Sampling bias created by ampicillin in isolation media for *Aeromonas*

Jennifer R. Huddleston, John C. Zak, and Randall M. Jeter

Abstract: Members of the bacterial genus *Aeromonas* are widely isolated from aquatic environments and studied in part for their ability to act as opportunistic pathogens in a variety of animals. All aeromonads, with the exception of *Aeromonas trota*, are generally thought to be resistant to ampicillin, so the antibiotic is frequently added to isolation medium as a selective agent. In this study, 282 aeromonads from environmental sources were isolated on a medium without ampicillin and their resistance to ampicillin determined. Of the 104 of these isolates that were judged to be independent (nonredundant), 18 (17.3%) were susceptible to ampicillin. A chi-square analysis was performed to determine the impact of ampicillin use on enumerating *Aeromonas* species from environmental samples. Our results indicate that, when ampicillin is used as a selective agent, a significant portion of the aeromonad population in at least some environments can be omitted from isolation.

Key words: Aeromonas, ampicillin, selective media.

Résumé : Les membres du genre bactérien *Aeromonas* sont largement isolés d'environnements aquatiques et étudiés entre autre pour leur capacité d'agir comme pathogène opportuniste chez une variété d'animaux. Tous les aéromonades, à l'exception de *Aeromonas trota*, sont généralement considérés comme résistants à l'ampicilline et de ce fait, cet antibiotique est fréquemment ajouté au milieu de culture lors de l'isolement comme agent de sélection. Dans cette étude, 282 aéromonades de sources environnementales ont été isolés sur du milieu sans ampicilline et leur résistance envers cet antibiotique a été déterminée. Des 104 isolats considérés indépendants (non-redondants), 18 (17,3 %) étaient sensibles à l'ampicilline. Une analyse par chi-carré a été réalisée afin de déterminer l'impact de l'utilization d'ampicilline pour la numération des espèces d'*Aeromonas* d'échantillons environnementaux. Nos résultats indiquent que, lorsque l'ampicilline est utilizée comme agent de sélection, une portion significative de la population d'aéromonades de certains environnements du moins, peut être omise lors de l'isolement.

Mots clés : Aeromonas, ampicilline, milieu de sélection.

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Introduction

Members of the genus *Aeromonas* are ubiquitous in aquatic environments and have been isolated from unpolluted as well as polluted waters, including chlorinated water distribution systems (Fox et al. 1990; Huys et al. 1997; Kühn et al. 1997*a*; Fernández et al. 2000;), freshwater–marine interfaces (Hazen et al. 1978), raw sewage (Holmes et al. 1996), and groundwater (McKeon et al. 1995). Several aeromonads cause diseases in a variety of endothermic and poikilothermic animals, including fish, amphibians, reptiles, and mammals. Aeromonads also cause serious opportunistic wound infections in humans (Janda and Abbott 1996), are associated with intestinal infections (Joseph 1996; Kühn et al. 1997*b*), and have been found to cause problems with extended-wear contact lenses (Pinna et al. 2004).

Environmental surveys of aeromonads are performed to determine their occurrence in freshwater, their correlation with water quality, and the potential of a water source to serve as a reservoir of infection (Hazen et al. 1978; Kühn et al. 1997*b*; Miranda and Castillo 1998; Fernández et al. 2000). In the primary isolation of aeromonads, a selective medium containing ampicillin is often used. Ampicillin resistance is common in most aeromonads owing to the production of chromosomally encoded β-lactamases (Jones and Wilcox 1995; Overman and Janda 1999). However, susceptibility to ampicillin is a distinguishing characteristic for one species, *Aeromonas trota* (Carnahan et al. 1991). Culture media that have been used to isolate environmental aeromonads include Rimler–Shotts medium (Shotts and Rimler 1973), mA medium (Rippey and Cabelli 1979), SGAP-10C agar (Huguet and Ribas 1991), blood agar with ampicillin (Vila et al. 2003), pril–ampicillin–dextrin–ethanol agar (Imziln et al. 1997), and ampicillin–dextrin agar (Havelaar et al. 1987). All of these media except for Rimler–Shotts medium contain ampicillin or penicillin as the selective agent.

