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The Activity of Ceftiofur Sodium for *Aeromonas* spp. Isolated from Ornamental Fish

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ABSTRACT: Our objective was to determine the activity of ceftiofur sodium against *Aeromonas hydrophila* and *A. sobria* isolated from a variety of domestic and imported tropical fish. Twelve antimicrobial drugs were tested for effectiveness against these aeromonads using the Kirby-Bauer Disk Diffusion technique and minimum inhibitory concentration determinations. Ceftiofur sodium was highly effective in vitro against aeromonads isolated from ornamental fish. Of the 42 isolates of *Aeromonas* spp. tested, none were resistant to ceftiofur sodium; however, all isolates were resistant to ampicillin, and 71% were resistant to tetracycline.

Key words: ceftiofur sodium activity, *Aeromonas*, ornamental fish.

It is well established in the pet fish industry that bacterial infections are responsible for heavy losses, from the farm level to the hobbyist tank. *Aeromonas* spp. has emerged as the most common bacterial pathogen; it causes infections in wounds, and opportunistic infections following the stresses of temperature change, handling, or poor water quality. Resistance of *Aeromonas* spp. to commonly used antibiotics is an emerging problem in the ornamental fish industry; for example, isolates of *Aeromonas* spp. from tropical fish imported from Singapore were resistant to tetracycline, sulfa drugs, erythromycin, and to a lesser degree to quinolone antibacterial drugs (Dixon et al., 1990). Antimicrobial resistance also has been documented in bacteria isolated from food fish. Aeromonad resistance to tetracycline (Terramycin®) and ormethoprim/sulfadimethoxine (Romet-30®), the only two antibiotics approved for use on food fish in the United States, has been reported by numerous authors (Bullock, 1984; MacMillan, 1985; Ganzhorn, 1987; Hastings and McKay, 1987; Taylor, 1987; Stamm, 1989).

Many of the commonly used antimicrobial drugs may no longer prove efficacious for the treatment of this Gram-negative fish pathogen (Dixon et al., 1990). It may be necessary at this time to begin screening antimicrobial compounds for efficacy against *Aeromonas* spp.

Naxcel®, the sodium salt of ceftiofur, is a broad spectrum B-lactamase-resistant cephalosporin. Naxcel® currently is marketed by the Upjohn Company (Kalamazoo, Michigan, USA) for treatment of bovine respiratory diseases associated with *Pasteurella hemolytica* and *P. multocida* infections. Ceftiofur also has excellent *in vitro* activity against Gram-negative bacteria such as *Escherichia coli, Salmonella typhimurium* and *Actinobacillus pleuropneumoniae* (Yancey et al., 1987).

Our objective was to determine the *in vitro* activity of ceftiofur sodium (Naxcel®) against *Aeromonas* spp. isolated from domestic and imported ornamental fish.

MATERIALS AND METHODS

To determine the efficacy of ceftiofur sodium, 50 freshwater ornamental fish were obtained from a local wholesaler (Pan Ocean Aquarium, Inc., Hayward, California, USA) over the course of 4 mo. The fish included representatives from the following families: Anabantidae, Callichthyidae, Cichlidae, and Cyprinidae. Samples of kidneys or any evident external lesions were streaked on Rimler-Shotts medium (Shotts and Rimler, 1973) for primary isolation of *Aeromonas* spp., and incubated at 30 C. Oxidase positive isolates were identified to genus according to Fish Health Section Bluebook guidelines (Amos, 1985) using the following tests; motility, sensitivity to novobiocin (5 mcg), growth in salt-free nutrient gelatin, and fermentation of glucose on OF medium. Suspect *Aeromonas* spp. were inoculated into the Nonfermenter Test strip (NFT) system (Analytab Products, Plainview, New York, USA) for species identification. Once identified, stock cultures of the isolates were maintained on tryptic soy agar slants (Remel, Sacramento, California). Stock isolates were grown for 24 hr at 30 C, matched to a 0.5

TABLE 1. Activity of Ceftiofur sodium for *Aeromonas* spp. isolated from freshwater domestic and imported ornamental fish.

Antibiotic	Number of resistant isolates	Percent of resistant isolates (n = 42)
Ampicillin	42	100
Tetracycline	30	71
Nalidixic acid	11	26
Triple sulfa	11	26
Erythromycin	8	19
Nitrofuradontoin	8	19
Romet-30® (ormetoprim-sulfadimethoxine)	5	12
Sulfamethoxazone-trimethoprim	5	12
Oxolinic acid	5	12
Neomycin	4	9.5
Trimethoprim	4	9.5
Ceftiofur sodium	0	0

McFarland Standard and streaked onto Mueller-Hinton agar (Remel, Sacramento, California). In vitro antibiotic sensitivity to 12 antimicrobial drugs was determined using the Kirby-Bauer disk diffusion technique (Bauer et al., 1966). Twelve susceptibility disks were used. Erythromycin (0.015 mg), nalidixic acid (0.03 mg), neomycin (0.03 mg), and sulfamethoxazole (25 mcg)/trimethoprim (25 mcg) were obtained from General Diagnostics (Morris Plains, New Jersey, USA). Nitrofurantoin (0.3 mg), triple sulfa (0.3 mg), tetracycline (0.03 mg), trimethoprim (5 mcg), and ampicillin (10 mcg) were obtained from Difco Laboratories (Detroit, Michigan). Oxolinic acid (2 mcg) and ormetoprim (1.2 mcg)/sulfadimethoxine (23.8 mcg) (Romet-30®) were sampled by Baltimore Biological Laboratories (Cockeysville, Maryland, USA). Ceftiofur sodium (30 mcg) was supplied by the Upjohn Company (Kalamazoo, Michigan). Following incubation at 30 C for 24 hr, inibition zone sites were measured. Minimum inhibitory concentrations (MIC) were determined by the Veterinary Diagnostic Laboratory System, University of California, Davis, California, using Sensititre susceptibility plates (Radiometer/Copenhagen Company, Westlake, Ohio, USA).

