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Evaluation of Aquarium Antibiotic Formulations

T. J. TRUST AND DIANE C. CHIPMAN

Department of Bacteriology and Biochemistry, University of Victoria, Victoria, British Columbia, Canada

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The antibacterial activity of eight products marketed for the therapy and prophylaxis of diseases of ornamental fishes was tested. The products contained erythromycin, neomycin, a nitrofuran, penicillin, sulfa compounds, streptomycin, or tetracycline. When used at the concentration recommended by the manufacturer, the products failed to inhibit the growth of bacterial species known to be potential pathogens of ornamental fishes and failed to reduce significantly the bacterial numbers in water containing fish. Concentrations of the products that were bacteriostatic were markedly higher than the in-use concentration recommended by the manufacturer. The danger presented by the unrestricted availability of antibiotic compounds frequently used in the treatment of human and animal disease is discussed.

Large numbers of a wide variety of species of ornamental fishes are sold throughout the world to hobbyists for home aquaria and are also used extensively in a number of areas of biological research (5). Many of these fish subsequently succumb to bacterial diseases, the most common being fin and tail rot, furunculosis, gill infections, piscine mycobacteriosis, columnaris disease, and infectious hemorrhagic septicemia (6). A number of products are manufactured and marketed for the therapy and prophylaxis of these diseases. Some of these products are liquid formulations containing such compounds as the acridines, formaldehyde, malachite green, methylene blue, potassium permanganate, quaternary ammonium compounds, or silver oxide, compounds that do not exhibit selective toxicity against bacterial cells. The antibacterial activity of 14 of these liquid formulations was tested, and it was shown that when used at the dilutions recommended by the manufacturers, the products failed to inhibit the growth of bacterial species known to be potential pathogens of freshwater fishes and failed to reduce significantly the bacterial numbers in water containing fish (18). Another group of products is available to treat diseases of ornamental fishes. These solid formulations are reputed to contain compounds known to exhibit selective toxicity against bacteria. These include erythromycin, neomycin, a nitrofuran, penicillin, streptomycin, the sulfa compounds, and tetracycline. Because the previous study had demonstrated the liquid formulations to be inadequate, it was thought desirable to continue the previous study to ascertain the effectiveness of these solid formulations.

MATERIALS AND METHODS

Products tested. Products 1, 2, 3, 4, 5, 6, and 8 were purchased from commercial outlets in Victoria, British Columbia; product 7 was supplied by the manufacturer. This latter product contained 6-hydroxymethyl-2-[2-(5-nitro-2-furyl)vinyl]pyridine (NFP). This compound has recently been marketed as an antibacterial specifically useful for fish culture. The products were marketed by three manufacturers in the United States for the therapy and prophylaxis of infections of aquarium fishes. The active antibacterial ingredients in each formulation, the weight of each antibacterial ingredient per capsule or tablet, and the manufacturers' recommended usage concentrations expressed in terms of the active antibacterial components are shown in Table 1. Additional information on the presence or absence of other active ingredients in product 7 was not available. Pure samples of the active, selectively toxic compounds present in the above formulations were also tested for comparative purposes. Erythromycin was purchased from Sigma Chemicals Co., and potassium penicillin, neomycin sulfate, sodium sulfamerazine, sodium sulfamethazine, sodium sulfathiazole, streptomycin sulfate, and tetracycline hydrochloride were purchased from Nutritional Biochemicals Corp. Pure samples of NFP were not available. All solids were dried to constant weight before use. Standard solutions were prepared according to the recommendations of the Association of Official Analytical Chemists (4) and Kavanagh (11, 12). Product 1 and neomycin sulfate solutions were both prepared in 0.05 M tris(hydroxymethyl)aminomethane buffer (pH 8.0); product 2 and tetracycline hydrochloride solutions were both prepared in 0.048 M KH_2PO_4 (pH 4.5). A 0.14 M KH_2PO_4-K_2HPO_4 buffer (pH 7.0) was used to prepare

