<td>—/100/96.3</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>100/85/84.6</td>
<td>100/85/93.3</td>
<td>100/89/100</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>100/22/80.8</td>
<td>100/15/80</td>
<td>100/64/100</td>
</tr>
</tbody>
</table>

— = data not available.
type strain. Therefore, in the modern era of taxonomy based on molecular identification, the knowledge of the distribution of chromosomally mediated \( \beta \)-lactamases among different Aeromonas genomospecies should be reevaluated. The \( \beta \)-lactamases intrinsically carried by Aeromonas species based on current knowledge are summarized in the Table 2, and the MBLs and AmpC \( \beta \)-lactamases are discussed in detail in the following sections.

**Metallo-\( \beta \)-lactamases**

The most commonly mentioned MBL in Aeromonas species is CphA, which has a very specific substrate profile, being active on penems and carbapenems only, but not on penicillins and cephalosporins. Other MBLs among aeromonads were identified, including ImiS, IMP-19, and VIM. The distribution of cphA among aeromonads is species-specific, mainly found in A. hydrophila, A. veronii, and A. jandaei, but not in A. caviae. We further noticed that the isolates of A. aquariorum, a recently described species that was initially isolated from ornamental fish aquaria in 2008, also carried cpha. A. aquariorum has been associated with a wide spectrum of human diseases, such as septicaemia, skin soft tissue infections, and gastroenteritis, and was widely distributed in clinical and environmental specimens.

**AmpC \( \beta \)-lactamases**

In general, AmpC \( \beta \)-lactamases can hydrolyze many \( \beta \)-lactam antibiotics, including cephamycins and third-generation cephalosporins, and are resistant to \( \beta \)-lactam inhibitors, such as clavulanic acid, tazobactam, and sulbactam. However, fourth-generation cephalosporins are not recognized by AmpC \( \beta \)-lactamases. Aeromonas AmpC \( \beta \)-lactamases ever reported included Cep$$_S$$ from A. veronii bv. sobria 163a (later reported to be A. hydrophila strain), A. jandaei, CepH from A. hydrophila, CAV-1 from A. caviae, MOX-4 from A. caviae, and recently described TRU-1 from A. enteropelogene.

| Table 2 | Species-specific distribution of three chromosome-mediated \( \beta \)-lactamases and reported extended-spectrum \( \beta \)-lactamase (ESBL) producing isolates among different Aeromonas species |
|------------------|------------------|------------------|------------------|------------------|------------------|
| **Chromosomally mediated** | **Acquired** |
| MBL | Class B | Class C | Class D | penicillinase | Class A |
| A. hydrophila | + | + | + | + | Ever reported |
| A. caviae | - | + | + | + | Ever reported |
| A. veronii bv. sobria | + | +/- | + | + | Ever reported |
| A. enteropelogene (formerly A. trota) | - | + | - | - | Not reported |

+ = present; - = absent; +/- = isolates with and without indicated \( \beta \)-lactamase were reported.
about $10^7$ to $10^9$, suggesting that a point mutation was responsible for the generation of mutants.14

The production of AmpC β-lactamase mediating resistance to third-generation cephalosporins poses a therapeutic challenge in managing Aeromonas infections. For example, the use of cefoperazone in a patient with A. caviae in the respiratory tract selected a mutant that constitutively produced β-lactamase.38 Reported was the emergence of a ceftaxime-resistant mutant from a wild A. hydrophila strain under ceftaxime treatment in a burn patient.39 The observations highlighted the concern of monotherapy with a third-generation cephalosporin for infections due to AmpC gene-carrying aeromonads. Currently, there is no ready-to-use method recommended by the CLSI for screening AmpC β-lactamases. Therefore, it is prudent to consider A. hydrophila, A. caviae, and A. enteropelogen as isolates as AmpC gene-carrying species, and monotherapy with cephalosporins other than fourth-generation cephalosporins for invasive infections due to the above Aeromonas species should be undertaken with caution.

