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REVIEW ARTICLE

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

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Aeromonas species, aquatic Gram-negative bacilli, distributed globally and ubiquitously in the natural environment, may be implicated in a variety of human diseases. They 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. However, conventional *in vitro* susceptibility tests may sometimes fail to detect these β-lactamases, and hence raise a therapeutic challenge. In this review article, two chromosomally mediated β-lactamases (i.e., AmpC β-lactamases and metallo-β-lactamases) and acquired extended-spectrum β-lactamases in aeromonads are reviewed, and the clinical implications of the complexity of β-lactamases are discussed.

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

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contaminated water or foods.[2] *A. hydrophila*, *A. caviae*, and *A. veronii* bv. *sobria* are the three principal *Aeromonas* species found to be associated with human diseases.[2]

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.[4] Three major classes of chromosomally mediated β-lactamases—Ambler class B, C, and D β-lactamases—have been recognized in *Aeromonas* species.[2,5] Metallo-β-lactamases (MBLs), AmpC β-lactamases, and penicillinases are the principal class B, C, and D β-lactamases harbored in aeromonads, respectively.[2] 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.[6,7] However, conventional *in vitro* susceptibility tests would sometimes fail to detect these β-lactamases,[6,8,9] 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*.[2] It is not clear whether those profiles can be extrapolated to other less frequently encountered taxa causing illness.[2] 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.[10] Of the three major *Aeromonas* species, some species-specific susceptibility variations have been found, as demonstrated by the summary of three previous reports (Table 1),[3,11,12] 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.[13] In a study by Fosse et al,[5] 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*.[14] 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,[12] Wu et al,[3] and Lamy et al[11]

Drugs	% of susceptible isolates in studies by Janda/Wu/Lamy		
	A. hydrophila	*A. caviae*	*A. veronii* bv. *sobria*
Ampicillin or amoxicillin	0/0/0	13/0/6.7	9/7/3.7
Ampicillin/sulbactam or Amoxicillin/clavulanate	75/0/15.4	80/0/40	100/7/11.1
Piperacillin	—/38/88.4	—/59/100	—/82/100
Piperacillin/tazobactam	—/90/88.4	—/89/100	—/92/100
Cephalothin	21/5/11.5	13/0/20	82/93/100
Cefuroxime	92/90/—	72/74/—	100/100/—
Cefotaxime or ceftriaxone	92/90/92.3	93/74/100	100/100/100
Cefepime	—/98/100	—/96/100	—/100/96.3
Aztreonam	—/98/—	—/89/—	—/100/—
Imipenem	—/73/84.6	—/96/93.3	—/64/37
Gentamicin	—/92/100	—/93/100	—/96/100
Amikacin	—/95/100	—/100/100	—/100/96.3
Ciprofloxacin	100/85/84.6	100/85/93.3	100/89/100
Co-trimoxazole	100/22/80.8	100/15/80	100/64/100

— = data not available.

type strain.[15] Therefore, in the modern era of taxonomy based on molecular identification, the knowledge of the distribution of chromosomally mediated β-lactamases among different *Aeromonas* genomospecies should be reevaluated. The β-lactamases intrinsically carried by *Aeromonas* species based on current knowledge are summarized in the Table 2, and the MBLs and AmpC β-lactamases are discussed in detail in the following sections.

Metallo-β-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.[16] Other MBLs among aeromonads were identified, including ImiS,[17] IMP-19,[18] and VIM.[19] The distribution of *cphA* among aeromonads is species-specific, mainly found in *A. hydrophila*, *A. veronii*, and *A. jandaei*, but not in *A. caviae*.[20,21] We further noticed that the isolates of *A. aquariorum*, a recently described species that was initially isolated from ornamental fish aquaria in 2008,[22] also carried *cphA*.[8] *A. aquariorum* has been associated with a wide spectrum of human diseases, such as septicemia, skin soft tissue infections, and gastroenteritis,[23] and was widely distributed in clinical and environmental specimens.[24,25] *A. aquariorum* and *A. hydrophila* subsp. *dhakensis* are closely related species based on a phylogenetic analysis of the *gyrB*, *rpoD*, and *rpoB* genes,[26,27] and an identical phenotypic profile of the inability to produce acid from L-arabinose.[28] Till now, the biological and clinical characteristics of *A. aquariorum* have not been well studied, and further studies are warranted.