TABLE 1. *Details of products tested*

Product[a]	Antibacterial ingredients[b]	Wt of antibacterial ingredients/capsule or tablet	Manufacturers' recommended usage concn (μg of active component/ml)
1	Neomycin sulfate	10	0.3
	Copper sulfate	27	0.8
2	Tetracycline hydrochloride	250	6.5
3	Streptomycin sulfate	10	0.3
	Merbromin	10	0.5
4	Sodium sulfathiazole	65	3.2
	Acriflavine	22	1.1
5	Sodium sulfathiazole	166	8.1
	Sodium sulfamerazine	166	8.1
	Sodium sulfamethazine	166	8.1
	Neomycin sulfate	10	0.3
6	Erythromycin	200	10.6
7	NFP[c]	7.5	0.2
8	Potassium penicillin	12 (200,000 U)	0.6

[a] Market name, control or lot number, and manufacturer are listed in laboratory protocol. Names available on request.

[b] Information obtained from manufacturers' packages.

[c] NFP, 6-Hydroxymethyl-2-[2-(5-nitro-2-furyl)vinyl]pyridine.

solutions of both erythromycin and product 6. All other stock solutions were prepared in distilled water.

Test organisms. *Aeromonas hydrophila* ATCC 9071, *Aeromonas liquefaciens* ATCC 14715, *Aeromonas salmonicida* ATCC 14174, a *Cytophaga* species, *Mycobacterium fortuitum* ATCC 9820, *Mycobacterium marinum* ATCC 927, *Pseudomonas fluorescens* ATCC 13525, *Pseudomonas putida* ATCC 12633, and *Vibrio anguillarum* ATCC 19264 were selected for study because these species cause infection in aquarium fishes (6). These species of *Aeromonas*, *Mycobacterium*, and *Pseudomonas* have also been isolated from a variety of clinical diseases in man (2, 10, 21).

Escherichia coli ATCC 11775, *Klebsiella pneumoniae* ATCC 10031-1, *Pseudomonas aeruginosa* ATCC 15442, *Salmonella typhi* ATCC 167-P, and *Staphylococcus aureus* ATCC 6538 and ATCC 6538 P were chosen for study because these species are recommended for assays of antibacterial compounds (4, 11, 12). These species are also potential human pathogens (22). Species of *Aeromonas*, *Pseudomonas*, and *Enterobacteriaceae* are common in aquarium water (19). *Bacillus subtilis* ATCC 6633 was chosen because of its ability to form spores and because *Bacillus* species are common in fish feeds (20). A spore suspension was used in assays.

Preparation of inocula. Different cultural conditions were required to prepare inocula of the test species for the antibacterial assays. Cultures of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus* were incubated at 37 C for 24 h in antibiotic assay broth (BBL); *A. hydrophila*, *A. liquefaciens*, *P. putida*, *P. fluorescens*, and *V. anguillarum* were incubated at 30 C for 24 h. *A. salmonicida* was grown at 30 C for 48 h. The *Cytophaga* species, *M. fortuitum*, and *M. marinum* were grown on Penassay agar slants at 30 C for 48 h, and the growth on each slant was suspended in antibiotic assay broth. The *B. subtilis* spore suspension was prepared by the method of the Association of Official Analytical Chemists (4). This suspension contained 3×10^8 viable spores per ml. All other cultures were adjusted to contain approximately 10^8 cells per ml. These cultures were then used to assay the bacteriostatic properties of the test formulations.

Bacteriostatic activity of products. The dilution of each test product that prevented the growth of each test species was determined by serial tube dilution, using the method described by Kavanagh (11). Duplicate twofold dilutions of the test product were prepared in broth. Compounds containing erythromycin, NFP, neomycin sulfate, penicillin, streptomycin sulfate, or tetracycline hydrochloride were assayed in antibiotic assay broth (pH 7.0). Mueller-Hinton broth (BBL) (pH 7.4) was used for assays of products containing sulfa compounds. Each 5-ml dilution was then inoculated with 0.03 ml of the appropriate inoculum culture and incubated at 30 C for 96 h. Assays with *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus* were incubated at 37 C for 96 h. Tubes were then examined for the presence or absence of growth of the test species.

