Antimicrobial susceptibility of *Aeromonas* spp. isolated from clinical and environmental sources to 26 antimicrobial agents

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Abstract

We determined the susceptibility 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 non-susceptible phenotype. A multi-non-susceptible 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 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 Standard Institute (CLSI) recently published recommendations (8). The objective of this study was to determine the antimicrobial susceptibility profile 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 (comprising 54 wounds, 33 blood, 34 stools and 23 isolates from miscellaneous specimens) and 49 environmental (isolated from water (43), fish (5) and one from crab meat) isolates. Strains were previously identified phenotypically by extensive biochemical testing (3) and their identity confirmed genotypically from their gyrB and rpoD gene sequences (2). Ten Aeromonas spp. were represented and included A. aquariorum (59 strains), A. veronii bt sobria (49), A. hydrophila (39), A. caviae (36), A. jandaei (3), A. media (3), A. salmonicida (2) and one strain each of A. allosaccharophila, A. bestiarum and A. schubertii.

Antimicrobial susceptibility testing was performed by the agar dilution break-point method as described by the CLSI (7). Antimicrobial agents tested included the following: amikacin, amoxicillin, amoxicillin-clavulanate, cephalothin, cefazolin, cefepime, cefoxitin, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, meropenem, moxifloxacin, nalidixic acid, nitrofurantoin, norfloxacin, piperclillin-tazobactam, tetracycline, ticarcillin-clavulanate,
tobramycin, trimethoprim and trimethoprim-sulphamethoxazole (Table 1). Susceptibility was
declared as absence of growth on solid media containing any of these antimicrobial agents.
Presence of growth indicated non-susceptibility. E-strips containing doxycycline (AB
Biodisk, Solna, Sweden), ampicillin, tigecycline and colistin (Biomérieux, Marcy-l’Etoile,
France) were used to determine minimum inhibitory concentrations (MICs). Interpretative
criteria for tigecycline and ampicillin were derived from those described for the
Enterobacteriaceae by the Food and Drug Administration (9) and by the CLSI (8) for
doxycycline as outlined in Table 1 of the E-strip package insert. Interpretative criteria for
colistin were from Fosse et al. (10) (MIC < 2 μg/mL was considered susceptible). MIC
breakpoints used were (μg/mL): tigecycline S, ≤ 2; I, 4; R, ≥ 8; doxycycline S, ≤ 4; I, 8; R, ≥
16; ampicillin S, ≤ 8; I, 16; R, ≥ 32. Escherichia coli ATCC 25922 was used as 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) 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 non-susceptible to ticarcillin-clavulanate (16/2
μg/mL). Of these, 8 (4.4%) were also non-susceptible 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, 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, for tigecycline from 0.064 to 3.0 μg/mL and for colistin from 0.094 to >256 μg/mL. Susceptibility to doxycycline and tigecycline was high in 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* compared to *A. aquariorum*, *A. caviae* and *A. hydrophila* (*p* = 0.0001). Nine clinical isolates (4.7%) were able to grow in agar plates containing 4 μg/mL of tobramycin including seven (19.4%) *A. veronii* bt *sobria*, one (2.9 %) *A. caviae* and one (50%) *A. media*. Multi-non-susceptible 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 (49.1%) environmental isolates. *A. caviae* was the most susceptible species (83.7%) compared 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%), cefoxitin (63.2%) while only five (10.2%) isolates were susceptible to cefazolin.

Differences in antimicrobial susceptibilities between clinical and environmental strains have been previously described (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 1) 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; 2) water sources are less polluted in Western Australia than other regions and 3) 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 multi-non-susceptible 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 (6, 17, 19). The three amoxicillin-susceptible isolates described here confirm that amoxicillin-susceptible strains other than *A. trota* (5) occur, as previously reported (1, 14), and their growth may be suppressed by amoxicillin-containing media.

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 vs 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 bt sobria* (61.7% vs 2.5%) and for *A. caviae* (16.2% vs 2.1%).

The number of isolates susceptible to piperacillin-tazobactam (97.4% and 98.9%) and ticarcillin-clavulanate (91.2% and 95.9%) was 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.
when combined with clavulanate thus increasing the activity of this agent. However, prolonged use of amoxicillin-clavulanate to treat infections caused by \textit{A. veronii bv sobria} has resulted in over-expression of carbapenemases and cephalosporinases (23).

All isolates were susceptible to meropenem. A single \textit{A. hydrophila} isolate that grew in all three agar dilution concentrations was susceptible by the E-strip method using two different inocula, $1.5 \times 10^8$ /ml and $3.0 \times 10^8$ /ml (results not shown). A large inoculum ($10^8$ CFU) has been recommended to detect carbapenemase production before antibiotic therapy using carbapenems is considered, as conventional \textit{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 multi-drug non-susceptible \textit{Aeromonas} in Western Australia remains low and 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.