Several previous studies, especially with clinical isolates of aeromonads, have reported virtually all of the isolates to

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be resistant to ampicillin. For example, Ko et al. (1996) studied 234 isolates that were obtained from several clinical microbiology laboratories and found 99.6% to be resistant to ampicillin. Morita et al. (1994) found that 100% of 182 aeromonads isolated from both clinical and environmental sources were ampicillin resistant. Unfortunately, no information about initial isolation procedures was presented in either of these reports. Vila et al. (2003) isolated 18 aeromonads as a cause of traveler's diarrhea from stool samples on blood agar supplemented with ampicillin and on cefsulodin–irgasan–novobiocin agar and concluded, not surprisingly, that all of the 16 isolates tested for antimicrobial susceptibility were resistant to ampicillin.

In contrast, other studies have suggested that the prevalence of ampicillin-resistant aeromonads in clinical samples may not be quite so high. Overman and Janda (1999) tested 56 clinical aeromonads for antimicrobial susceptibility and found two of 17 (12%) isolates of *Aeromonas jandaei* to be susceptible to ampicillin. No information about initial isolation procedures was given. A United States Navy study reported 43% of *Aeromonas* isolates from the Philippines to be susceptible to ampicillin (Kilpatrick et al. 1987). Abbott et al. (2003) tested 193 clinical aeromonads, including 13 type strains and 37 reference strains, for their biochemical properties and antimicrobial susceptibility. They found that, excluding *A. trota*, 9 of 69 (13%) strains representing six genomic species were susceptible to ampicillin. In addition, Palú et al. (2006) reported 8 of 28 clinical isolates (29%) to be susceptible to ampicillin plus sulbactam.

Surveys of aeromonads from nonclinical sources have also been conducted in which the bacteria were isolated on media without ampicillin. However, ampicillin resistance for these isolates was not reported (Hazen et al. 1978; Fernández et al. 2000). Goñi-Urriza et al. (2000) did report that 99% of 138 nonredundant aeromonads isolated from polluted European rivers were resistant to ampicillin but did not specify whether the isolation medium contained ampicillin. Sharma et al. (2005) found that all of 30 (100%) isolates from a river in India were resistant to ampicillin. The level of ampicillin resistance among aeromonads isolated from North American environmental sources is unclear.

Whether or not it is appropriate to use a selective medium that contains ampicillin to assess aeromonad densities and occurrence in natural water systems has not been evaluated. If the antibiotic is omitted, the background growth of other organisms makes determining which bacterial colonies are *Aeromonas* more difficult. However, if the medium does contain ampicillin, the accuracy of the occurrence and density estimates becomes questionable if the habitat contains a high percentage of ampicillin-susceptible *Aeromonas* populations.

The issue of whether the use of ampicillin in selective media significantly reduces the diversity and density estimates of *Aeromonas* assemblages from environmental samples was addressed in this study. The objectives were to (*i*) isolate pure cultures of environmental aeromonads on a culture medium without the addition of ampicillin, (*ii*) determine ampicillin susceptibility of the isolates, and (*iii*) establish whether the proportion of ampicillin-susceptible environmental aeromonads is low enough to justify the use of ampicillin as a selective agent in an isolation medium for *Aeromonas* species.

Materials and methods

Sampling

Surface water and sediment samples were collected from four urban playa lakes in Lubbock, Texas, two rural playa lakes near Shallowater, Texas, the Pecos River in Carlsbad, New Mexico, the North Fork of the Brazos River in Lubbock, Texas, and the South Fork of the Rio Hondo in Taos Ski Valley, New Mexico from March 2002 to July 2002. Four surface water samples were taken from each location at each sampling time using a 500 mL polyethylene sampling cup attached to a 2 m pole after dipping into the water twice to rinse it. Each sample was transferred to an 8 ounce (1 ounce = 28.413 062 cm^3) disposable polyethylene container with a snap-on lid (Fisher Scientific, Pittsburgh, Penn.). Four sediment samples from each location at each sampling time were collected with a trowel that was also rinsed twice before sampling. The samples were taken from sediment surface that was submerged 10–15 cm below the surface of the water. Approximately 200 g of sediment were taken and transferred to an 8 ounce disposable polyethylene container with a snap-on lid. Samples were kept at 25 °C and processed within 12 h of collection.

