Behaviour against β-lactams in Aeromonas spp. isolated from extraintestinal infections

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The role of Aeromonas spp. in a variety of human illnesses has been well documented during the last decades. The spectrum of infectious diseases caused by Aeromonas species includes gastrointestinal and extraintestinal infections. Aeromonas gastrointestinal infections are generally self-limiting and antibiotic therapy is unnecessary. However, for gastrointestinal infections in immunocompromised patients and extraintestinal infections, it is necessary to implement an appropriate antibiotic treatment. Since the early 1990’s, an increase in antimicrobial resistance of Aeromonas spp. has been observed worldwide. ß-lactam antibiotics are currently the most commonly used antibiotics in developing areas because of their cost, safety and selectivity in the treatment of infections caused by bacterial pathogens. Our results support the hypotheses that it is possible that the therapeutic failures of ß-lactams in the treatment of infections caused by Aeromonas spp. could be associated with the inoculum effect, the expression of inducible ß-lactamases and the emergence of derepressed mutants in vivo.

Keywords Aeromonas; β-lactams; extraintestinal infections

1. Introduction

Aeromonas species are recognized as etiological agents of a wide variety of human illnesses, including intestinal and extraintestinal diseases and syndromes, ranging from systemic and local infections in both immunocompetent and immunocompromised hosts [1].

Gastrointestinal infections with Aeromonas are generally self-limiting and antibiotic therapy is either unnecessary or at least remains controversial. However, antimicrobial therapy should be initiated in patients with extraintestinal diseases or risk factors for extraintestinal spread of infection [2].

Since the early 1990’s an increase in antimicrobial resistance of Aeromonas spp. has been observed worldwide. In particular, resistance to ß-lactams represents a serious problem for the therapeutic management of Aeromonas infections. It has been reported that the bacterial inoculum and the production of multiple ß–lactamases in Aeromonas spp. are critical for the manifestation of a resistance phenotype [3-4].

2. The inoculum size effect

Antibiotic susceptibility assays constitute a fundamental part of microbiological practices; however, in vitro procedures do not often reflect what happens in vivo.

One of the factors that can generate discrepancies in this aspect is the size of the bacterial inoculum, since the bacterial concentrations in the site of infection can be much higher than that of the inoculum used in the laboratory assays. This allows the bacteria to reduce the activity of some antimicrobial agents and cause faults in the implemented treatment.

It has been observed that an increase in inoculum size can be correlated with an increase in ß-lactam minimum inhibitory concentrations (MICs) and a decrease in efficacy in vivo. Factors that could contribute to these effects are the number, type, and amount of ß-lactamases, the outer membrane permeability, the efflux, the number and susceptibility of penicillin-binding protein targets, the phase of growth, and even species dependence [5-6].

In our experience, when using an inoculum size of 10⁴ cfu/ml (normal inoculum), the results of ß-lactam susceptibility of 21 extraintestinal clinical isolates of Aeromonas spp. were comparable to those observed by others [7-9]. All the Aeromonas strains were uniformly resistant to ampicillin and sensitive to third- and fourth-generation cephalosporins and carbapenems. The isolates showed a high level of resistance to cephalotin (85.7%) and ampicillin-sulbactam (81.0%). When the bacterial inoculum was increased to 10⁸ cfu/ml (high inoculum), all the ß-lactams studied were affected (Table 1). Results showed a strong inoculum size effect on carbapenem MIC, as also observed by Rossolini et al. [3] and Martín Talavera et al. [10]. In higher inoculum tests, imipenem was dramatically affected, with susceptibility decreasing from 100% in standard inoculum tests to 28.5% of isolates in the higher inoculum tests. The MIC of cefepime showed the smallest inoculum effect of all the compounds tested.
**Table 1** Inoculum size effect on the MIC and susceptibility to β-lactams of 21 *Aeromonas* spp. recovered from extraintestinal infections.

