β-Lactam Resistance of Motile Aeromonas Isolates from Clinical and Environmental Sources

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The MICs of various β-lactams for 182 isolates of Aeromonas species, i.e., A. hydrophila (n = 101), A. sobria (n = 69), and A. caviae (n = 12), from clinical and environmental sources were determined by an agar dilution technique. All strains were resistant to ampicillin and susceptible to aztreonam. A. sobria and A. caviae demonstrated lower resistance rates than A. hydrophila. Penicillin-hydrolyzing β-lactamases were detected in all strains.

Species of motile Aeromonas, an inhabitant of water environments, are increasingly being reported as important pathogens causing gastroenteritis and severe extraintestinal diseases, such as septicemia and peritonitis, in immunocompromised hosts as well as healthy individuals (2, 4, 5, 7, 8). The role of antibiotic therapy in the management of Aeromonas infections has not yet been defined (14, 15), although the antibiotic resistance in motile Aeromonas spp. is an important problem for therapy directed to these organisms. Most clinical isolates of motile Aeromonas spp. resistant to β-lactams are resistant to penicillins (16). However, some interspecies differences in susceptibility were observed with cefalosporins (9, 15).

New β-lactams that possess greater activity against gram-negative and gram-positive bacteria have been developed. In particular, penems and carbapenems such as imipenem inhibit a broad range of microorganisms, including motile Aeromonas spp. (3, 11). However, some investigators recently reported the appearance of imipenem-resistant isolates of Aeromonas spp. caused by inducible β-lactamases that are active against carbapenems (1, 6, 16). The present study examined the species-associated β-lactam susceptibility patterns and β-lactamase production of clinical and environmental isolates of A. hydrophila, A. sobria, and A. caviae.

**Organisms.** A total of 182 Aeromonas isolates consisting of 109 strains from the feces of patients with diarrhea acquired in Japan, Southeast Asia, and the People’s Republic of China and 73 strains from environmental sources such as food, fresh water, and seawater collected in the Tokyo metropolitan and Kanagawa Prefecture areas were examined in the present study.

The species of the Aeromonas isolates were determined by the criteria of Popoff (13) and Janda et al. (8).

**Susceptibility testing.** MICs were determined by an agar dilution technique with sensitivity disk agar (Eiken Chemical Co.) containing graded concentrations of antibiotics and an inoculum of approximately 10⁴ cells per spot, which was applied with a multi-inoculator. Plates were incubated at 35°C for 18 h. The following standard antibiotic powders were tested: ampicillin and imipenem (Banyu Pharmaceutical Co.), ticarcillin and ticarcillin-clavulanate (SmithKline Beecham Pharmaceutical Co.), piperacillin (Sankyo Co.), cefaloridine and moxalactam (Shionogi Pharmaceutical Co.), cefoperazone (Toyama Chemical Co.), cefoxitin (Daiichi Pharmaceutical Co.), cefuroxime (Nippon Glaxo Co.), cefotaxime (Chugai Pharmaceutical Co.), ceftriaxone (Nippon Roche Co.), and aztreonam (Eisai Co.). Breakpoint concentrations for susceptibility and resistance were based on the criteria of the National Committee for Clinical Laboratory Standards (10).

**β-Lactamase testing.** β-Lactamase production was determined by the benzylpenicillin substrate method with benzylpenicillin disks (Beta-Lactamase Detection Paper; Oxoid, Basingstoke, United Kingdom) and was also determined by the chromogenic cephalosporin substrate method (12) with nitrocefin disks (Cefinase disk; BBL Microbiology Systems, Cockeysville, Md.).

The results of the susceptibility tests are shown in Table 1. All strains were uniformly resistant to ampicillin and were susceptible to aztreonam. Piperacillin showed variable activity against isolates of each species and was more active than the other penicillins tested. The ticarcillin-clavulanate combination was active, and the MIC of this combination for 90% of the strains was fourfold lower than that of ticarcillin alone. Of three species tested, the MICs of cefalosporins, monobactam, and carbapenem for 90% of A. hydrophila isolates tested were two- to eightfold higher than those for A. sobria and A. caviae isolates. Eight of the 101 A. hydrophila strains (8%) and 2 of the 69 A. sobria strains (3%) were resistant to imipenem. No differences in susceptibility were observed between environmental and clinical isolates of A. hydrophila, A. sobria, and A. caviae (data not shown).

