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β-Lactam Resistance in *Aeromonas* spp. Caused by Inducible β-Lactamases Active against Penicillins, Cephalosporins, and Carbapenems

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Use of cefoperazone in a patient with *Aeromonas caviae* in the respiratory tract selected a mutant that constitutively produced β-lactamase. This mutant, in contrast to its parental strain with an inducible β-lactamase, showed enhanced resistance to newer cephalosporins and aztreonam. This observation suggested that species of *Aeromonas*, like those of other genera with inducible β-lactamases, may pose therapeutic problems associated with the rapid development of multiple β-lactam resistance. Thus, a study was designed to identify the β-lactamases in 12 strains representing four species of *Aeromonas* and assess their role in drug resistance. Eleven strains possessed inducible β-lactamases. One strain showed no detectable activity. An analysis of substrate and inhibitor profiles, isoelectric points, and β-lactam susceptibility patterns revealed the presence of at least four distinguishable inducible β-lactamases. These enzymes were involved in the resistance of strains within the genus to penicillins, cephalosporins, aztreonam, and imipenem but not cefoxitin. Unlike most other organisms with inducible β-lactamases, all four strains of *A. caviae*, one of four strains of *A. sobria*, and one of three strains of *A. hydrophila* possessed two distinct inducible β-lactamases. Furthermore, substrate and inhibitor profiles revealed that many of these *Aeromonas* β-lactamases were distinct from inducible enzymes that have been characterized in other genera of gram-negative bacteria.

Although little is known about *Aeromonas* β-lactamases, a recent report by Shannon et al. (25) suggested that individual *Aeromonas* strains may possess multiple distinct β-lactamases. These β-lactamases appear to be inducible (12, 24, 25, 31) and have various isoelectric points (25). The substrate profiles for the *Aeromonas* β-lactamases reported by various investigators have differed somewhat with hydrolytic activity demonstrated against penicillins (24, 31), early cephalosporins (12, 24, 25, 31), and carbapenems (23). One possible explanation for the conflicting results concerning β-lactamase activity in *Aeromonas* spp. may be related to the failure of previous studies to distinguish between the various species of this genus (12, 24, 25, 30).

Recent studies by Popoff et al. (14, 15) and Hickman-Brenner and co-workers (9) suggest that at least four distinct species should be recognized within the genus *Aeromonas*. The four species include *Aeromonas caviae*, *A. hydrophila*, *A. sobria*, and *A. veronii*. Collectively these species are often referred to as the *A. hydrophila* complex. All four species have been implicated as human pathogens causing a variety of clinical infections. These include gastroenteritis, cellulitis, and bacteremia (3, 6, 7, 27). Resistance to penicillins and the early cephalosporins has usually been observed with all four species characterized to date (9, 13, 16, 17).

Recently we had an opportunity to examine the mechanism(s) responsible for the development of resistance to expanded spectrum cephalosporins in a clinical isolate of *A. caviae*. Due to the limited literature on *Aeromonas* β-lactamases, the study was expanded to include representative strains of all four *Aeromonas* species in an attempt to answer the following questions: (i) are there differences in susceptibility to β-lactam antibiotics among the various species, and (ii) what types of β-lactamases are associated with these species?

MATERIALS AND METHODS

Bacteria. Eleven *Aeromonas* strains were kindly provided by Michael Janda, Microbial Diseases Laboratory, Berkeley, Calif. These included one isolate recovered from a guinea pig (*A. caviae* ATCC 15468), one environmental isolate (*A. sobria* AER83), and nine isolates recovered from specimens obtained from humans. Two clinical isolates of a single strain of *A. caviae* (DLS4 and DLS5) were provided by Dennis L. Stevens, Veterans Administration Medical Center, Boise, Idaho. Species identification of each strain was performed as recommended by Janda et al. (10) and Hickman-Brenner et al. (9). The species of one strain, *A. hydrophila* AER84, could not be determined precisely, but it was grouped with the species as it had originally been identified (5). Each strain was kept frozen in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.)-horse serum (1:1, vol/vol) at −70°C until used. The following species were studied: *A. caviae* (four strains), *A. hydrophila* (three strains), *A. sobria* (four strains), and *A. veronii* (one strain).