References


Table 1. Antimicrobial susceptibility of 193 *Aeromonas* species (% susceptible)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Symbol</th>
<th>Break-points (μg/mL)</th>
<th>All isolates (n = 193)</th>
<th>Clinical (n = 144)</th>
<th>Environmental (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>AMX</td>
<td>8</td>
<td>1.6 (3)</td>
<td>0.7 (1)</td>
<td>4.0 (2)</td>
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<td>16.5 (32)</td>
<td>6.25 (9)</td>
<td>46.9 (23)</td>
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<td>Norfloxacin</td>
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<td>100</td>
<td>100</td>
<td>100</td>
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<td>CIP</td>
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<td>100</td>
<td>100</td>
<td>100</td>
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<td>Nitrofurantoin</td>
<td>NIT</td>
<td>32</td>
<td>99.5 (192)</td>
<td>99.3 (143)</td>
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<td>Trimethoprim</td>
<td>TMP</td>
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<td>92.7 (179)</td>
<td>90.1 (131)</td>
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<td>27.4 (53)</td>
<td>20.8 (30)</td>
<td>46.9 (23)</td>
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<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Meropenem</td>
<td>MEM</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td>MEM</td>
<td>4</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
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<td>4</td>
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<td>99.3 (143)</td>
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<td>Tobramycin</td>
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<td>4</td>
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<td>93.8 (135)</td>
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<td>100</td>
<td>100</td>
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<tr>
<td>Ceftriaxone</td>
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<td>1</td>
<td>96.9 (187)</td>
<td>95.8 (138)</td>
<td>100</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>CAZ</td>
<td>0.5</td>
<td>97.4 (188)</td>
<td>96.5 (139)</td>
<td>100</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>CAZ</td>
<td>4</td>
<td>99.5 (192)</td>
<td>99.3 (143)</td>
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<td>Aztreonam</td>
<td>ATM</td>
<td>4</td>
<td>99.5 (192)</td>
<td>99.3 (143)</td>
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<td>Ticarcillin-clavulanate</td>
<td>TIM</td>
<td>16/2</td>
<td>91.2 (176)</td>
<td>88.9 (128)</td>
<td>97.9 (48)</td>
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<tr>
<td>Ticarcillin-clavulanate</td>
<td>TIM</td>
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<td>95.9 (185)</td>
<td>95.1 (137)</td>
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<td>Trimethoprim-sulphamethoxazole</td>
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<td>98.6 (142)</td>
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<td>98.9 (191)</td>
<td>98.6 (142)</td>
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<tr>
<td>Cefepime</td>
<td>FEP</td>
<td>8</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Nalidixic acid</td>
<td>NAL</td>
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<td>96.9 (187)</td>
<td>97.9 (141)</td>
<td>93.8 (46)</td>
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<tr>
<td>Cefotixin</td>
<td>FOX</td>
<td>8</td>
<td>65.2 (126)</td>
<td>65.9 (95)</td>
<td>63.2 (31)</td>
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<tr>
<td>Piperilnin-tazobactam</td>
<td>TZP</td>
<td>16/4</td>
<td>97.4 (188)</td>
<td>96.5 (139)</td>
<td>100</td>
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<tr>
<td>Piperlicillin-tazobactam</td>
<td>TZP</td>
<td>64/4</td>
<td>98.9 (191)</td>
<td>98.6 (142)</td>
<td>100</td>
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<td>Moxifloxacin</td>
<td>MXF</td>
<td>1</td>
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<td>99.3 (143)</td>
<td>97.9 (48)</td>
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<td>Tetracycline</td>
<td>TET</td>
<td>4</td>
<td>94.3 (182)</td>
<td>95.1 (137)</td>
<td>81.6 (40)</td>
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<tr>
<td>Cefalozin</td>
<td>CFZ</td>
<td>2</td>
<td>20.7 (40)</td>
<td>8.2 (100)</td>
<td>10.2 (5)</td>
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<tr>
<td>Doxycycline MIC(^1) (μg/mL)</td>
<td>DOX</td>
<td>S ≤ 4, I 8, R ≥ 16</td>
<td>97.9 (189)</td>
<td>97.2 (140)</td>
<td>100</td>
</tr>
<tr>
<td>Tigecycline MIC (μg/mL)</td>
<td>TGC</td>
<td>S ≤ 2, I 14, R ≥ 8</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Colistin</td>
<td>CST</td>
<td>S &lt; 2</td>
<td>86 (44.5)</td>
<td>39.5 (57)</td>
<td>49.1 (29)</td>
</tr>
</tbody>
</table>

1MIC, minimum inhibitory concentration; 2109 strains tested;