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Antimicrobial Susceptibilities of *Aeromonas* Strains Isolated from Clinical and Environmental Sources to 26 Antimicrobial Agents

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We determined the susceptibilities of 144 clinical and 49 environmental *Aeromonas* strains representing 10 different species to 26 antimicrobial agents by the agar dilution method. No single species had a predominantly nonsusceptible phenotype. A multidrug nonsusceptible pattern was observed in three (2.1%) clinical strains and two (4.0%) strains recovered from diseased fish. Common clinical strains were more resistant than the corresponding environmental isolates, suggesting that resistance mechanisms may be acquired by environmental strains from clinical strains.

Aeromonas species are globally distributed Gram-negative, oxidase-positive fermentative rods, found in aquatic environments (15), foods (12), and the microflora of fish (16). Antimicrobial resistance in these organisms is usually chromosomally mediated, but β-lactamases produced by aeromonads may occasionally be encoded by plasmids (11, 22) or integrons (4). These enzymes have activity against most β-lactam antimicrobial agents, including cefepime and other extended-spectrum cephalosporins. Antimicrobial susceptibility reporting for *Aeromonas* generally followed guidelines for the *Enterobacteriaceae* until the Clinical and Laboratory Standards Institute (CLSI) recently published recommendations (9). The objective of this study was to determine the antimicrobial susceptibility profiles of commonly used agents against a collection of *Aeromonas* species from clinical, fish, and environmental sources.

Aeromonas spp. used in this study included 144 clinical isolates (comprising 54 wound specimens, 33 blood specimens, 34 stool specimens, and 23 isolates from miscellaneous specimens) and 49 environmental isolates (from water [$n = 43$], fish [$n = 5$], and crab meat [$n = 1$]). Strains were previously identified phenotypically by extensive biochemical testing (3) and their identities confirmed genotypically from their *gyrB* and *rpoD* gene sequences (2). Ten *Aeromonas* spp. were represented: *A. aquariorum* (58 strains), *A. veronii* bv. sobria (49 strains), *A. hydrophila* (39 strains), *A. caviae* (36 strains), *A. jandaei* (3 strains), *A. media* (3 strains), *A. salmonicida* (2 strains), and one strain each of *A. allosaccharophila*, *A. bestiarum*, and *A. schubertii*.

Antimicrobial susceptibility testing was performed by the agar dilution breakpoint method as described by the CLSI (8). Antimicrobial agents tested included the following: amikacin, amoxicillin, amoxicillin-clavulanate, cephalothin, cefazolin, cefepime, cefoxitin, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, meropenem, moxifloxacin, nalidixic acid, nitrofurantoin, norfloxacin, piperacillin-tazobactam, tetracycline, ticarcillin-clavulanate, tobramycin, trimethoprim, and trimethoprim-sulfamethoxazole (Table 1). Susceptibility was defined as absence of growth on solid medium containing any of these antimicrobial agents. Presence of growth indicated nonsusceptibility. E-strips containing doxycycline (AB Biodisk, Solna, Sweden), ampicillin, tigecycline, and colistin (bioMérieux, Marcy l'Etoile, France) were used to determine MICs. Interpretative criteria for tigecycline and ampicillin were derived from those described for the *Enterobacteriaceae* by the Food and Drug Administration (5), and those for doxycycline were derived from those described by the CLSI (9), as outlined in Table 1 of the E-strip package insert. Interpretative criteria for colistin were from Fosse et al. (10) (MIC of <2 μg/ml was considered susceptible). MIC breakpoints used were as follows (in μg/ml): for tigecycline, susceptible (S), ≤2; intermediate (I), 4; resistant (R), ≥8; for doxycycline, S, ≤4; I, 8; R, ≥16; and for ampicillin, S, ≤8; I, 16; R, ≥32. *Escherichia coli* ATCC 25922 was used as a quality control organism for both E-strip MICs and agar dilution tests. Statistical analyses were conducted with Fisher's exact method of contingency table analysis using statistical software (Prism version 5.0; GraphPad, Inc., San Diego, CA).