Antibiotics. Working solutions were prepared on the day of use from laboratory standard powders of all compounds tested according to the manufacturers' specifications. All isolates were tested for susceptibility to the following compounds: benzylpenicillin (Sigma Chemical Co., St. Louis, Mo.), ampicillin trihydrate (Bristol Laboratories, Syracuse, N.Y.), cephalothin (Eli Lilly and Co., Indianapolis, Ind.), cefoxitin and imipenem (Merck Sharp & Dohme, West Point, Pa.), cefuroxime and ceftazidime (Glaxo Group Research, Ltd., Greenford, United Kingdom), aztreonam (E. R. Squibb & Sons, Princeton, N.J.), and potassium clavulanate (Beecham Laboratories, Bristol, Tenn.).

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Susceptibility tests. Antibiotic susceptibility tests were performed by serial twofold dilution tests in Mueller-Hinton II agar (BBL). Inocula of 10^5 CFU per spot were applied with a Steers multiple-inoculum replicator (26). The MIC was defined as the lowest antibiotic concentration preventing growth after incubation for 18 h at 35°C in air. The presence of three colonies or fewer was ignored. MICs of ampicillin alone and in combination with 2 μg of potassium clavulanate per ml were determined in tests performed simultaneously with use of the same inoculum.

β-Lactamase induction. All 13 bacterial isolates were examined for the presence of inducible β-lactamases (20). Each isolate was grown overnight in Mueller-Hinton broth at 37°C in air. A 1:20 dilution of each culture was made into fresh Mueller-Hinton broth, and the diluted culture was shaken at 37°C in air for 90 min. Inducer at a concentration equal to 0.125 MIC for cefoxitin or imipenem or 100 μg of ampicillin per ml was then added. After incubation for another 120 min, 8-hydroxyquinoline (Sigma) was added to each culture. The cells were harvested by centrifugation and washed once in phosphate buffer (0.1 M, pH 7.0). Cell-free sonic extracts were prepared with the same phosphate buffer. The cells were disrupted with a Soniprep 150 ultrasonic disintegrator (MSE Scientific Instruments, Sussex, United Kingdom), and the crude sonic extracts were dialyzed overnight in 0.1 M phosphate buffer at 4°C. The protein concentration of each sonic extract was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.). The rate of hydrolysis of cephalothin, cefuroxime, and imipenem was measured with a Beckman DU-6 UV-visible spectrophotometer with readings recorded at 10-s intervals for 5 min at the wavelength for maximal absorbance for each drug. The rate of hydrolysis of benzylpenicillin was measured at 233 nm with a Beckman DU-7 visible spectrophotometer. Each measurement was corrected for protein concentration, and the rate of hydrolysis was expressed as substrate hydrolyzed per minute per milligram of protein. Each induction experiment was performed in triplicate.

Isoelectric focusing. β-Lactamases were focused in 7% polyacrylamide (Sigma) gels containing pH 3 to 10 ampholytes (Sigma) by the method of Vecoli et al. (28). The gels were focused across the width at 4°C and 1,600 V for 90 min with a Multiphor 2197 power unit (LKB Instruments, Rockville, Md.). The focused gels were overlaid with molten agar containing 50 μg of nitrocephin per ml either directly or after a 20-s overlay of 1 mM potassium clavulanate, cloxacillin, or aztreonam (18). Each gel was photographed with a Polaroid MP-4 camera with a Tiffen 58 dark green filter and type 51 high-contrast film (22). The following bacterial strains, with their plasmids and well-characterized β-lactamases in parentheses, were kindly provided for use as marked by A. A. Medeiros (Brown University, Providence, R.I.): *Escherichia coli* RTEM(R6K; TEM-1), *E. coli* 1752E(RP1; TEM-2), *E. coli* J53(R1010; SHV-1), *Pseudomonas aeruginosa* PU21(RPL11; PSE1), and *P. aeruginosa* PU21(pMG19; PSE4).