Antibacterial activity of products in aquaria holding fish. The ability of the products to reduce the viable aerobic bacterial count in the aquarium water in the presence of goldfish (*Carassius auratus*) was generally tested at one, two, and four times the concentration recommended by the manufacturers (18). The effect of the control antibacterial compounds on the viable bacterial numbers was also tested. The concentrations chosen were based on the highest concentrations added to commercial test disks used to determine the antibiotic susceptibility of

gram-negative species. Hence, assays of the control antibacterial compounds were generally performed at one, two, and four times this concentration. In several experiments, concentrations higher than these were also tested. The fish were acclimatized in a holding aquarium for at least 7 days before the assays. The bioassays were performed as recommended by the American Public Health Association (3) in 6-liter round glass jars, each containing 5 liters of distilled water (adjusted to pH 7). The jars were aerated at 1 liter/min and held at 22 C. Ten goldfish (mean weight, 1.2 g; range of weights, 0.9 to 1.6 g) were placed in each of four assay jars. The fish were placed in the test squarium 48 h before the addition of the test formulation and were not fed until the end of the bioassay. The pH of the water in the aquaria was between pH 6.5 and pH 6.8 immediately before addition of the test formulation. The formulation under test was then added at the appropriate concentration to each of three aquaria. The fourth was maintained as a control. In assays with NFP or tetracycline, the jars were covered with aluminum foil to minimize photodecomposition of the antibacterial compound. Each jar was sampled immediately before addition of the product (zero time) as well as 0.5, 1, 3, 6, 12, 24, 48, and 72 h after addition of the product. The viable bacterial load present in these water samples was determined by the drop pipette method of Miles and Misra (13). The count procedure used standard methods agar (BBL) plates and duplicate serial dilutions in sterile buffered water. The plates were incubated at 20 C for 72 h, and the colonies were then counted.

RESULTS

The concentration of active ingredients of each formulation required to inhibit growth of the test species is shown in Table 2. None of the products inhibited growth of all test species when used at the concentration recommended by the manufacturers. The concentrations of the active ingredients of product 1 required to inhibit growth of all the test species were 25 μg of neomycin per ml and 67.6 μg of $CuSO_4$ per ml. The recommended usage concentration of tetracycline in product 2 inhibited the growth of all species except *B. subtilis*, *M. marinum*, and *P. aeruginosa*. These species were inhibited by the tetracycline in product 2 at 100 μg/ml. Product 3 required more than 25 μg of streptomycin and 50 μg of merbromin per ml to inhibit the test species, whereas product 4 required 125 μg of sulfathiazole and 50 μg of acriflavine per ml. With this assay technique, product 5 failed to inhibit the test species even when used at 225 μg of each sulfa compound per ml and 9.3 μg of neomycin per ml. Product 6 produced bacteriostasis with 500 μg of erythromycin per ml. In product 7 more than 1.6 μg of NFP per ml was required to inhibit all the test

TABLE 2. *Bacteriostatic activity of aquarium antibacterial compounds*

Product	Manufacturers' recommended usage concn (μg of active ingredient/ml)	Concn of active ingredients required to inhibit growth (μg/ml)															
		A. hydrophila 9071	*A. liquefaciens* 14715	*A. salmonicida* 14174	*B. subtilis* 6633 (spores)	*Cytophaga* sp.	*K. pneumoniae* 10031-1	*M. fortuitum* 9820	*M. marinum* 927	*P. aeruginosa* 15442	*P. fluorescens* 13525	*P. putida* 12633	*S. aureus* 6538	*S. aureus* 6538-P	*V. anguillarum* 19264	*E. coli* 11775	*S. typhi* 167-P
1	Neomycin (0.3)	12.5	12.5	25	6.3	6.3	6.3	12.5	25	12.5	25	25	12.5	12.5	12.5	10	5
	$CuSO_4$ (0.8)	33.8	33.8	67.6	17	17	17	33.8	67.6	33.8	67.6	67.6	33.8	33.8	33.8	27	13.5
2	Tetracycline (6.5)	0.6	1.3	1.3	10	1.3	1.3	5	>50	50	5	5	0.2	0.2	0.6	5	2.5
3	Streptomycin (0.3)	10	10	10	10	5	5	10	0.3	>25	10	10	5	1.3	6.25	12.5	12.5
	Merbromin (0.5)	20	20	20	20	10	10	20	0.6	>50	20	20	10	2.5	12.5	25	25
4	Sulfathiazole (3.2)	25	25	25	125	50	20	12.5	25	125	100	100	50	25	10	100	25
	Acriflavine (1.1)	10	10	10	50	20	10	5	10	50	40	40	20	10	4	40	10
5	Sulfathiazole (8.1)	112.5	45	56.3	11.3	84	56.3	112.5	112.5	>225	112.5	112.5	45	45	105	105	84
	Sulfamethazine (8.1)	112.5	45	56.3	11.3	84	56.3	112.5	112.5	>225	112.5	112.5	45	45	105	105	84
	Sulfamerazine (8.1)	112.5	45	56.3	11.3	84	56.3	112.5	112.5	>225	112.5	112.5	45	45	105	105	84
	Neomycin (0.3)	4.2	1.7	2.1	0.4	3.1	2.1	4.2	4.2	>9.3	4.2	4.2	1.7	1.7	3.9	3.9	3.1
6	Erythromycin (10.6)	125	250	50	25	100	125	20	500	250	250	250	5	5	25	125	250
7	NFP[a] (0.2)	0.8	0.8	0.4	>1.6	>1.6	0.8	>1.6	>1.6	>1.6	>1.6	0.8	1.6	1.6	0.2	0.8	0.8
8	Penicillin (0.6)	>30	>30	3	30	0.15	>30	>30	>30	>30	>30	>30	0.15	0.15	7.5	15	1.5