We sampled bacteria from the zone of inhibition around the ceftiofur sodium disk. As a control, we sampled bacteria from the same plate in an area without antibiotic exposure. The samples were fixed in 2% glutaldehyde for 30 min, rinsed twice in 0.05 M sodium cacodylate buffer for 10 min each at pH 7.2, fixed for 60 min in 1% osmium tetroxide, and rinsed twice in 2% sodium cacodylate buffer for 10 min each. The samples were stained for 30 min with 2% uranyl acetate made up in 30% ethanol. Dehydration was completed with 50% ethanol for 10 min, 70% ethanol for 10 min, 95% ethanol twice for 10 min each, and 100% ethanol three times for 10 min each. The samples were embedded in Spurr's resin (Ted Pella, Inc., Redding, California); sections were taken with a glass knife. The sections were stained with uranyl acetate and lead citrate, and examined using a Hitachi HS-8 transmission electron microscope (Hitachi Scientific Instruments, Mountain View, California).

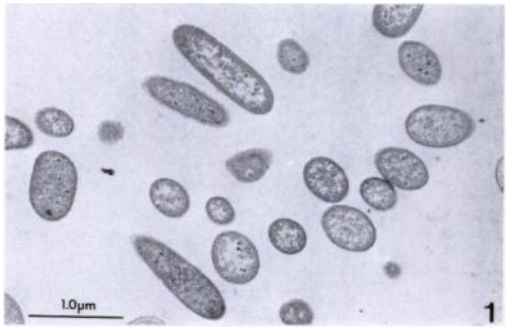

FIGURE 1. *Aeromonas sobria* unexposed controls; 24-hr culture grown on Mueller-Hinton agar at 30 C. Note uniform rod shaped cells.

RESULTS

Twenty-four of the 42 isolates were identified as *A. sobria*, 12 as *A. hydrophila* and six as *Aeromonas* spp. None of the isolates were resistant to ceftiofur sodium as determined by zone of inhibition size measurements. Zone sizes ranged from 19 to 35 mm. Minimum inhibitory concentrations ranged from 0.25 to 1.0 μg/ml, well within the range of susceptibility. However, all 42 isolates were resistant to ampicillin, and 30 also were resistant to tetracycline (Table 1).

Control cells appeared as uniform rod-shaped cells (Fig. 1). In contrast, aeromonad cells exposed to ceftiofur sodium appeared elongate with several indentations along the cell wall (Fig. 2).

DISCUSSION

Ceftiofur sodium caused in vitro inhibition of *Aeromonas* spp. isolated from do-

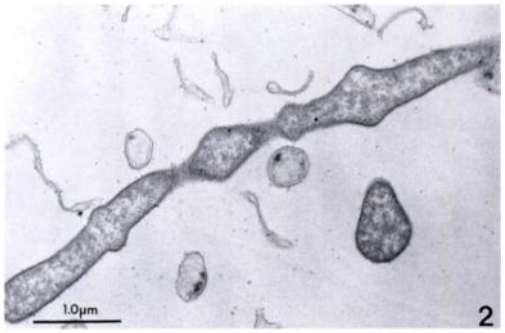

FIGURE 2. *Aeromonas sobria* exposed to 30 mcg of ceftiofur sodium; 24-hr culture grown on Mueller-Hinton agar at 30 C. Note indentations along the cell wall.

mestic and imported ornamental fish. Naxcel®, the trade name for ceftiofur sodium, previously was shown to be very effective in the control of Gram-positive and Gram-negative bacterial pathogens of veterinary importance both in vivo and in vitro (Yancey et al., 1987). Ceftiofur sodium, like other cephalosporins, is bacteriocidal by inhibiting cell wall synthesis. Aeromonad cells exposed to ceftiofur appeared elongate with the presence of numerous indentations of the cell wall (Fig. 2) compared with uniform control cells (Fig. 1).

In 1988, Naxcel® was approved by the Food and Drug Administration for the treatment of respiratory disease of cattle (U.S. Food and Drug Administration, 1988). Its broad spectrum activity partly is attributed to its resistance to bacterial B-lactamase (Jaglan et al., 1989).

Antibiotic resistant bacteria have been described from both food fish and shrimp culture (Brown, 1989). *Aeromonas* spp. resistant to several antimicrobial drugs have been observed from natural environments, including Chesapeake Bay (Maryland) and areas in Asia (McNicol et al., 1980). Dixon et al., (1990) reported that over half of 70 isolates of *Aeromonas* spp. isolated from pet fish imported from Singapore were resistant to seven of 12 antimicrobial drugs tested.

Because of the bacterial resistance to antibiotics already observed in human and veterinary medicine, bacterial resistance to antibiotics is a potential problem in the domestic and imported ornamental pet fish industry. Further in vivo study is needed to determine the potential for ceftiofur sodium as an efficacious treatment for aeromonad infections of ornamental fish.

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