The CphA MBL production is not easily detected by conventional *in vitro* susceptibility tests with EDTA-based combination disk diffusion, E-test, or agar dilution methods with standard inocula, unless large inocula is adopted.[8,9] In a study testing 34 *cphA*-carrying *Aeromonas* blood isolates, all but one (33, 97%) isolates were susceptible to imipenem tested by the disk diffusion, E-test, and agar dilution (10^4 CFU spot inocula) with standard inocula, while 33 (97%) isolates had imipenem MICs of $\geq$16 μg/ml, higher than the susceptible breakpoint (4 μg/ml), by the agar dilution test using large inocula (10^7 CFU). This inoculum effect on imipenem MIC was not observed in aeromonads without *cphA*.[8] The modified Hodge test (MHT), recommended for the detection of carbapenemases in Enterobacteriaceae by the CLSI,[29] is another method to detect CphA carbapenemases, since 97% of *cphA*-carrying *Aeromonas* blood isolates were MHT-positive.[8]

The clinical relevance of CphA MBL in *Aeromonas* species remained obscure. Theoretically, carbapenem monotherapy would fail to inhibit MBL-producing aeromonads in infectious diseases with high bacterial burdens, such as peritonitis/abdominal sepsis or necrotizing fasciitis. Moreover, the production of CphA would increase in the presence of a β-lactamase inducer, such as benzylpenicillin or imipenem.[16] The emergence of imipenem-resistant *Aeromonas* isolates during carbapenem treatment or antecedent amoxicillin–clavulanate treatment were reported.[8,30,31] These observations highlight the controversy of carbapenem therapy for infectious diseases caused by *cphA*-carrying *Aeromonas* isolates. Therefore, it is advisable to perform the susceptibility test with a large inoculum or the MHT before considering a carbapenem-based chemotherapy for *Aeromonas* infections.[8,9]

AmpC β-lactamases

In general, AmpC β-lactamases can hydrolyze many β-lactam antibiotics, including cephamycins and third-generation cephalosporins, and are resistant to β-lactamase inhibitors, such as clavulanic acid, tazobactam, and sulbactam.[32] However, fourth-generation cephalosporins are not recognized by AmpC β-lactamases. *Aeromonas* AmpC β-lactamases ever reported included CepS from *A. veronii* bv. *sobria* 163a (later reported to be *A. hydrophila* strain),[15,33] AsbA1 from *A. jandaei*,[34] CepH from *A. hydrophila*,[35] CAV-1 from *A. caviae*,[36] MOX-4 from *A. caviae*,[37] and recently described TRU-1 from *A. enteropelogene*.[13] These accumulated findings are in accordance with Fosse's observation that AmpC β-lactamases were distributed among *A. hydrophila*, *A. caviae*, and *A. enteropelogene* isolates.

As other bacteria carrying AmpC genes, aeromonads with AmpC genes do not always express AmpC β-lactamases and may display cefotaxime susceptibility. The mechanisms involved in the expression of AmpC β-lactamases include inducible β-lactamase production in the presence of suitable inducers (cefoxitin or imipenem)[33] or development of depressed mutation which leads to a constitutive high-level production of β-lactamases.[14] The frequency of *in vitro* production of resistant mutants in *Aeromonas* isolates was

Table 2 Species-specific distribution of three chromosome-mediated β-lactamases and reported extended-spectrum β-lactamase (ESBL) producing isolates among different *Aeromonas* species

	Chromosomally mediated			Acquired
	Class B MBL	Class C AmpC	Class D penicillinase	Class A ESBL
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 β-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 cephalosporin 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 cefotaxime-resistant mutant from a wild *A. hydrophila* strain under cefotaxime 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. enteropelogene* 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 bla_{TEM-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 bla_{CTx-3} gene in 2010.[37] Environmental ESBL-producing isolates included several isolates with bla_{PER-1}, bla_{PER-6}, bla_{SHV-12}, bla_{VEB-1a}, bla_{TLA-2}, or bla_{GES-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 bla_{PER-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, cefepime-based tests, such as cefepime—clavulanate combination disk and cefepime—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 microenvironment. Such clinical settings as necrotizing 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 Fosse'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 EBSL 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 cefepime—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* genomospecies are needed.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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