[a] NFP, 6-Hydroxymethyl-2-[2-(5-nitro-2-furyl)vinyl]pyridine.

species, whereas product 8 required more than 30 μg of penicillin per ml. In all cases, these bacteriostatic concentrations were markedly higher than the recommended concentrations, ranging from 8 to 83 times that recommended.

The minimal inhibitory concentrations of the standard reference antibacterial compounds are shown in Table 3. Inhibition of all test species was obtained with erythromycin at 500 μg/ml, neomycin at 250 μg/ml, streptomycin at 500 μg/ml, or tetracycline at 105 μg/ml. With the assay technique used, 500 μg each of sulfamerzine, sulfamethazine, or sulfathiazole per ml, singly or the three combined, failed to inhibit bacterial growth. Comparison of the titers obtained with the test formulations (Table 2) and the control antibacterial compounds (Table 3) indicates that the concentrations of antibacterial compounds reputed to be present in products 2, 6, and 8 were accurately stated by the manufacturer. In most cases identical results, or results differing by only a single dilution tube, were obtained. It is apparent that addition of $CuSO_4$ to the neomycin in product 1 enhances the antibacterial activity of the formulation, whereas merbromin enhances the antibacterial activity of streptomycin in product 3. Acriflavine appears to enhance the antibacterial activity of sulfathiazole in product 4, and the combination of three sulfa compounds with neomycin in product 5 has markedly greater antibacterial activity than that displayed by the individual compounds or by triple sulfa alone.

The numbers of viable bacteria in the water of aquaria containing goldfish were reduced by product 1 from 10^7 to 10^3 per ml within 6 h (Table 4). However, when used at the concentration recommended by the manufacturer, five of the assay fish were dead within 90 min of the addition of the formulation to the aquarium. An additional four dead fish were removed after 6 h, and the remaining fish was dead at 12 h. At the higher concentrations tested, all the fish were dead 6 h after addition of the formulation. Fish mortalities were also obtained when the experiment was repeated with a separate batch of fish. In this second experiment, three assay fish were dead after 24 h in the aquarium containing the concentration recommended by the manufacturer. Another two fish were dead after 48 h. At twice this concentration, seven fish died in the first 6 h and the remainder were dead at 12 h. At four times the recommended concentration, all 10 fish were dead within 4 h. When product 2 was used at four, eight, and sixteen times the recommended concentration, the viable bacterial numbers were only lowered

