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Short Communication

The Rapid Induction of Carbapenem-Resistance in an *Aeromonas dhakensis* Blood Isolate

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SUMMARY: Meropenem-susceptible and -resistant *Aeromonas dhakensis* isolates from blood cultures of a fatal case of septicemia were analyzed. The two isolates were homologous and gene expression of metallo-β-lactamase in the resistant strain was upregulated. Physicians should be aware of the possibility of the induction of carbapenem-resistance, following the use of carbapenems in the treatment of *Aeromonas* infection.

In an earlier study, conducted at a hospital in Japan, we observed that *Aeromonas* septicemia occurred frequently in hospitalized patients with underlying diseases, including malignancy (1). In this report, a fatal case of *Aeromonas* septicemia in a girl with neutropenia is described. Two strains of *Aeromonas veronii* biovar sobria were isolated from blood cultures obtained before and after treatment with carbapenem. The strain isolated at the onset of sepsis was sensitive to carbapenem; however, the strain isolated after treatment, was resistant. *Aeromonas* species have chromosomal genes, such as *cphA* and *imiS*, encoding metallo-β-carbapenemase, which are not commonly expressed, but inducible. These genes have similar sequences and the enzymes can hydrolyze carbapenems efficiently (2,3). To understand the mechanism underlying the development of resistance to carbapenem, we analyzed the genetic homology between the blood isolates and the gene expression levels of the chromosomal metallo-β-lactamase.

The two *Aeromonas* strains (meropenem-susceptible, MIC $\leq 0.5\,\mu$g/mL; meropenem-resistant, MIC $\geq 32\,\mu$g/mL) isolated from the patient were cultured on sheep blood agar overnight. Bacterial DNA was extracted using a QIAamp DNA blood and tissue mini-kit (Qiagen, Hilden, Germany). Homology analysis was performed using Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR, with the primer ERIC1 (5′-GTGAATCCCCAGGAGCTTACAT-3′) and the primer ERIC2 (5′-AAGTAAGTGACTGGGGTGAGC G-3′) (4,5), to confirm whether the 2 isolates were the same strain. A similar pattern was observed in the 2 isolates, using gel electrophoresis with 1.6% Seakem GTG agarose (LONZA Japan, Tokyo, Japan).

We evaluated the gene expression levels of metallo-β-lactamase. Each strain was suspended at about 5×10^7 cfu/mL in 3 mL of Luria-Bertani broth and incubated with shaking at 250 rpm and a temperature of 37°C for 6 hours; the cell pellet was suspended using ISOGEN (Nippon Gene, Tokyo, Japan). After RNA extraction using the RNA Mini Kit (Life technologies Japan, Tokyo, Japan), cDNA was obtained by reverse-transcription, using SuperScript III reverse transcriptase (Life technologies Japan) and a random hexamer. Gene expression levels of *cphA/imiS* (sense primer, 5′-GATC TGATGAAGAGCGACTGG-3′; antisense primer, 5′-CAGCTTCTCCTTGAGGATGC-3′) and *16SrRNA* (sense primer, 5′-AGTTGGAAACGACTGCT-3′; antisense primer, 5′-CCAGCAGATATTAGCTACTG-3′) were quantified using the LightCycler480 (Roche Diagnostics, Tokyo, Japan). The gene expression level of *cphA/imiS* was corrected using the expression level of *16SrRNA* (6). Compared with the meropenem-susceptible strain, the expression of metallo-β-lactamase in the meropenem-resistant strain was upregulated by 13.5-fold ($n = 3$).

Aeromonas infections are typically observed in subtropical zones. While they are not common in temperate zones, the rare cases in these regions are often invasive and life threatening. Immunocompromised conditions can be important factors of *Aeromonas* septicemia and hepatobiliary infections (1,7). Fluoroquinolones or β-lactams are generally used for the treatment of *Aeromonas* infections; however, clinical concern regarding drug resistance to these antibiotics is not widely recognized. The induction of β-lactamases can lead to treatment failure because *Aeromonas* species have chromosomal β-lactamases (i.e., AmpC β-lactamase and metallo-β-lactamase).