Assay of outer membrane proteins. Outer membrane proteins of *A. caviae* DLS4 and DLS5 were analyzed by vertical slab gel electrophoresis as described by Lugtenberg et al. (11). Cell envelopes were prepared from overnight cultures in Mueller-Hinton broth by the method of Ames (2). Purified outer membrane preparations were obtained by treatment of the cell envelopes with sodium-*N*-lauroyl sarcosinate (23). A 20-μl sample (ca. 20 μg of protein) of each outer membrane preparation was applied to the gel and electrophoresed with an LKB Multiphor 2197 power unit at 15 mA of constant current until the dye front reached the bottom of the gel. Protein bands were stained with Coomassie brilliant blue (Imperial Chemical, London, United Kingdom), and the destained gel was photographed with a Polaroid MP-4 camera with a Tiffen 8 yellow-2 filter and type 52 film.

RESULTS

Case history. A 67-year-old male was admitted to the Boise Veterans Administration Medical Center complaining of left-sided pleuritic chest pain and hemoptysis. Two days before admission a sputum culture had been collected, and the patient had been placed on amoxicillin-potassium clavulanate. The sputum culture contained *A. hydrophila* DLS4. This isolate was resistant to ampicillin and cephalothin but susceptible to cefoperazone. Therapy was changed to intravenous cefoperazone for the next 24 h. At this time the diagnosis was changed from lobar pneumonia to pulmonary emboli, and cefoperazone was discontinued. The patient's condition deteriorated over the next 24 h, and a repeat sputum culture was obtained. Cefoperazone therapy was resumed. *A. hydrophila* was recovered again in pure culture, but the isolate (DLS5) was now resistant to cefoperazone. Intravenous trimethoprim-sulfamethoxazole was added to the therapeutic regimen. Antibiotics were continued for another 10 days, and the patient was discharged after 18 days of hospitalization. He was readmitted 2 weeks later with pulmonary emboli.

The determination of species of *A. hydrophila* DLS4 and DLS5 was repeated in Omaha with supplementary tests described by Janda et al. (10). Results of these tests indicated both isolates to be *A. caviae*. Plasmid profiles of the two isolates were identical, as were outer membrane proteins. Eleven additional *Aeromonas* isolates were collected and examined in further detail with *A. caviae* DLS4 and DLS5 for their antibiotic susceptibility and β-lactamase content.

Antibiotic susceptibility. All 13 *Aeromonas* isolates were resistant to penicillin G and ampicillin (MIC, ≥128 μg/ml). No enhancement of the activity of ampicillin was noted when potassium clavulanate was present. All but three isolates were also resistant to cephalothin (Table 1). *A. sobria* appeared to be more susceptible than *A. hydrophila* and *A. caviae* to cefoxitin, cefuroxime, and ceftazidime. All isolates were highly susceptible to aztreonam. *A. caviae* DLS5 was less susceptible to cefuroxime, ceftazidime, and aztreonam than was *A. caviae* DLS4. All isolates were susceptible to imipenem, although MICs for some *A. sobria* and *A. veronii* isolates were somewhat higher than MICs obtained in tests with the other two species (Table 1).

β-Lactamase. Only one isolate, *A. caviae* DLS5, produced detectable β-lactamase when grown in the absence of inducer (Table 2). The hydrolytic activities of sonic extracts from *A. caviae* DLS5 before and after induction with cefoxitin were similar, suggesting constitutive β-lactamase expression in this isolate. Among the remaining 12 isolates, all but one appeared to possess inducible β-lactamase activity against benzylpenicillin (Table 2). No β-lactamase activity was found in sonic extracts of *A. sobria* AER28 regardless of the substrate or inducer tested.