TABLE 3. *Bacteriostatic activity of standard antibacterial compounds*

Antibacterial compound	Concn of antibacterial compound inhibiting growth[a] (μg/ml)														
	A. hydrophila 9071	*A. liquefaciens* 14715	*A. salmonicida* 14174	*B. subtilis* 6633 (spores)	*Cytophaga* sp.	*K. pneumoniae* 10031-1	*M. fortuitum* 9820	*M. marinum* 927	*P. aeruginosa* 15442	*P. fluorescens* 13525	*P. putida* 12633	*S. aureus* 6538	*S. aureus* 6538-P	*V. anguillarum* 19264	*E. coli* 11775	*S. typhi* 167-P
Erythromycin	100	200	50	25	50	50	100	500	500	500	250	10	2.5	25	125	250
Neomycin	100	100	50	2.5	10	10	125	250	50	50	10	10	12.5	25	25	25
Penicillin	>30	>30	6	0.75	0.6	>30	30	>30	>30	>30	>30	0.2	0.2	>30	15	1.5
Streptomycin	100	100	100	500	1.25	10	100	500	100	100	10	50	50	50	25	125
Tetracycline	1.25	1.25	1.25	10	2.5	2.5	5	>50	50	105	2.5	0.6	0.6	0.3	5	5
Triple sulfa[b]	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500

[a] Expressed in terms of active antibacterial component(s).
[b] Equal weights of sulfamerazine, sulfamethazine, and sulfathiazole.

TABLE 4. *Effect of antimicrobial formulations on viable bacterial numbers in water containing goldfish*

Antimicrobial agent	Concn of active ingredients tested (μg/ml)	Estimated no. of bacteria in aquarium water (×10⁵/ml) after addition of test formulation								
		0 h	0.5 h	1 h	3 h	6 h	12 h	24 h	48 h	72 h
Product 1 (neomycin, $CuSO_4$)[a]	0, 0	136	137	137	150	197	93	85	23	—[b]
	0.3, 0.8	165	2	0.3	0.08	0.02	0.04	1	300	—
	0.6, 1.6	270	2	0.2	0.04	0.01	0.001	1	0.3	—
	1.2, 3.2	140	1	0.5	0.1	0.02	0.005	0.004	0.03	—
Product 2 (tetracycline)	0	49	48	37	46	42	21	6	5	4
	23	53	42	16	19	10	7	5	3	8
	46	42	38	19	17	12	5	6	7	120
	92	61	38	24	19	12	2	2	26	130
Product 3 (streptomycin, merbromin)	0, 0	84	101	118	130	132	190	56	13	—
	0.3, 0.5	160	92	81	81	74	135	195	62	—
	0.5, 1.0	320	160	130	190	120	110	175	120	—
	1.1, 4.0	200	86	23	34	31	21	165	270	—
Product 4 (sulfathiazole, acriflavine)	0, 0	150	150	139	118	150	45	65	5	1
	3.2, 1.1	100	63	61	48	37	40	36	13	16
	6.8, 2.2	108	13	13	8	9	18	42	27	32
	12.8, 4.4	71	39	20	14	32	24	50	26	97
Product 5 (sulfamerazine, sulfamethazine, sulfathiazole, neomycin)	0, 0, 0, 0	116	89	95	84	120	42	11	6	0.6
	8.1, 8.1, 8.1, 0.3	36	39	26	31	32	42	20	5	2
	16.2, 16.2, 16.2, 0.6	43	25	28	26	39	20	150	34	3
	32.4, 32.4, 32.4, 1.2	41	24	25	19	33	50	170	40	5
Product 6 (erythromycin)	0	59	66	67	50	63	35	23	16	2
	10.6	92	76	55	72	69	40	39	16	44
	21.2	190	140	130	96	120	78	43	27	53
	42.4	108	88	78	73	68	45	56	56	84
Product 7 (NFP)[c]	0	3	3	3	4	2	3	9	26	2
	0.4	1	0.4	0.6	6	11	1	25	1	6
	0.8	5	0.4	0.6	3	2	6	5	11	22
	1.7	2	0.2	0.04	0.5	8	11	5	18	52
Product 8 (penicillin G)	0	3	3	1	4	6	5	24	26	4
	0.6	5	7	5	18	11	14	44	60	1
	1.2	3	0.9	0.6	2	68	98	62	33	70
	2.4	10	9	22	36	49	96	312	176	320

[a] Active ingredients.
[b] —, Not tested.
[c] NFP, 6-Hydroxymethyl-2-[2-(5-nitro-2-furyl)vinyl]pyridine.