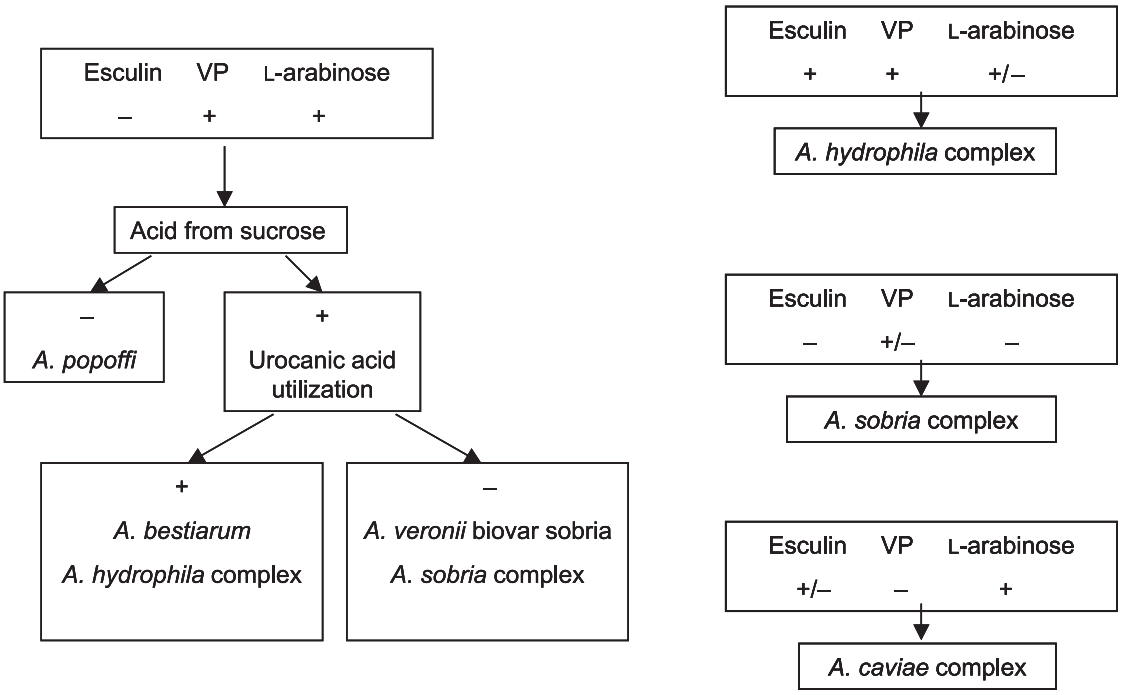
Isolation media and growth conditions

All environmental samples were inoculated directly onto the isolation medium without any prior enrichment. The water samples were diluted in sterile 0.85% NaCl to 10^{-2}, 10^{-3}, and 10^{-4}; the sediment samples were diluted in sterile 0.85% NaCl to 10^{-3}, 10^{-4}, and 10^{-5}; and 0.1 mL volumes of each dilution were plated in quadruplicate onto *Aeromonas* isolation medium without the addition of ampicillin. The medium consisted of 4 g of soluble starch (Becton Dickinson, Sparks, Md.), 0.25 g of NH_4Cl (Fisher Scientific, Pittsburgh, Penn.), 1 g of Bacto™ tryptone (Becton Dickinson), 0.5 g of Bacto™ yeast extract (Becton Dickinson), 40 mg of the pH indicator bromothymol blue (Fisher Scientific), 15 g of granulated agar (Becton Dickinson), and 1 L of distilled water. The pH was adjusted to 8.0 with 1 mol/L KOH. After autoclaving and cooling, 100 mg of sodium desoxycholate (Becton Dickinson) was added as a solid powder, and 5 mL each of 0.41% L-tryptophan (Sigma-Aldrich, St. Louis, Mo.) and 0.99% L-phenylalanine (Sigma-Aldrich) solutions were added aseptically. After inoculation and incubation at 30 °C for 36 h, *Aeromonas* appeared as light yellow, circular colonies 1–3 mm in diameter.