Variations in substrate profiles were noted both between and within species. Two isolates of *A. caviae* (AER5 and AER51) showed predominantly penicillinase activity (Table 2). The remaining three isolates of this species showed broad-spectrum β-lactamase activity, readily hydrolyzing both benzylpenicillin and cephalothin. Two isolates of *A.*

TABLE 1. β-Lactam susceptibility of four species of *Aeromonas*

Isolate	MIC (μg/ml)					
	Cephalothin	Cefoxitin	Cefuroxime	Ceftazidime	Aztreonam	Imipenem
A. caviae						
AER5	>128	8	2	0.12	≤0.015	0.12
AER51	>128	8	2	0.25	≤0.015	0.12
DLS4	>128	4	1	0.06	≤0.015	0.12
DLS5	>128	8	16	4	0.5	0.12
ATCC 15468	>128	8	1	0.06	≤0.015	0.06
A. sobria						
AER14	>128	4	0.12	≤0.03	≤0.015	0.5
AER28	1	0.12	≤0.06	≤0.03	≤0.015	1
AER83	>128	2	≤0.06	≤0.03	≤0.015	0.5
AER178	0.5	0.12	≤0.06	≤0.03	≤0.015	0.25
A. hydrophila						
AER19	>128	4	0.5	0.06	≤0.015	0.25
AER64	>128	>128	0.5	0.06	≤0.015	0.25
AER84	>128	8	0.5	0.06	≤0.015	0.12
A. veronii ATCC 35624	8	1	2	0.25	0.06	1

sobria (AER14 and AER83) hydrolyzed imipenem more readily than benzylpenicillin and cephalothin. A third isolate of *A. sobria* (AER178) and *A. veronii* ATCC 35624 hydrolyzed imipenem and benzylpenicillin equally but did not hydrolyze cephalothin (Table 2). All three isolates of *A. hydrophila* showed broad-spectrum β-lactamase activity that included imipenem. None of the isolates examined hydrolyzed cefuroxime.

To compare the β-lactamase inducer potential of cefoxitin with that of ampicillin and imipenem, one strain from each *Aeromonas* species was selected for further study. Sonic extracts obtained from cefoxitin-induced cells of *A. caviae*, *A. hydrophila*, and *A. sobria* consistently displayed the greatest activity against benzylpenicillin, cephalothin, and imipenem, although both ampicillin and imipenem did induce enzyme in these strains (Table 3). In contrast, both ampicillin and imipenem were more potent inducers of β-lactamase than cefoxitin in *A. veronii* (Table 3).

Isoelectric focusing was performed on sonic extracts prepared from each strain before and after induction. Examples of patterns observed with each of the four species are shown in Fig. 1. Sonic extracts from seven isolates showed multiple bands, whereas those from five isolates showed only a single β-lactamase band. A band of pI 8.0 appeared to be common to *A. sobria*, *A. hydrophila*, and *A. veronii* but was not found in sonic extracts of the *A. caviae* isolates (Fig. 1). All bands appeared inducible except for those in sonic extracts of *A. caviae* DLS5.

TABLE 2. β-Lactamase activity in four species of *Aeromonas*

Isolate	β-Lactamase activity[a] (mean ± SD)		Relative rate of hydrolysis[b]			pI[c]
	Uninduced	Induced	Benzylpenicillin	Cephalothin	Imipenem	
A. caviae						
AER5	–[d]	727 ± 260	100	23		7.3, 6.7
AER51	–	617 ± 156	100	25		7.3, 6.7
DLS4	–	84 ± 16	100	67		6.8, 6.7
DLS5	453 ± 95	486 ± 129	100	60		6.8, 6.7
ATCC 15468	–	108 ± 44	100	152		7.3, 6.7
A. sobria						
AER14	–	118 ± 49	100	98	163	8.0
AER28	–	–				
AER83	–	133 ± 12	100	82	201	8.0, 6.9
AER178	–	54 ± 28	100		100	8.0
A. hydrophila						
AER19	–	300 ± 73	100	80	76	8.0, 7.0
AER64	–	174 ± 55	100	154	28	8.0
AER84	–	244 ± 37	100	143	42	8.0
A. veronii ATCC 35624	–	11 ± 2	100		85	8.0

[a] Activity in uninduced or cefoxitin-induced sonic extracts. Results are shown as nanomoles of benzylpenicillin hydrolyzed per minute per milligram of protein.
[b] Based upon activity in induced sonic extracts relative to that of benzylpenicillin.
[c] Values given only for major bands.
[d] –, No activity detected. Minimal detectable activity was 9 nmol/min for benzylpenicillin, 1 nmol/min for cephalothin, and 1 nmol/min for imipenem.