to 10^5 viable cells per ml at 24 h. This was also the case when products 3, 4, 5, 6, 7, and 8 were added to the aquaria at four times the recommended concentration. Moreover, four dead fish were removed from the aquarium containing 42.4 μg of erythromycin per ml at the end of the 72-h assay period. It should be noted that although the total count was generally only reduced to 10^5 viable cells per ml in the aquaria, there was an effect on the bacterial species present. Generally, one or two colony types were found to predominate in aquaria to which formulations had been added, whereas numerous colony types were present in the control aquaria. It should also be noted that in several assays, the viable bacterial numbers in the water of the control aquaria decreased during the assay period. This probably reflects the attachment of many of the organisms to the surfaces of the aquaria.

Table 5 shows the effect of the standard antibacterial compounds on the viable bacterial numbers in test aquarium water. Even when added to the water at concentrations six times higher than that recommended in the case of erythromycin and tetracycline and 167 times higher in the case of streptomycin, the antibacterial compounds failed to reduce the viable

TABLE 5. *Effect of standard antibacterial compounds on viable bacterial numbers in water containing goldfish*

Antibacterial compound	Concn tested[a] (μg/ml)	Estimated no. of bacteria in aquarium water (×10⁵/ml) after addition of test compound								
		0 h	0.5 h	1 h	3 h	6 h	12 h	24 h	48 h	72 h
Erythromycin	0	15	15	23	17	14	105	4	3	2
	15	16	11	11	13	8	6	9	12	4
	30	22	11	8	10	8	3	8	11	8
	60	87	30	21	21	27	13	33	20	21
Neomycin	0	17	16	19	58	18	10	24	14	48
	30	80	10	10	45	8	14	55	12	224
	60	24	0.06	0.04	0.02	0.02	2	50	12	480
	120	88	1	0.8	0.7	0.4	5	34	10	136
Penicillin G	0	6	7	7	9	9	14	40	26	6
	0.6	5	7	5	4	3	5	3	5	7
	1.2	4	6	5	4	5	6	8	16	29
	2.4	6	5	5	4	5	9	10	31	74
Sulfathiazole	0	10	10	10	10	10	10	18	10	3
	300	10	10	10	11	1	7	9	5	1
Sulfamerazine, sulfamethazine, sulfathiazole	0	3	3	3	4	2	3	9	26	2
	300	3	0.4	0.3	1	7	11	16	21	27
Streptomycin	0	26	37	37	41	29	28	77	24	32
	12.5	81	21	8	4	4	0.5	39	62	47
	25	79	4	5	1	4	0.5	41	25	40
	50	100	14	18	10	14	0.7	40	38	45
Tetracycline	0	29	4	25	34	22	17	8	3	4
	30	12	0.7	6	3	5	2	1	3	4
	60	16	0.8	6	5	5	2	4	6	3
	130	39	2	16	16	11	2	24	10	8

[a] Expressed in terms of active antibacterial component(s).

bacterial numbers to much less than 10^5 per ml. In addition, neomycin was toxic to the assay fish. In the aquarium containing 30 μg/ml, one fish was dead at 24 h, another was dead at 48 h, and an additional four were dead at 72 h. In the aquarium with 60 μg of neomycin per ml, one fish was dead at 48 h and another six were dead at 72 h. In the aquarium containing 120 μg of neomycin per ml, one fish was dead at 24 h, another two fish were dead at 48 h, and another four were dead after 72 h.

DISCUSSION

The resistance of bacteria to antibiotics and other synthetic chemotherapeutic agents has been recognized for many years (9). In the last decade, strains of bacteria resistant to many antibacterial agents have been isolated, and the incidence of these antibacterial-resistant organisms in the environment appears to be on the increase (15). Not unexpectedly, possession of the property of antibiotic resistance by bacteria causing disease in man has become a matter of considerable concern.

There seems little doubt that the incidence of such antibiotic-resistant strains reflects the widespread use of antibiotics (15). The resistance can arise by a number of mechanisms (9, 15), but whatever the route whereby resistance appears in a hitherto susceptible cell, selection pressure most operate if that cell is to become a major component of the bacterial population. There is no doubt that once a resistant cell has emerged, selection with appropriate antibiotics when they are present will lead to the displacement of the susceptible population by a resistant population (15). Conversely, withdrawal of the antibiotics commonly leads to a decrease in the number of resistant organisms encountered (15).