Results of analysis of β-lactam cross-resistance are given in Table 2. Three A. hydrophila strains resistant to cefotaxime, ceftriaxone, and moxalactam were uniformly resistant to the other cefalosporins tested. Twenty-five percent or more of imipenem-resistant A. hydrophila strains were resistant to cefotaxime, ceftriaxone, cefoperazone, and moxalactam, while the two imipenem-resistant strains of A. sobria were susceptible to these four cefalosporins.

These differences in the resistance patterns against newer β-lactams suggest that imipenem resistance in A. hydrophila and A. sobria is associated with some distinct β-lactamase activities (16). In our results of β-lactamase testing, penicillin-hydrolyzing β-lactamase production was observed in all strains of A. hydrophila, A. sobria, and A. caviae. Recently, we found and analyzed some inducible or stably derepressed imipenem-hydrolyzing β-lactamases in imipenem-resistant isolates of A. hydrophila and A. sobria used in the present study (data not shown and unpublished data).

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### TABLE 1. Antimicrobial activities of β-lactams against motile *Aeromonas* isolates

| Species (no. of isolates) and antimicrobial agent | MIC (µg/ml)* | % Resistantb | Breakpoint concn (µg/ml)c |
|-------------------------------------------------|--------------|--------------|--------------------------|
|                                                 | Range        | 50%          | 90%                      |                          |
| *A. hydrophila* (n = 101)                       |              |              |                          |                          |
| Ampicillin                                      | 32->256      | >256         | >256                     | 100                      | 32                        |
| Piperacillin                                    | 2->256       | 64           | 128                      | 32                       | 128                       |
| Ticarcillin                                     | 32->256      | 128          | 128                      | 59                       | 128                       |
| Ticarcillin-clavulanate (2 µg)                  | 2-128        | 4            | 32                       | 4                        | 128                       |
| Cefaloridine                                    | 8->256       | 64           | >256                     | 84                       | 32                        |
| Cefoxitin                                       | 0.12-128     | 8            | 64                       | 28                       | 32                        |
| Cefuroxime                                      | 0.12-128     | 2            | 16                       | 11                       | 32                        |
| Cefotaxime                                      | 0.12-128     | 2            | 8                        | 3                        | 64                        |
| Ceftriaxone                                     | ≤0.06-64     | 1            | 8                        | 3                        | 64                        |
| Cefoperazone                                    | 0.12-128     | 2            | 16                       | 5                        | 64                        |
| Moxalactam                                      | ≤0.06-64     | 1            | 8                        | 3                        | 64                        |
| Aztreonam                                       | ≤0.06-16     | ≤0.06        | 0.5                      | 0                        | 32                        |
| Imipenem                                        | ≤0.06-64     | 0.5          | 4                        | 8                        | 16                        |
| *A. sobria* (n = 69)                            |              |              |                          |                          |                           |
| Ampicillin                                      | 32->256      | 128          | >256                     | 100                      | 32                        |
| Piperacillin                                    | 1-128        | 16           | 128                      | 16                       | 128                       |
| Ticarcillin                                     | 16->256      | 64           | 128                      | 36                       | 128                       |
| Ticarcillin-clavulanate (2 µg)                  | 1-128        | 1            | 32                       | 3                        | 128                       |
| Cefaloridine                                    | 0.5-128      | 2            | 64                       | 21                       | 32                        |
| Cefoxitin                                       | ≤0.06-32     | 1            | 8                        | 4                        | 32                        |
| Cefuroxime                                      | ≤0.06-32     | 0.5          | 4                        | 0                        | 64                        |
| Cefotaxime                                      | ≤0.06-32     | 1            | 4                        | 0                        | 64                        |
| Ceftriaxone                                     | ≤0.06-32     | 1            | 4                        | 0                        | 64                        |
| Cefoperazone                                    | ≤0.06-32     | 1            | 4                        | 0                        | 64                        |
| Moxalactam                                      | ≤0.06-32     | 1            | 4                        | 0                        | 64                        |
| Aztreonam                                       | ≤0.06-1      | ≤0.06        | 0.12                     | 0                        | 32                        |
| Imipenem                                        | ≤0.06-64     | 0.5          | 2                        | 3                        | 16                        |
| *A. caviae* (n = 12)                            |              |              |                          |                          |                           |
| Ampicillin                                      | 64->256      | >256         | >256                     | 100                      | 32                        |
| Piperacillin                                    | 16->256      | 32           | 128                      | 25                       | 128                       |
| Ticarcillin                                     | 32->256      | 128          | 128                      | 67                       | 128                       |
| Ticarcillin-clavulanate (2 µg)                  | 4-64         | 16           | 32                       | 0                        | 128                       |
| Cefaloridine                                    | 8-128        | 32           | 128                      | 83                       | 32                        |
| Cefoxitin                                       | 1-32         | 4            | 16                       | 8                        | 32                        |
| Cefuroxime                                      | 0.5-8        | 2            | 4                        | 0                        | 32                        |
| Cefotaxime                                      | 0.25-8       | 1            | 4                        | 0                        | 64                        |
| Ceftriaxone                                     | 0.12-4       | 1            | 4                        | 0                        | 64                        |
| Cefoperazone                                    | 0.5-8        | 1            | 8                        | 0                        | 64                        |
| Moxalactam                                      | 0.25-4       | 1            | 4                        | 0                        | 64                        |
| Aztreonam                                       | ≤0.06-0.1    | ≤0.06        | 0.12                     | 0                        | 32                        |
| Imipenem                                        | ≤0.06-0.5    | 0.25         | 0.5                      | 0                        | 16                        |