Presumptive identification as aeromonads

As presumptive tests for assignment as an aeromonad, Gram-negative rod isolates were screened for oxidase activity using Oxidase™ test reagent (Becton Dickinson), DNase using Difco™ DNase test agar (Becton Dickinson) supplemented with 0.05 g/L of methyl green (Manufacturing Chemists, Rochester, N.Y.), growth in Difco™ nutrient broth (NB; Becton Dickinson) and absence of growth in NB supplemented with 6% NaCl (EM Science, Gibbstown, N.J.), resistance to 10 and 150 μg of vibriostatic agent 0/129 (Oxoid, Ogdensburg, N.Y.), and anaerobic growth on 20% tryptic soy agar consisting of 8 g of Difco™ tryptic soy agar (Becton Dickinson) supplemented with 12 g of granulated agar in 1 L of distilled water. Anaerobic growth was tested in an

Fig. 1. Aeromonad complex identification schemes based on biochemical tests (Popoff 1984; Abbott et al. 2003). Only patterns of esculin hydrolysis, Voges–Proskauer (VP), and L-arabinose results pertaining to the *Aeromonas* isolates in this study are shown.



anaerobic chamber (Model 1025 Anaerobic System; Forma Scientific, Marietta, Ohio) at 30 °C for 72 h. *Aeromonas hydrophila* American Type Culture Collection (ATCC) 7965, *Aeromonas veronii* bv. sobria ATCC 9071, *Aeromonas caviae* ATCC 15468^T, *Vibrio fischeri* 345 (Presque Isle Cultures, Presque Isle, Penn.), and *Escherichia coli* DH5α were controls.

The *gyrB* gene from three of the presumptive aeromonad isolates was sequenced to confirm their identities as *Aeromonas* species. The *gyrB* gene was amplified from each isolate using primers GyrB3F (5′-TCCGGCGGTCTGCACGGCGT-3′) and GyrB14R (5′-TTGTCCGGGTTGTACTCGTC-3′) (Yañez et al. 2003) and TaKaRa Ex Taq™ reagents (Fisher Scientific) in a GeneAmp™ PCR System 9600 (Perkin-Elmer, Wellesley, Mass.). Each reaction consisted of 25 pmol of each primer, 5.0 μL of 10× TaKaRa Ex Taq™ buffer, 2.0 mmol/L of each dNTP, 100 ng of template DNA, and 1 U of TaKaRa Ex Taq™ polymerase made up to 50 μL with sterile water. The PCR conditions were as follows: initial denaturation at 94 °C for 2 min, 30 subsequent cycles of denaturation at 93 °C for 30 s, annealing at 60 °C for 30 s, and elongation at 72 °C for 1 min with no final extension. Amplicons were extracted and purified using QIAEX II™ gel extraction kit (Qiagen, Valencia, Calif.) according to the manufacturer's instructions. Amplicons were sequenced by the Texas Tech University Center for Biotechnology and Genomics. The sequences were compared to known aeromonad *gyrB* sequences deposited in GenBank using Vector NTI Suite 9.

Identification of isolates

Biochemical tests to determine membership in the *A. hydrophila*, *A. caviae*, and *A. sobria* complexes were based on data from Popoff (1984) and Abbott et al. (2003). Flow charts with the identification schemes are shown in Fig. 1. These tests included esculin hydrolysis, Voges–Proskauer, acid from L-arabinose, and acid from sucrose fermentation. The *Aeromonas* ATCC cultures listed in the previous section were used as controls to ensure accurate complex designations.

Organisms from field isolations were identified to genus and species, when possible, using Biolog MicroLog™ 3, release 4.20 (Biolog, Hayward, Calif.). Cultures were grown overnight at 30 °C on Columbia agar base (EMD Chemicals Inc, Gibbstown, N.J.) supplemented with 5% defibrinated sheep blood (Colorado Serum Company, Denver, Colo.). The colonies were resuspended in pre-warmed Biolog GN/GP-IF (inoculating fluid) and adjusted turbidometrically within the recommended range, and 150 μL of the suspensions were added to each well of a 96-well Biolog GN2 MicroPlate™ test panel. Plates were read with an automated Biolog MicroStation Reader™ after incubation at 30 °C for 16–24 h. Identifications were made using the Biolog Gram-negative database (release 4.20).