TABLE 3. Induction of β-lactamase in four species of *Aeromonas*

Isolate	Substrate	Relative β-lactamase activity[a] after induction with:		
		Cefoxitin	Ampicillin	Imipenem
A. caviae AER5	Benzylpenicillin	1.0	0.4	0.1
	Cephalothin	1.0	0.5	0.2
	Imipenem	–[b]	–	–
A. hydrophila AER19	Benzylpenicillin	1.0	0.4	0.2
	Cephalothin	1.0	0.4	0.2
	Imipenem	1.0	0.3	0.2
A. sobria AER83	Benzylpenicillin	1.0	0.8	0.6
	Cephalothin	1.0	0.7	0.7
	Imipenem	1.0	0.6	0.6
A. veronii ATCC 35624	Benzylpenicillin	1.0	4.3	3.6
	Cephalothin	–	–	–
	Imipenem	1.0	8.6	8.6

[a] Expressed as the ratio of activity (nanomoles of substrate hydrolyzed per minute per milligram of protein) after induction with drug indicated divided by activity after induction with cefoxitin.
[b] See footnote *d* of Table 2.

Sonic extracts from isolates of *A. caviae* showed a major band of pI 6.7. Other prominent bands were observed at pI 6.8 (*A. caviae* DLS4 and DLS5) and 7.3 (*A. caviae* AER51, AER5, and ATCC 15468). Two minor bands of pI 5.8 and 6.4 were also observed in sonic extracts of *A. caviae* AER5 (Fig. 1). Sonic extracts from most of the other *Aeromonas* isolates showed a single major band of pI 8.0. In addition, sonic extracts from *A. hydrophila* AER19 and *A. sobria* AER83 showed second major bands of pIs 7.0 and 6.9, respectively (Fig. 1). Isoelectric focusing patterns were the same for sonic extracts produced from cells induced with cefoxitin, imipenem, or ampicillin.

Polyacrylamide gels were overlaid with various β-lactamase inhibitors to further evaluate the nature of the enzyme bands observed with each species (Fig. 2). Potassium clavulanate inhibited the enzyme bands of pI 7.3 in *A. caviae* AER5 and pI 8.0 in *A. hydrophila* AER19, *A. sobria*

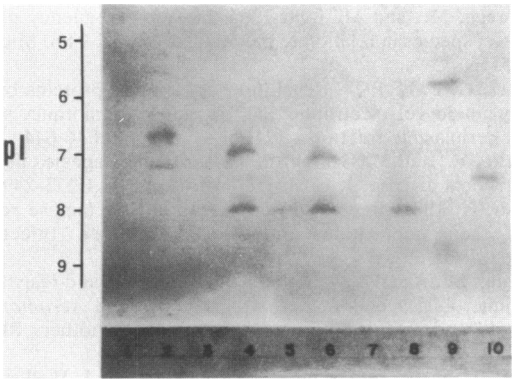

FIG. 1. Analytical isoelectric focusing patterns of *Aeromonas* β-lactamases. Lanes 1, 3, 5, and 7 contain uninduced samples; lanes 2, 4, and 6 contain samples of strains induced with cefoxitin; and lane 8 contains a sample induced with imipenem. Lanes: 1 and 2, *A. caviae* AER5; 3 and 4, *A. hydrophila* AER19; 5 and 6, *A. sobria* AER83; 7 and 8, *A. veronii* ATCC 35624. β-Lactamase markers with known pI values are shown in lanes 9 (PSE-1; pI 5.7) and 10 (SHV-1, pI 7.7). β-Lactamase activity was detected with nitrocephin (21).