As a result of the increased awareness that the development of antibiotic-resistant bacteria impairs the effectiveness of antibiotics in treat-

ing human and animal disease, controls have been imposed on the sales of antibacterial agents for the therapy and prophylaxis of human and animal disease (7, 14, 17). Such restrictions do not apply to the sale of antibiotics for the therapy and prophylaxis of ornamental fishes. People can purchase products containing erythromycin, neomycin, nitrofuran, penicillin, streptomycin, sulfa compounds, or tetracycline from a large number of retail outlets in North America for use in their household aquaria. Even if these formulations were effective in controlling fish disease, the lack of restrictions would not be desirable and obviously deserves consideration by the appropriate authorities.

The method most commonly used to treat diseases of ornamental fishes is to bathe the fish in the water-soluble antibacterial compound (8). Our results show that the prescribed levels are likely to be subtherapeutic since they fail to inhibit the growth of bacterial species known to cause infections in aquarium fishes and species commonly found in aquarium water. The results also show that markedly higher levels of the formulations often fail to significantly decrease the numbers of viable bacteria in the aquarium water. Moreover, one of the more effective antibacterial formulations was toxic to the fish. It is worth noting that control of bacterial growth in household aquaria may further be complicated by the presence of sand, plants, and food, since these will reduce the efficiency of the antibacterial compound by direct inactivation or by mechanically protecting the bacteria from attack. In view of the apparent lack of effect on the bacteria in the aquarium water, the formulations would probably be unable to reduce tha viable bacterial numbers of surface infections of the fish. In addition, although few studies have directly compared drug levels attained by different routes of administration, experience has taught that therapeutic tissue levels can rarely be attained by bathing fish in chemotherapeutics (8). For example, less than 3% of the neomycin reaching the alimentary tract is absorbed through the gut wall.

Various antibiotics and synthetic chemotherapeutics have been used for cultured fish as feed additives in various contries to prevent and treat various infections. They have sometimes been administered directly into fish pond water to control fish infections. Fish pathogens carrying transferable drug resistance factors have been reported. (1). It is likely that bacteria carrying transferable drug resistance factors will also emerge either in household aquaria containing ornamental fish or in the aquaria of the distributors of the ornamental fishes if antibiotics are used to treat the fish in these situations. This is undesirable, especially since water containing ornamental fish has been found to contain bacterial species that are potential pathogens of man (19). The potential public health risk of water containing drug-resistant bacteria can readily be seen since aquaria containing ornamental fish are found in schools, eating establishments, shopping centers, retails stores, hospital wards, homes for the elderly, and doctors' and dentists' offices.

In addition to the potential for rapid emergence of strains of multidrug-resistant bacteria by virtue of their acquisition of transferable drug resistance factors, a rapid emergence of resistance to streptomycin in vitro can also occur by the so-called "one-step" resistance pattern (9). Gram-negative species and *Mycobacterium* in particular can be made to yield mutants with a high resistance to streptomycin (9). In fact, aquarium-borne mycobacterioses that are refractory to chemotherapy have already been reported (2, 10). Therefore, it seems undesirable to continue to allow the unrestricted sale of streptomycin and other antibiotics for aquarium use.

The philosophy of the use of bacteriostatic antibiotics to bathe fish also needs to be reevaluated. The in vivo success of chemotherapy with bacteriostatic compounds often depends on the ability of these compounds to control bacterial growth until the immune response can cope with the invaders. This is not likely to be applicable when poikilothermic species are bathed in such bacteriostatic agents. Not only is it unlikely that sufficient tissue levels of antibacterial agents will be attained, but the immune system of the poikilothermic species is not as efficient as that of the homeotherms. Antibody titers of poikilotherms are generally low and attained slowly (16).

In view of the questionable value of these formulations in the therapy and prophylaxis of infections of ornamental fishes, and since it seems wise that all efforts should be directed towards reducing the selection pressures favoring antibiotic-resistant bacteria (15), it would seem desirable to withdraw from the market products containing antibiotics used in human chemotherapy. This would contribute to the reduction in the reservoir of resistant bacteria in the environment.