All isolates were inhibited by amikacin, cefepime (8 μg/ml), ciprofloxacin, meropenem, norfloxacin, and tigecycline. Susceptibility to amoxicillin was demonstrated in three (1.6%) isolates (one clinical and one environmental *A. veronii* bv. sobria and one environmental *A. aquariorum* isolate) by agar dilution and confirmed by the E-strip method, with MIC values of 8 μg/ml for all three isolates. Thirty-two isolates (16.5%) failed to grow in the presence of amoxicillin-clavulanate, while 17 (8.8%) were nonsusceptible to ticarcillin-clavulanate (16/2 μg/ml). Of these, 8 (4.4%) were also nonsusceptible to the higher concentration of ticarcillin-clavulanate (64/2 μg/ml). Susceptibility to cephalothin and cefazolin was observed in 53 (27.4%) and 40 (20.7%) isolates, respectively. A moderate level of susceptibility was detected with cefoxitin (126 isolates, 65.2%) and colistin (86 isolates, 44.5%). The majority of the isolates were susceptible to the remaining antimicrobial agents (Table 1). The MICs for doxycycline ranged from 0.064 to 24.0 μg/ml, those for tigecycline ranged from 0.064 to 3.0 μg/ml, and those for colistin ranged from 0.094 to >256 μg/ml. Susceptibility to doxycycline and tigecycline was high in

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TABLE 1 Antimicrobial susceptibilities of 193 *Aeromonas* species

Antimicrobial agent	MIC breakpoint(s) (μg/ml)	Percentage (no.) of strains susceptible		
		All isolates ($n = 193$)	Clinical isolates ($n = 144$)	Environmental isolates ($n = 49$)
Amoxicillin (AMX)	8	1.6 (3)	0.7 (1)	4.0 (2)
Amoxicillin-clavulanate (AMC)	8/4	16.5 (32)	6.25 (9)	46.9 (23)
Norfloxacin (NOR)	4	100	100	100
Ciprofloxacin (CIP	1	100	100	100
Nitrofurantoin (NIT)	32	99.5 (192)	99.3 (143)	100
Trimethoprim (TMP)	8	92.7 (179)	91 (131)	97.9 (48)
Cephalothin (CEF)	8	27.4 (53)	20.8 (30)	46.9 (23)
Meropenem (MEM)	0.25	100	100	100
	1	100	100	100
	4	100	100	100
Gentamicin (GEN)	4	99.5 (192)	99.3 (143)	100
Tobramycin (TOB)	4	95.3 (184)	93.8 (135)	100
Amikacin (AMK)	16	100	100	100
Ceftriaxone (CRO)	1	96.9 (187)	95.8 (138)	100
Ceftazidime (CAZ)	0.5	97.4 (188)	96.5 (139)	100
	4	99.5 (192)	99.3 (143)	100
Aztreonam (ATM)	4	99.5 (192)	99.3 (143)	100
Ticarcillin-clavulanate (TIM)	16/2	91.2 (176)	88.9 (128)	97.9 (48)
	64/2	95.9 (185)	95.1 (137)	97.9 (48)
Trimethoprim-sulfamethoxazole (SXT)	2/38	98.9 (191)	98.6 (142)	100
Cefepime (FEP)	0.5	98.9 (191)	98.6 (142)	100
	8	100	100	100
Nalidixic acid (NAL)	16	96.9 (187)	97.9 (141)	93.8 (46)
Cefoxitin (FOX)	8	65.2 (126)	65.9 (95)	63.2 (31)
Piperacillin-tazobactam (TZP)	16/4	97.4 (188)	96.5 (139)	100
	64/4	98.9 (191)	98.6 (142)	100
Moxifloxacin (MXF)	1	98.9 (191)	99.3 (143)	97.9 (48)
Tetracycline (TET)	4	94.3 (182)	95.1 (137)	81.6 (40)
Cefazolin (CFZ)	2	20.7 (40)	8.2 (9)[a]	10.2 (5)
Doxycycline (DOX)	S, ≤4; I, 8; R, ≥16	97.9 (189)	97.2 (140)	100
Tigecycline (TGC)	S, ≤2; I, 4; R, ≥8	100	100	100
Colistin (CST)	S, <2	44.5 (86)	39.5 (57)	59.1 (29)

[a] One hundred nine strains tested.