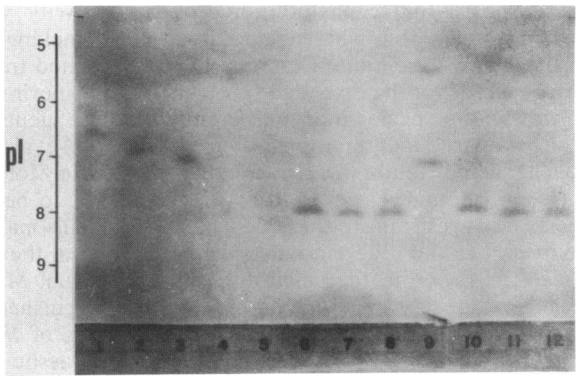

FIG. 2. Isoelectric focusing patterns of *Aeromonas* β-lactamases after overlay with 1 mM potassium clavulanate (lanes 1 through 4), cloxacillin (lanes 5 through 8), and aztreonam (lanes 9 through 12). Lanes: 1, 5, and 9, *A. caviae* AER5; 2, 6, and 10, *A. hydrophila* AER19; 3, 7, and 11, *A. sobria* AER83; 4, 8, and 12, *A. veronii* ATCC 35624.

AER83, and *A. veronii* ATCC 35624. These were not inhibited by aztreonam. Only the β-lactamase of pI 7.3 in *A. caviae* AER5 was inhibited by cloxacillin. In contrast, the β-lactamase bands of pIs 6.7 in *A. caviae* AER5, 6.9 in *A. sobria* AER83, and 7.0 in *A. hydrophila* AER19 were inhibited by aztreonam and cloxacillin but not by potassium clavulanate.

DISCUSSION

Species of *Aeromonas* are becoming increasingly recognized as a cause of human infections in immunologically normal as well as immunocompromised hosts. Although the majority of infections in the literature have been cases of diarrhea, several recent reports have implicated *Aeromonas* species as the cause of serious wound infections or bacteremia in immunocompromised hosts (1, 3, 5, 6, 27, 29). In the pulmonary disease of our patient, the pathogenetic role of *A. caviae* was uncertain, since other etiologies were not definitively ruled out. Nevertheless, the use of cefoperazone in this patient was associated with the selection of a derepressed mutant of *A. caviae* showing multiple β-lactam resistance. Thus, use of the newer cephalosporins to treat *Aeromonas* infections in other patients may carry the risk of clinical failure and emergence of resistance similar to that seen with other genera possessing inducible β-lactamases (21).

The MICs obtained with the various species of *Aeromonas* examined in this study showed certain genus- and species-associated trends. All of the *Aeromonas* isolates examined were highly resistant to benzylpenicillin and ampicillin. This is in agreement with reports published previously (4, 9, 13, 17, 25, 31). The good inducer activity of these penicillins coupled with the susceptibility of the drugs to hydrolysis by *Aeromonas* β-lactamases probably explains some of the penicillin resistance in this genus. However, potassium clavulanate did not reduce MICs of ampicillin into the susceptible range despite sensitivity of many of the *Aeromonas* β-lactamases to this inhibitor. This disparity has also been reported by Zemelman et al. (31). It probably results from a high intrinsic resistance of the genus to the penicillins, since the single β-lactamase-negative strain examined in this study was still highly resistant to both penicillin and ampicillin (MIC, 128 μg/ml).

The resistance of *Aeromonas* species to cephalothin was also associated with the presence of β-lactamase. All 10

isolates possessing cephalothin-hydrolyzing activity were resistant to this drug, whereas the three isolates lacking this activity were susceptible. Certain species-associated trends were observed for the extended spectrum cephalosporins. *A. sobria* was the species most susceptible to these agents—a finding similar to that reported previously by Motyl et al. (13) and Chang and Bolton (4). However, in contrast to Motyl et al. (13), we found *A. caviae*, not *A. hydrophila*, to be the species most resistant to these cephalosporins. This may be due in part to the different antibiotics examined in the two studies. Also due to the small differences in actual MICs, these two species may be more readily distinguished in strain-to-strain comparisons than in comparisons of MICs for 50% or 90% of isolates. Although the species-associated trends observed in the current study were most obvious with cefuroxime, no hydrolysis of this β-lactam was detected in tests with any strain. However, increased resistance to cefuroxime, ceftazidime, and aztreonam associated with derepression of β-lactamase in *A. caviae* DLS5 suggests that the enzyme may be involved in resistance to these drugs as well.