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LITERATURE CITED

1. Aoki, T., S. Egusa, Y. Ogata, and T. Watanabe. 1971. Detection of resistance factors in fish pathogen *Aeromonas liquefaciens*. J. Gen. Microbiol. **65**:343-349.
2. Adams, R. M., J. S. Remington, J. Steinberg, and J. Seibert. 1970. Tropical fish aquariums. A source of *Mycobacterium marinum* infections resembling sporotrichosis. J. Amer. Med. Ass. **211**:457-461.
3. American Public Health Association. 1965. Standard methods for the examination of water and wastewater, 12th ed., p. 545-563. American Public Health Association Inc., New York.
4. Association of Official Analytical Chemists. 1970. Official methods of analysis of the A.O.A.C., 11th ed. Washington, D.C.
5. Axelrod, H. A. 1973. Tropical aquarium fishes as laboratory animals. Trans. Amer. Fish. Soc. **102**:249-250.
6. Bullock, G. L., D. A. Conroy, and S. F. Snieszko. 1971. Diseases of fishes, p. 7-9. *In* S. F. Snieszko and H. A. Axelrod (ed.), Bacterial diseases of fishes. THF Publications, Jersey City, N.J.
7. Health and Welfare Canada. 1973. The use of antimicrobials in animal feeds. Information letter 387. Health and Welfare Canada, Ottawa.
8. Herman, R. L. 1972. The principles of therapy in fish diseases, p. 141-152. *In* L. E. Mawdesley-Thomas (ed.), Diseases of fish. Symposia of the Zoological Society of London, no. 30. Academic Press Inc., London.
9. Hughes, W. H., and H. C. Stewart. 1970. Concise antibiotic treatment, p. 83-85. Butterworth and Co., Ltd., London.
10. Janssen, W. A. 1970. Fish as potential vectors of human bacterial diseases, p. 284-290. *In* S. F. Snieszko (ed.), A symposium on diseases of fishes and shellfishes. Amer. Fish. Soc. Spec. Publ. no. 5. American Fisheries Society, Washington, D.C.
11. Kavanagh, F. 1963. Dilution methods of antibiotic assays, p. 125-140. *In* F. Kavanagh (ed.), Analytical microbiology, vol. 1. Academic Press Inc., New York.
12. Kavanagh, F. 1973. Analytical microbiology, vol. 11. Academic Press Inc., New York.
13. Miles, A. A., and S. S. Misra. 1938. The estimation of the bactericidal power of the blood. J. Hyg. **38**:732-749.
14. New Scientist. 1972. U.S. restricts antibiotics in feeds. New Scientist **53**:413.
15. Richmond, M. H. 1972. Some environmental consequences of the use of antibiotics: or "what goes up must come down." J. Appl. Bacteriol. **35**:155-176.
16. Saunders, G. C. 1970. Development of the immune response, p. 93-135. *In* P. Abramoff and M. F. La Via (ed.), Biology of the immune response. McGraw-Hill Book Co., New York.
17. Swann Committee. 1969. Report of the joint committee on the use of antibiotics in animal husbandry and veterinary medicine. Her Majesty's Stationery Office, London.
18. Trust, T. J. 1972. Inadequacy of aquarium antibacterial formulations for the inhibition of potential pathogens of freshwater fish. J. Fish. Res. Bd. Can. **29**:1425-1430.
19. Trust, T. J., and K. H. Bartlett. 1974. Occurrence of potential pathogens in water containing ornamental fishes. Appl. Microbiol. **28**:35-40.
20. Trust, T. J., and V. G. Money. 1972. Bacterial population of diets for aquarium fishes. J. Fish. Res. Bd. Can. **29**:429-433.
21. Von Graevenitz, A., and J. Weinstein. 1971. Pathogenic significance of *Pseudomonas fluorescens* and *Pseudomonas putida*. Yale J. Biol. Med. **44**:265-273.
22. Wilson, G. S., and A. A. Miles. 1964. Topley and Wilson's principles of bacteriology and immunity, 5th ed., vol 1. Edward Arnold Ltd., London.