Enterobacterial repetitive intergenic consensus (ERIC)–PCR

Amplification of ERIC sequences was performed on the isolates to demonstrate strain uniqueness. ERIC–PCR has been used previously to type aeromonads (Sechi et al. 2002; Soler et al. 2003; Szczuka and Kaznowski 2004; Aguilera-Arreola et al. 2005). The following primers were used: ERIC 1R (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and ERIC 2 (5′-AAGTAAGTGACTGGGGGT-3′) (Vila et al. 1996). The reactions consisted of 50 pmol of each primer, 2.5 μL of 10× TaKaRa Ex Taq™ buffer, an additional 1.5 mmol/L $MgCl_2$, 4.0 U mmol/L of each dNTP, 125 ng of template DNA, and 2 of TaKaRa Ex Taq™ polymerase made up to 25 μL with sterile water. The PCR conditions were as follows: 30 cycles of denaturation at 94 °C for 1 min 30 s, annealing at 52 °C for 1 min 45 s, elongation at 65 °C for 8 min 13 s, and a final extension of 65 °C for 16 min. The PCR denaturation,

annealing, and elongation times were extended beyond those of the aeromonad ERIC–PCR studies mentioned above because the reactions were carried out in a Robocycler™ Gradient 96 (Stratagene, La Jolla, Calif.). The products were electrophoresed on a 2.0% agarose gel at a voltage of 70 V for 2 h. Amplifications of 20% of the isolates were performed in triplicate to demonstrate reproducibility.

Ampicillin susceptibility testing

Ampicillin susceptibility testing was originally performed in conjunction with testing of other antibiotics, drugs, metals, and metalloids under a uniform set of conditions. A complete description of these results will be published in a separate report (Huddleston et al. 2006). All presumptive aeromonad isolates were grown on 20% tryptic soy agar. The isolates were incubated for 24 ± 2 h at 30 °C. Isolated colonies were picked from the overnight cultures, inoculated into 4.5 mL of 40% NB, pH 7.0, and adjusted to an A_{560} value of 0.12 ± 0.02 (0.5 McFarland standard). Then, 5.5 µL of the culture were inoculated into 5.5 mL of 40% NB (a 1:1000 dilution). After mixing, 100 µL of the suspensions were inoculated into wells of Costar™ 96-well cell-culture cluster plates (Fisher Scientific) with 200 µL of 40% NB supplemented with ampicillin sodium salt (Sigma-Aldrich). Ampicillin concentrations were set up in twofold serial dilutions from 0 to 64 µg/mL. The plates were incubated at 30 °C for 24 ± 2 h and then read using an automated plate reader (Bio-Tek EL311sc Auto Reader, Winooski, Vt.). An A_{550} value exceeding 0.1 indicated growth. *Aeromonas hydrophila* ATCC 7965 and *Pseudomonas aeruginosa* ATCC 27853 were used as positive (ampicillin-resistant) controls, and *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as negative (ampicillin-susceptible) controls. Ten percent of the isolates were retested for ampicillin susceptibility under the same conditions to verify reproducibility and accuracy of the test results.

Isolates were also tested by the reference broth microdilution procedure as recommended by the Clinical and Laboratory Standards Institute, M7-A7, (2006) to verify the accuracy of the 40% NB tests. Isolated colonies of each strain were grown in Mueller–Hinton broth (Becton Dickinson), pH 7.3, adjusted with cations for a final concentration of 411.7 µmol/L $MgCl_2 \cdot 6H_2O$ (Fisher Scientific), which is 10 mg Mg^{2+}/L, and 500.6 µmol/L $CaCl_2 \cdot 2H_2O$ (Fisher Scientific), which is 20 mg Ca^{2+}/L, and incubated for 24 ± 2 h at 37 °C. Overnight cultures were diluted in 0.9% NaCl and adjusted to an A_{560} value of 0.12 ± 0.02 (0.5 McFarland standard). The suspensions were inoculated into the wells of Costar™ 96-well cell-culture cluster plates for a final concentration of 5×10^5 CFU/mL per well in 150 µL of cation-adjusted Mueller–Hinton broth (CAMHB) with and without a supplement of 32 µg/mL ampicillin sodium salt (Sigma-Aldrich). The plates were incubated at 37 °C for 16–20 h and then read using an automated plate reader as stated above. Isolates found to be susceptible to ampicillin, including the control strains, were re-tested using the Bauer–Kirby method with cation-adjusted Mueller–Hinton agar and BBL Sensi-Disc™ ampicillin susceptibility discs (Becton Dickinson).