clinical strains, at 97.2 and 100%, respectively. There was no statistically significant difference in antimicrobial susceptibility between clinical and environmental isolates of *A. aquariorum*. In contrast, clinical isolates of *A. veronii* bv. sobria were less susceptible than environmental strains ($P = 0.0226$). Other statistically significant differences were observed for amoxicillin-clavulanate between *A. aquariorum* and *A. hydrophila* ($P = 0.0036$) (*A. aquariorum* was less susceptible than *A. hydrophila*) and between *A. aquariorum* and *A. veronii* bv. sobria ($P = 0.0053$) (*A. veronii* bv. sobria was less susceptible than *A. aquariorum*) but not between *A. aquariorum* and *A. caviae*. Further, susceptibility to cephalothin was significantly higher in *A. veronii* bv. sobria than in *A. aquariorum*, *A. caviae*, and *A. hydrophila* ($P = 0.0001$). Nine clinical isolates (6.2%) were able to grow in agar plates containing 4 μg/ml of tobramycin, including seven (14.2%) *A. veronii* bv. sobria, one (2.7%) *A. caviae*, and one (33.3%) *A. media* isolate. Multidrug nonsusceptible patterns were observed in three isolates. Of these, *A. caviae* strain 138 was less susceptible to most β-lactams, including aztreonam. *A. veronii* bv. sobria strain 189 was the only isolate to grow in the presence of both gentamicin and tobramycin. Susceptibility to colistin was recorded in 57 (39.5%) clinical and 29 (59.1%) environmental isolates. *A. caviae* was the most susceptible species (83.7%), next to *A. aquariorum* (31.0%). Most environmental isolates were susceptible to tetracycline (81.6%) and nalidixic acid (93.8%). Moderate susceptibility was observed with amoxicillin-clavulanate (46.9%), cephalothin (46.9%), and cefoxitin (63.2%), while only five (10.2%) isolates were susceptible to cefazolin.

Differences in antimicrobial susceptibility between clinical and environmental strains have been described previously (19, 20). The resistance observed in environmental aeromonads has been associated with heavily polluted waters as the source of multiple resistance plasmids (13). In contrast, our results suggest that (i) environmental strains are not the principal source of resistance but that antibiotic resistance in clinical isolates may be due to the selective pressure to which these organisms may have been exposed, (ii) water sources are less polluted in Western Australia than other regions, and (iii) environmental strains may have acquired resistance determinants from clinical strains.

In general, growth of *Aeromonas* was inhibited by most antimicrobial agents, with few isolates showing a multidrug nonsusceptible profile. Susceptibility to tetracycline was high (94.36%), consistent with previous reports from Australia and the United States (18, 20). In contrast, tetracycline resistance in up to 49% of isolates has been reported in studies from the Asian region (7, 17, 19). The three amoxicillin-susceptible isolates described here con-

firm that amoxicillin-susceptible strains other than *A. trota* (6) occur, as previously reported (1, 14), and that their growth may be suppressed by amoxicillin-containing medium.

Susceptibility to cephalothin was high in *A. veronii* bv. sobria, a feature that has been reported by others and proposed as a phenotypic marker to differentiate this species from other aeromonads (18, 20). Similarly, susceptibility to colistin was proposed as an identifying marker for *Aeromonas* (10). Our results were consistent with those obtained by a previous study (10) for *A. hydrophila* (61.7% resistance in this study, versus 85.8%) and *A. jandaei* (100% resistance in both studies). However, MIC results obtained in this report differed from the previous study for *A. veronii* bv. sobria (61.7% versus 2.5%) and for *A. caviae* (16.2% versus 2.1%). The numbers of isolates susceptible to piperacillin-tazobactam (97.4% and 98.9%) and ticarcillin-clavulanate (91.2% and 95.9%) were much higher than those susceptible to amoxicillin-clavulanate (16.5%), suggesting that the former two antimicrobials could be considered for the treatment of infections caused by *Aeromonas.* Zemelman et al. (24) reported that, depending on the strain, the MIC to amoxicillin decreased from 2- to 8-fold in combination with clavulanate, thus increasing the activity of this agent. However, prolonged use of amoxicillin-clavulanate to treat infections caused by *A. veronii* bv. sobria has resulted in overexpression of carbapenemases and cephalosporinases (23).

All isolates were susceptible to meropenem. A single *A. hydrophila* isolate that grew in all three agar dilution concentrations was susceptible by the E-strip method using two different inocula, 1.5×10^8 CFU/ml and 3.0×10^8 CFU/ml (results not shown). A large inoculum (3×10^8 CFU/ml) has been recommended to detect carbapenemase production before antibiotic therapy using carbapenems is considered, as conventional *in vitro* susceptibility testing may fail to detect the presence of carbapenemases in otherwise carbapenemase-susceptible phenotypes (21).

In conclusion, this study shows that the number of multidrug nonsusceptible *Aeromonas* species in Western Australia remains low and that clinicians have a wide choice of antimicrobial agents to treat infections with these species, consistent with other reports (17, 25). However, antimicrobial susceptibility testing for clinically significant strains is highly recommended, as resistance to antibacterial agents may be strain dependent.

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