### Extended-spectrum β-lactamases

ESBLs, belonging to the class A β-lactamases according to Ambler’s classification, confer resistance to all penicillins, cephalosporins, and monobactams, but not to cephamycins or carbapenems, and are inactivated by β-lactamase inhibitors.40 ESBL-producing aeromonads have been increasingly reported in recent years. Clinical cases included a pediatric patient with A. hydrophila sepsis in 2005,41 two isolates with blaTEM-24 gene from diarrheal feces and wound in 2003 and 2004, respectively,42,43 and an aged patient with pneumonia caused by A. caviae with blaCTX-3 gene in 2010.37 Environmental ESBL-producing isolates included several isolates with blaPER-1, blaPER-6, blaSHV-12, blaVEB-1a, blaTLA-2, or blaGES-7 from the Seine River,7 and the isolates form an urban river in China.44 In one study investigating 156 Aeromonas blood isolates in southern Taiwan, four (2.6%) exhibited the ESBL phenotype, and two A. caviae isolates possessed blaPER-3 gene located in both chromosomes and plasmids.6 Unlike chromosomally encoded MBL and AmpC β-lactamases, the acquisition of ESBL genes in aeromonads may result from horizontal gene transfer by mobile genetic elements between aeromonads and coexistent bacteria in aquatic microenvironments.6

To screen for ESBL production among Aeromonas isolates, nonsusceptibility of third-generation cephalosporins is probably the laboratory clue. Previous studies adopted the clavulanate-based synergy test as the ESBL phenotype among aeromonads;41,43 as those recommended for phenotypic confirmation of ESBL-producing Enterobacteriaceae by CLSI.29 However, the ESBL phenotype may be difficult to detect using third-generation cephalosporins as ESBL substrates among AmpC-β-lactamase-producing bacteria.45 It is possible that antagonism by clavulanate on ESBL producers may be masked by the coexistence of AmpC β-lactamases in A. hydrophila and A. caviae strains. Therefore, ceftazime-based tests, such as ceftazime–clavulanate combination disk and ceftazime–clavulanate ESBL E-test are suggested for the screening of ESBL-producing among aeromonads.6

Prior administration of antibiotics is a well-known risk factor for infections caused by other community-onset ESBL-producing Enterobacteriaceae bacteremia and urinary tract infections.46–48 However, the association of prior exposure of antibiotics with development of ESBL-producing Aeromonas infections not evident in the previous study.6 The optimal therapy for ESBL-producing Aeromonas infections also remains undefined due to the rarity of clinical reports.6 With initial non-carbapenem antimicrobial therapy for two patients with pneumonia and one with necrotizing fasciitis failed,37,41,43 whereas was effective for three with bacteremia.6 The differences in the severity of illness at the time of antibiotic initiation and in the toxin expression from aeromonads and bacterial loads might have contributed to the different outcomes in these cases. 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 CphA MBL in A. hydrophila, A. veronii, and A. jandaei isolates.

Induction potential or the selection of resistant mutants among AmpC-carrying bacteria does not necessarily correlate with clinical risk, because a rapid bactericidal action will kill the organisms before a sufficient quantity of enzymes has been induced.49 However, it would be a concern in infected patients with a heavy load of aeromonads in subinhibitory antibiotic concentrations due to ischemic microenvironement. Such clinical settings as nectrotizing fasciitis, burn wounds, or abscesses formation, would favor the emergence of resistant mutants.39 In infections with high inocula, clinical use of β-lactams, which are hydrolyzed by AmpC β-lactamases or MBLs, should be pursued with caution. Therefore, according to Fosses’s scheme based on the distribution of β-lactamases, treatment failure is possible in severe infections due to A. hydrophila with third-generation cephalosporins or carbapenem monotherapy, or those due to A. caviae with third-generation cephalosporin monotherapy, or those due to A. veronii with carbapenem monotherapy. For severe infection due to AmpC β-lactamase- and MBL-carrying aeromonads, fourth-generation cephalosporin would be an effective β-lactam agent. However, if the causative isolates turn out to be ESBL producers, the drug of choice will be limited. In summary, given the current susceptibility data, the induction potential of multiple intrinsic β-lactamases and the possibility of the acquisition of ESBL genes, empirical therapy for severe Aeromonas infections would consist of a broad-spectrum cephalosporin in combination with gentamicin or amikacin,11 or one of the fluoroquinolones to avoid the complexity of β-lactamase production. Later, definite therapy can be adjusted according to the susceptibility profile and accurate species identification. More susceptibility tests, such as cephamycin–clavulanate synergy tests and the MHT, should be performed in selected Aeromonas isolates and clinical conditions.6,8

Species-specific distribution of chromosomally mediated AmpC β-lactamases and MBL, and the acquisition of ESBL among aeromonads raise the therapeutic concern of broad-spectrum cephalosporins as monotherapy for severe
**Aeromonas** infections. Due to limited data, the optimal antibiotic for such infections is not conclusive. Moreover, recent advances in **Aeromonas** taxonomy have led to the reclassification of aeromonads with the emergence of new species. More clinical studies to reveal intrinsic β-lactamase profile and therapeutic outcome in the cases of infections due to recently recognized **Aeromonas** genomicspecies are needed.

**Conflicts of interest**

All contributing authors declare no conflicts of interest.

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