Statistical analysis

The minimum sample size needed to detect a difference in *Aeromonas* numbers with and without ampicillin was determined by the equation: $N_{min} = [(z_c \sqrt{pq}) / e]^2$ where p = 0.993 (Goñi-Urriza et al. 2000), q = 0.004, and z_c = 1.96 for 95% confidence. The tolerance limit or effect size, e, was 0.016. After the minimum sample size was determined, the frequencies of ampicillin-resistant and ampicillin- susceptible aeromonads were counted and compared with the expected frequencies using a χ^2 test. The complete expression used was

$$\chi^2 = \sum [(\text{observed count} - \text{expected count})^2 / \text{expected count}]$$

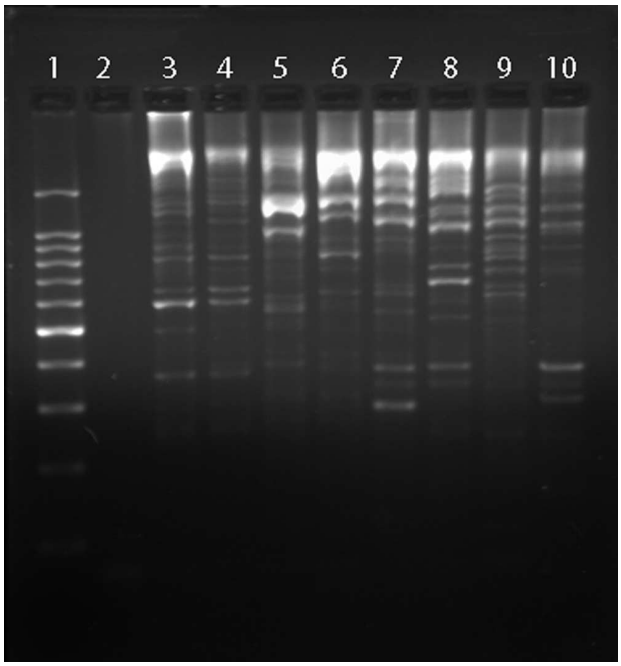


Fig. 2. Enterobacterial repetitive intergenic consensus (ERIC)–PCR of eight different *Aeromonas* strains. Lanes: 1, 100 bp DNA molecular size markers; 2, no DNA template control; 3, *Aeromonas hydrophila* complex; 4, *A. hydrophila* complex; 5, *A. hydrophila* complex; 6, *Aeromonas caviae* complex; 7, *A. caviae* complex; 8, *A. caviae* complex; 9, *Aeromonas sobria* complex; 10, *A. sobria* complex.

Results and discussion

Two hundred eighty-two aeromonad isolates were obtained from March to July 2002 from eight different sampling locations across a geographical area of 83 000 km^2 in West Texas and New Mexico. A total of 28 samples were taken and ten to 11 aeromonads were isolated from each sample. All of these isolates were new and did not overlap with any of those characterized in a previous report (Warren et al. 2004). At least 104 isolates were judged to be independent strains (not clones) based on differences in sampling location, sampling date, antimicrobial resistance patterns, plasmid profiles, and phenotypic characteristics. ERIC–PCR results supported this conclusion. All strains varied in their ERIC patterns by one or more major bands. The amplifications were reproducible. A sample gel with eight different patterns is shown in Fig. 2. All 104 isolates were identified by the

Biolog system as belonging to the genus *Aeromonas*. Among the 104 aeromonad isolates, 18 were susceptible to ampicillin. The ampicillin-susceptible aeromonads were isolated from seven different locations and from both sediment and freshwater samples. Three isolates, including one that was susceptible to ampicillin, were confirmed to be *Aeromonas* based on the DNA sequence of the *gyrB* gene (Yañez et al. 2003).