The species that possessed imipenem-hydrolyzing β-lactamases were most resistant to this drug, although MICs were still in the susceptible range. This may be due to the low level of enzyme produced coupled with the rapid permeation of imipenem into the gram-negative cell (30), which may help to minimize the protective effect of β-lactamase. A similar lack of association between imipenem resistance in *Aeromonas* species and the presence of imipenem-hydrolyzing β-lactamase has been previously reported by Shannon et al. (25).

The diverse nature of the β-lactamases found in *Aeromonas* species, apparent from previously published literature (8, 12, 24, 25, 31), was confirmed in this study. However, from data presented in this and the previous studies, there appears to be a limited number of discrete enzymes characteristic for this genus. Although no study to date has precisely identified the genetic basis for their expression, these enzymes appear to be chromosomally mediated due to their inducibility and the relative paucity of conjugative plasmids in *Aeromonas* species (4). Each distinct β-lactamase appears to be inducible by benzylpenicillin, ampicillin, imipenem, or cefoxitin (24, 25, 31).

From a comparison of substrate profiles and isoelectric points, there appears to be a minimum of four distinct *Aeromonas* β-lactamases. Enzyme A, found in *A. veronii* and *A. sobria*, hydrolyzes imipenem and benzylpenicillin. Enzyme B, found in *A. hydrophila* and *A. sobria*, hydrolyzes imipenem, benzylpenicillin, and cephalothin. Both enzymes A and B are susceptible to inhibition by clavulanic acid but not cloxacillin or aztreonam and have an isoelectric point of 8.0. At least two additional distinct enzymes can be inferred from the data obtained with *A. caviae*. In this species, no enzyme of pI 8.0 was found, and no hydrolysis of imipenem occurred. Thus, there must be at least two enzymes that hydrolyze benzylpenicillin and/or cephalothin, one (enzyme C) which is susceptible to inhibition by clavulanic acid and another (enzyme D) which is not.

The presence of two distinct inducible β-lactamases in a single strain was noted in all *A. caviae* strains and one strain each of *A. sobria* and *A. hydrophila*. This has been reported previously only in *Pseudomonas maltophilia* (18, 19), two strains of *Enterobacter cloacae*, and a single strain of *Serratia marcescens* (A. A. Medeiros and R. S. Hare, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 116, 1986). Interestingly, in each of these organisms, one of the two inducible β-lactamases hydrolyzed imipenem, whereas the other was a characteristic class I cephalosporinase. In *A. caviae*, such cannot be the case since imipenem was not hydrolyzed by any isolate of this species. However, a distinct imipenem-hydrolyzing β-lactamase may account for one of the two enzymes found in *A. sobria* AER83 and *A. hydrophila* AER19.

Although determination of the substrate profile of each enzyme within the same *Aeromonas* strain will require purification, differences in the inhibitor profiles for each band observed on focusing gels clearly indicate the presence of two distinct β-lactamases. For each strain producing two distinct β-lactamases, one enzyme had the inhibitor profile characteristic for inducible class I cephalosporinases (3a, 22); i.e., resistance to potassium clavulanate and susceptibility to cloxacillin and aztreonam. These β-lactamases had pIs of 6.7, 6.9, and 7.0, respectively. The second enzyme in *A. hydrophila* AER19 and *A. sobria* AER83 of pI 8.0 had the inhibitor profile characteristic for class III, IV, or V β-lactamase (3a, 22); i.e., susceptibility only to potassium clavulanate. However, these classes of enzymes are not characteristically inducible. The second enzyme in *A. caviae* (pI 7.3) had the inhibitor profile characteristic for the class I oxyiminocephalosporinase of *Proteus vulgaris* (3a). However, the *Aeromonas* enzyme did not hydrolyze cefuroxime. Thus, it appears that within the genus *Aeromonas* there are a number of distinct enzymes and that many of these differ from β-lactamases that have been characterized in other genera of gram-negative bacteria.

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