ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1994, p. 353–355
0066-4804/94/$04.00+0

Vol. 38, No. 2

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

KOJI MORITA, NOBORU WATANABE, SATOSHI KURATA, AND MASATO KANAMORI*

Department of Microbiology, Kyorin University School of Health Sciences, 476, Miyashita, Hachioji, Tokyo 192, Japan

Received 10 March 1993/Returned for modification 15 March 1993/Accepted 8 November 1993

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 cephalosporins (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 10^4 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 cephalosporins, 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 cephalosporins 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 cephalosporins.

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).

* Corresponding author.

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

Species (no. of isolates) and antimicrobial agent	MIC (μg/ml)[a]			% Resistant[b]	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	1	8	1	32
Cefotaxime	≦0.06–32	1	4	0	64
Ceftriaxone	≦0.06–32	0.5	2	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

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.
[b] Resistance was determined at the breakpoint concentration.
[c] On the basis of the criteria of the National Committee for Clinical Laboratory Standards (10).

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]												
	AMP	PIP	TIC	T+C	CER	CFX	CXM	CTX	CTR	CPZ	MOX	AZT	IPM
A. hydrophila													
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 (69)	100	16	36	3	22	4	1	0	0	0	0	0	3
PIP (11)	100	100	100	0	82	18	0	0	0	0	0	0	0
TIC (25)	100	44	100	8	40	12	4	0	0	0	0	0	8
CER (15)	100	33	67	0	100	20	7	0	0	0	0	0	7
A. caviae													
AMP (12)	100	25	67	0	84	8	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

[a] 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.

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.

We thank Y. Kudoh, S. Horiuchi, M. Fukuyama, and N. Hiruta for kindly supplying the strains used in the study.

This work was supported by funds provided for microbiological research by medical research funds from Kyorin University.

REFERENCES

1. **Bakken, J. S., C. C. Sanders, R. B. Clark, and M. Hori.** 1988. β-Lactam resistance in *Aeromonas* spp. caused by inducible β-lactamases active against penicillins, cephalosporins, and carbapenems. Antimicrob. Agents Chemother. **32:**1314–1319.
2. **Freij, B. J.** 1987. Extraintestinal *Aeromonas* and *Plesiomonas* infections of humans. Experientia **43:**359–360.
3. **Ganguly, A. K., A. Afsono, V. M. Girjavallabhan, and S. McCombie.** 1985. Synthesis and preliminary in vitro profile of SCH 34343—a new penem antibacterial agent. J. Antimicrob. Chemother. **15:**1–4.
4. **George, W. L., M. M. Nakata, J. Thompson, and M. L. White.** 1985. *Aeromonas*-related diarrhea in adults. Arch. Intern. Med. **145:**2207–2211.
5. **Gracey, M., V. Burke, and J. Robinson.** 1982. *Aeromonas*-associated gastroenteritis. Lancet **ii:**1304–1306.
6. **Iaconis, J. P., and C. C. Sanders.** 1990. Purification and characterization of inducible β-lactamases in *Aeromonas* spp. Antimicrob. Agents Chemother. **34:**44–51.
7. **Janda, J. M., E. J. Bottone, and M. Reitano.** 1983. *Aeromonas* species in clinical microbiology: significance, epidemiology, and speciation. Diagn. Microbiol. Infect. Dis. **1:**221–228.
8. **Janda, J. M., M. Reitano, and E. J. Bottone.** 1984. Biotyping of *Aeromonas* isolates as a correlate to delineating a species-associated disease spectrum. J. Clin. Microbiol. **19:**44–47.
9. **Motyl, M. R., G. McKinley, and J. M. Janda.** 1985. In vitro susceptibilities of *Aeromonas hydrophila*, *Aeromonas sobria*, and *Aeromonas caviae* to 22 antimicrobial agents. Antimicrob. Agents Chemother. **28:**151–153.
10. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. **Neu, H. C., N. Chin, and N. M. Neu.** 1987. In vitro activity and β-lactamase stability of a new penem, CGP 31608. Antimicrob. Agents Chemother. **31:**558–569.
12. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shindler.** 1970. Novel method for detection of beta-lactamases by using chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1:**283–288.
13. **Popoff, M.** 1984. Genus III. *Aeromonas* Kluyver and Van Niel 1936, 398, p. 545–548. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
14. **Richardson, C. J. L., J. O. Robinson, L. B. Wagener, and V. Burke.** 1982. In-vitro susceptibility of *Aeromonas* spp. to antimicrobial agents. J. Antimicrob. Chemother. **9:**267–274.
15. **San Joaquin, V. H., R. K. Scribner, D. A. Pickett, and D. F. Welch.** 1986. Antimicrobial susceptibility of *Aeromonas* species isolated from patients with diarrhea. Antimicrob. Agents Chemother. **30:**794–795.
16. **Shannon, K., A. King, and I. Phillips.** 1986. β-Lactamases with high activity against imipenem and Sch 34343 from *Aeromonas hydrophila*. J. Med. Microbiol. **35:**363–367.