*50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

Resistance was determined at the breakpoint concentration.

On the basis of the criteria of the National Committee for Clinical Laboratory Standards (10).
Resistance to various antibiotics has previously been observed, especially in *A. hydrophila* isolates in comparison with other species of *Aeromonas* (9). This tendency of β-lactam resistance was also observed in our study.

These data suggest that the species-associated β-lactam resistance of motile *Aeromonas* spp. has important implications in the selection of definitive species-oriented therapy of infectious diseases caused by motile *Aeromonas* spp.

Our results demonstrate that aztreonam has good in vitro activity against motile *Aeromonas* spp.

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### TABLE 2. Rates of cross-resistance

| Species and subset of isolates resistant to β-lactam (no. of isolates) | % of isolates cross-resistant to β-lactam* |
|---------------------------------------------------------------|------------------------------------------|
| **A. hydrophila**                                              | **AMP** **PIP** **TIC** **T+C** **CER** **CFX** **CXM** **CTX** **CTR** **CPZ** **MOX** **AZT** **IPM** |
| AMP (100)                                                     | 100 32 59 4 84 28 11 3 3 5 3 0 8               |
| PIP (32)                                                      | 100 100 84 9 94 41 22 3 3 19 0 0 21           |
| TIC (60)                                                      | 100 43 100 7 92 35 18 5 5 8 5 0 10            |
| CER (85)                                                      | 100 34 67 5 100 28 13 4 4 13 8 0 9            |
| CFX (28)                                                     | 100 46 75 4 100 100 53 11 11 18 11 0 25        |
| CXM (11)                                                      | 100 73 100 0 100 100 100 27 27 91 27 0 45      |
| CPZ (11)                                                      | 100 55 100 0 100 100 91 27 27 100 27 0 46      |
| IPM (8)                                                       | 100 88 63 0 100 88 63 25 25 63 25 0 100        |
| **A. sobria**                                                 | **AMP** **PIP** **TIC** **T+C** **CER** **CFX** **CXM** **CTX** **CTR** **CPZ** **MOX** **AZT** **IPM** |
| AMP (69)                                                      | 100 16 36 3 22 4 1 0 0 0 0 0 3                |
| PIP (11)                                                      | 100 100 100 0 82 18 18 0 0 0 0 0 0             |
| TIC (25)                                                      | 100 44 100 8 40 12 4 0 0 0 0 0 0               |
| CER (15)                                                      | 100 33 67 0 100 20 7 0 0 0 0 0 0               |
| **A. caviae**                                                 | **AMP** **PIP** **TIC** **T+C** **CER** **CFX** **CXM** **CTX** **CTR** **CPZ** **MOX** **AZT** **IPM** |
| AMP (12)                                                      | 100 25 67 0 84 8 0 0 0 0 0 0 0 0               |
| TIC (8)                                                       | 100 38 100 0 100 0 0 0 0 0 0 0 0               |
| CER (10)                                                      | 100 30 90 0 100 10 0 0 0 0 0 0 0               |

*Abbreviations for β-lactams: AMP, ampicillin; PIP, piperacillin; TIC, ticarcillin; T+C, ticarcillin-clavulanate; CER, cefaloridine; CFX, cefoxitin; CXM, cefuroxime; CTX, cefotaxime; CTR, ceftriaxone; CPZ, cefoperazone; MOX, moxalactam; AZT, aztreonam; IPM, imipenem.*