<table>
<thead>
<tr>
<th>β-lactams</th>
<th>Inoculum size (cfu/ml)</th>
<th>MIC (µg/ml)</th>
<th>Susceptibility (%)</th>
<th>Nº of the strains affected by the inoculum size/ total of the strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>AMN</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>32-256</td>
<td>128</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>32-1024</td>
<td>1024</td>
<td>0</td>
</tr>
<tr>
<td>AMS</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>4/2-128/64</td>
<td>64/32</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>16/8-128/64</td>
<td>128/64</td>
<td>0</td>
</tr>
<tr>
<td>CEPH</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>8-512</td>
<td>256</td>
<td>14.3</td>
</tr>
<tr>
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<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>16- ≥1024</td>
<td>≥1024</td>
<td>0</td>
</tr>
<tr>
<td>CAZ</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.015-2</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.125-8</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>CTX</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.015-0.5</td>
<td>0.25</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.06-8</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>FEP</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.03-0.5</td>
<td>0.125</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.06-2</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>IMP</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.06-4</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.5-64</td>
<td>32</td>
<td>28.5</td>
</tr>
</tbody>
</table>


cfu: colony-forming units

### 3. The production of β-lactamases

It has been reported that *Aeromonas* spp. produce several chromosomally mediated inducible β-lactamases [11]. Three principal classes of β–lactamases are recognized in *Aeromonas* species: a class C cephalosporinase (AmpC β-lactamase), a class D penicillinase, and a class B metallo-β-lactamase [11-14], which are under a single mechanism of coordinate expression [15]. The expression of one or more inducible β-lactamases is considered the main resistance mechanisms for most aeromonads [1].

Like in many *Enterobacteriaceae* [16], AmpC expression is low in *Aeromonas* spp. but inducible in response to β-lactam exposure.

In a previous study [17], we have demonstrated the presence of inducible β–lactamases in more than 50% of the strains by phenotypic methods using impenem and cefoxitin as inducers. The most important clinical aspect of the production of inducible β-lactamases is the emergence of resistant mutants [18-20]. For microorganisms with the potential for high-level AmpC β-lactamase production by mutation, the development of resistance upon therapy is a concern, as reported in various studies [16].

In the presence of suboptimal or low antibiotic concentrations of an inducer, producers of inducible β-lactamases might be genetically derepressed giving them survival advantage to their selection and clinical treatment failure [20].

It is important to distinguish induction from derepression. Induction is a temporary phenomenon, whereas genetic derepression is permanent, where the derepressed mutant strains produce high levels of enzymes constitutively.

In another study [21], we have observed that *in vitro* and under antibiotic pressure with cefotaxime, resistant variants of *Aeromonas* spp. showing a cross-resistant behavior with other β-lactam antibiotics including imipenem can be isolated.

As expressed by Livermore [22], the weak inducer activity of cefotaxime often helps to maintain its activity against inducible cells. However, it has been reported that there are cases in which stably-derepressed mutants overrun inducible populations of bacteria in patients undergoing therapy with β-lactamase-labile weak inducers.

The MICs of mutant strains of β-lactam included in our study [21] were higher than the MICs obtained in tests with the wild-type strains (Table 2). Among the cephalosporins tested, we observed that the *in vitro* activity of ceftazidime was the least affected.

Not all the mutants increased the MIC of imipenem enough to be considered as resistant ones.

It has been reported that this may be due to the low level of enzyme produced coupled with the rapid permeation of imipenem into the gram-negative cell, which may help to minimize the protective effect of β-lactamase [23].

In *Aeromonas* spp., production of class B metallo-β-lactamase (MBL) is not able to confer a carbapenem-resistant phenotype in wild type isolates and it could not be readily recognized in conventional *in vitro* susceptibility testing or phenotypic test. We observed this situation in our study: in the wild-type isolates we did not detect the presence of MBL by phenotypic methods [21] whereas in the derepressed mutants we demonstrated MBL production (Table 2).
### Table 2
Detection of metallo-β-lactamase production by phenotypic methods and MICs of extraintestinal *Aeromonas* wild-type isolates and their derepressed mutants.

<table>
<thead>
<tr>
<th>Isolates (n=6)</th>
<th>MIC_{90} (µg/ml)</th>
<th>Phenotypic methods:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMN</td>
<td>AMS</td>
</tr>
<tr>
<td>Wild-type</td>
<td>64</td>
<td>32/16</td>
</tr>
<tr>
<td>Derepressed mutants</td>
<td>≥512</td>
<td>128/64</td>
</tr>
</tbody>
</table>

| WIld-type | (-) | (-) | (-) |
| Derepressed mutants | (+) | (+) | (+) |


### 4. Conclusion
In view of our results, we consider that the inoculum size effect should be borne in mind if imipenem is selected as a therapeutic alternative to treat *Aeromonas* infections. Thus, the use of cefotaxime to treat *Aeromonas* infections may carry the risk of emergence of resistance similar to that seen with other genera producing inducible β-lactamases.

Our results suggest that it might be prudent for clinicians who are considering the use of cefotaxime or imipenem to treat cases of serious infections caused by *Aeromonas* spp. to be prepared to monitor patients closely for signs of treatment failure.

### References


