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).

We evaluated the gene expression levels of metallo-β-lactamase. Each strain was suspended at about 5 × 10⁸ 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'–GATCTGATGAAGAGCGACTGG-3'; antisense primer, 5’–CAGCCTTCTCCTGAGGATGC-3') and 16SrRNA (sense primer, 5’–AGTTGAAACGACTGCT-3'; antisense primer, 5’–CCACGAGATATTAGCCTACTG-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).

Carbenapenem-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
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 septicaemia. 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.

REFERENCES


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

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Isolate</th>
<th>Antimicrobial agent, previous use</th>
<th>Therapeutic antimicrobial agent</th>
<th>MIC (μg/mL)</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meropenem</td>
<td>Imipenem</td>
<td>Ciprofloxacin</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>Sepsis</td>
<td>A. veronii biovar sobria</td>
<td>Meropenem Vancomycin Micafungin Biapenem Meropenem Penicillin</td>
<td>Ciprofloxacin</td>
<td>≥32</td>
<td>≥32</td>
<td>≤0.5</td>
</tr>
<tr>
<td>79</td>
<td>F</td>
<td>Sepsis</td>
<td>A. hydrophila</td>
<td>Vancomycin Micafungin Biapenem (end-of-life care)</td>
<td>—</td>
<td>≤0.5</td>
<td>8</td>
<td>≤0.5</td>
</tr>
<tr>
<td>82</td>
<td>M</td>
<td>Enterogastritis</td>
<td>A. hydrophila</td>
<td>Vancomycin Micafungin Biapenem Meropenem Moxalactam</td>
<td>Ciprofloxacin</td>
<td>&gt; 32</td>
<td>&gt; 32</td>
<td>≤0.5</td>
</tr>
<tr>
<td>47</td>
<td>M</td>
<td>Enterogastritis</td>
<td>A. caviae</td>
<td>Unknown</td>
<td>Ciprofloxacin + Metronidazole</td>
<td>16</td>
<td>8</td>
<td>≤0.5</td>
</tr>
</tbody>
</table>

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.
Carbapenem-Resistant *Aeromonas* septicemia