Carbapenem-resistant *Aeromonas* are rare. To our knowledge, a fatal case of acquired carbapenem-resistant *Aeromonas* septicemia has not yet been reported. A case report on hepatobiliary tract infection with carbapenem-resistant *A. veronii* biovar sobria, due to the induction of chromosomal carbapenemase, was first reported in 2009 (6). Verona integrin-encoded metallo-β-lactamase-producing *Aeromonas* was also isolated in a surveillance study conducted in Israel (8). In our

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Table 1. Carbapenem-resistant *Aeromonas* infections (n = 4), 2000–2013, Nagasaki University Hospital

Age (yr)	Sex	Diagnosis	Isolate	Antimicrobial agent, previous use[1)]	Therapeutic antimicrobial agent	MIC (μg/mL)			Outcome	Reference
						Meropenem	Imipenem	Ciprofloxacin		
15	F	Sepsis	*A. veronii* biovar sobria[2)]	Meropenem Vancomycin Micafungin	Ciprofloxacin	≥32	≥32	≤0.5	Died	(1) and the present study
79	F	Sepsis	*A. hydrophila*	Biapenem Meropenem Penicillin Vancomycin	— (end-of-life care)	≤0.5	8	≤0.5	Died	(1)
82	M	Enterogastritis	*A. hydrophila*	Imipenem Clindamycin	Ciprofloxacin	>32	>32	≤0.5	Cured	The present study
47	M	Enterogastritis	*A. caviae*	Unknown	Ciprofloxacin +Metronidazole	16	8	≤0.5	Cured	The present study

[1)]: Antimicrobial use during 30 days before the isolation of carbapenem-resistant *Aeromonas*.
[2)]: The isolate was re-identified as *A. dhakensis* by *rpoD* sequencing.
F, female; M, male.

study, the meropenem-susceptible and -resistant strains were homologous but the metallo-β-lactamase expression in the meropenem-resistant strain was upregulated. Using the microbiology laboratory database (2000–2013) in our hospital, we identified 4 patients with carbapenem-resistant (MIC ≥8 μg/mL) *Aeromonas* infections (Table 1). Three of these patients had a previous history of carbapenem administration. Two patients with gastroenteritis were cured using fluoroquinolone treatment. These findings, suggest that previous use of carbapenem can be a risk factor for carbapenem-resistant *Aeromonas*, and that fluoroquinolone can be an alternative antibiotic.

Aeromonas clinical isolates generally show good susceptibility to carbapenems but can develop carbapenem-resistance under certain conditions, such as antimicrobial pressure. The rapid induction of carbapenem-resistance is a clinically important finding, which may indicate that the acceleration of gene expression is easily induced by carbapenem exposure. Another possibility, linked to the host immune condition, is that neutropenia may contribute to a reduced clearance of bacteria and that the remaining bacteria may grow with gene expression for meropenem resistance. In addition, the large bacterial load in the infected sites may be involved in the induction of carbapenem-resistance because metallo-β-lactamase can be induced under high inoculum of bacteria in vitro (9).

The isolates in this study were re-identified by *rpoD* sequencing as *A. dhakensis* (formerly *A. aquariorum*) (10), because the phenotypical differentiation of the genus *Aeromonas* or its species has been recognized as difficult. *A. dhakensis* can be a causative bacterium of *Aeromonas* septicemia. *A. dhakensis* is often misidentified as *A. hydrophila* or *A. veronii* biovar sobria in the routine phenotype-based test, however, *A. dhakensis* can be more virulent than *A. hydrophila* (10,11). Therefore, it is important to be aware of high virulence and drug-resistance, when treating patients with severe *Aeromonas* infection.

Aeromonas infection is typically observed in an unsanitary water environment; however, sporadic cases have also occurred in developed nations. Physicians should focus on the possibility of drug-resistant infection among patients undergoing advanced medical treatment because these patients are often immunocompromised. Physicians treating patients with infectious diseases should be aware of the induction of carbapenem resistance in *Aeromonas* species because appropriate antimicrobial treatment is critically important for these patients.

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Conflict of interest None to declare.

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