Identification to genus by Biolog was accurate. However, the biochemical tests used to differentiate the isolates into the three major aeromonad complexes were not always consistent with the Biolog species identifications. Other researchers have also concluded that Biolog can correctly identify *Aeromonas* to genus, but not always to species (Ciapini et al. 2002; Tokajian and Hashwa 2004). For the 31 isolates determined biochemically to belong to the *A. sobria* complex, Biolog identified 19 (61.3%) as species belonging to this complex. For the *A. hydrophila* complex, Biolog identified 13 of 39 (36.8%) of these isolates as belonging to this complex. In the *A. caviae* complex, four of 26 (15.4%) of these isolates had the same identifications by both the biochemical tests and Biolog. Furthermore, 31 of 104 (29.8%) of the isolates were identified by Biolog only to genus, not to species.

Susceptible Gram-negative, non-*Enterobacteriaceae* bacteria are inhibited at less than 32 μg/mL of ampicillin (Clinical and Laboratory Standards Institute 2006). Of the isolates tested, 86 of 104 (82.6%) were resistant to concentrations of ampicillin exceeding 64 μg/mL. Ampicillin susceptibility was found to occur in 18 of 104 (17.3%) of the isolates. Ampicillin susceptibility testing in CAMHB at 37 °C gave comparable results to tests performed in 40% NB at 30 °C with the exception of one isolate. Isolates presumed to be ampicillin susceptible from both the 40% NB and CAMHB tests were confirmed as susceptible using the Bauer–Kirby method. Nine of the 18 susceptible isolates were inhibited at concentrations of less than 4 μg/mL. One isolate was inhibited at 8 μg/mL, two isolates at 16 μg/mL, and six isolates at 32 μg/mL. The ampicillin-susceptible *Aeromonas* were identified as belonging to seven different species as determined by Biolog. None of the isolates were determined to be *A. trota* by either Biolog or biochemical tests. None of the strains that were negative for esculin hydrolysis, Voges–Proskauer test, and L-arabinose fermentation were able to both ferment cellobiose and utilize citrate, as *A. trota* is expected to do (Carnahan et al. 1991). Only one isolate was identified as by Biolog as *A. jandaei,* which has been shown to occasionally be ampicillin susceptible (Overman and Janda 1999).

To determine if there was a significant difference in the percentage of ampicillin-susceptible aeromonads in the environmental population versus that previously observed by Goñi-Urriza et al. (2000), the minimum sample size needed to detect an effect of ampicillin on the structure of the *Aeromonas* assemblage was determined to be 60 aeromonad isolates based on a normal distribution. In our study, the sample size was 104 aeromonads.

Using the results of Goñi-Urriza et al. (2000) as a low estimate of aeromonad ampicillin resistance, if an unbiased sampling procedure is assumed and 137 of 138 (99.3%) of aeromonads are resistant to ampicillin, then a 95% confidence interval would indicate that resistant aeromonads will comprise 98.0%–100.0% of the population. The observed frequencies of resistance and susceptibility to ampicillin for the 104 aeromonad isolates in this study were 86 resistant and 18 susceptible. Using 0.993 as the proportion for aeromonad resistance (Goñi-Urriza et al. 2000), the number of isolates expected to be resistant out of 104 isolates should be 103, while only one should be susceptible. For the chi-square analysis, the null hypothesis was $\chi^2 \leq 10.8$ and the research hypothesis was $\chi^2 > 10.8$. The experimental χ^2 was determined to be $\chi^2 = 291.8$ with one degree of freedom, $\alpha_c = 0.05$ and $p = < 0.001$. The chi-square analysis rejected the null hypothesis that the number of resistant *Aeromonas* isolates was similar to that obtained by Goñi-Urriza et al. (2000).

Thus, the results from this study indicate that less than 99.3% of environmental aeromonads are resistant to ampicillin. The observed 17.3% of ampicillin-susceptible environmental *Aeromonas* strains is greater than that previously reported for most aeromonad isolates of known environmental origin. Our results indicate that ampicillin should not be used as a selective agent in isolation medium for *Aeromonas* when a complete analysis of *Aeromonas* diversity and density is desired. The addition of ampicillin to an isolation medium could likely inhibit the growth of a significant portion of *Aeromonas* assemblages in samples and provide an underestimation of densities and species diversity. We also recommend that researchers reporting ampicillin resistance in *Aeromonas* specify whether or not ampicillin was used in the isolation medium. If ampicillin was used as a selective agent, then ampicillin resistance of the resulting isolates should not be reported since this information is redundant and can be misleading.

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