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Antibiotic Resistance of Bacteria in Water Containing Ornamental Fishes

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Water containing ornamental fishes was found to frequently contain countable numbers of bacteria that were resistant to one or more antibiotic or chemotherapeutic agents. The multidrug-resistant strains most commonly isolated were lactose-fermenting *Citrobacter freundii*. The overall resistance of these aquaria strains was greater than the previously described resistance of clinical isolates of *C. freundii*. Although the strains examined appeared to lack R-factors, this pool of resistant bacteria may have public health implications.

Large numbers of ornamental fish are cultured and shipped internationally to be offered for sale to the public. During culture and shipping, the water housing the fish is often treated with antibiotics or other chemotherapeutic compounds in an attempt to reduce fish mortality. The compounds commonly used include chloramphenicol (Cm), kanamycin (K), penicillin (P), sulfonamides, and tetracycline (Te) (9).

Aquaria containing these fish can be found in homes, school classrooms, medical and dental offices, eating establishments, department stores, nursing homes, and even in hospital wards. Fish in these aquaria can be further treated with antibacterial agents since products containing neomycin (Ne), nitrofurans, streptomycin (Sm), sulfonamides, and tetracycline can be purchased by the public. It was recently demonstrated that the dosage levels recommended for these treatments are likely to be subtherapeutic (8). Richmond (5) has shown that the use of antibiotics at subtherapeutic levels leads to an increase in the frequency of drug-resistant strains of bacteria. The presence of multidrug-resistant bacteria in aquaria has public health implications since Trust and Bartlett (7) have demonstrated that water containing ornamental fish contain bacteria that are potential pathogens of humans.

The present study was initiated to determine the extent to which drug-resistant bacteria are associated with the aquarium environment and to determine the patterns of resistance present. In addition, the opportunity was taken to more fully characterize those multidrug-resistant species of *Enterobacteriaceae* that were most commonly isolated from the aquarium environment.

MATERIALS AND METHODS

Water samples. Ornamental fish were purchased from 14 retail outlets in Victoria, British Columbia, Canada. The fish were supplied in plastic bags containing water taken from the aquaria in which the fish had been housed. The water in each container was sampled for bacteriological examination immediately upon arrival at the laboratory, and the fish were transferred to holding aquaria. There was a total of 40 water samples, each representing a single aquarium and including a single fish species. The number of fish purchased ranged from one to five and included neon tetras (*Hyphessobrycon innesi*), puffer fish (*Tetraodom fluviatilis*), white clouds (*Tanichthys albonubes*), guppies (*Lebistes reticulatus*), Jack Dempsey (*Cichlasoma biocellatum*), angels (*Pterophyllum eimekei*), zebras (*Brachydanio rerio*), catfish (*Corydoras aneus*), and common goldfish (*Carassius auratus*).

A population of the goldfish collected from the above sources was also maintained at the University of Victoria. Goldfish were transferred from the holding aquarium to test aquaria. These test aquaria were 6-liter, round glass jars, each containing 5 liters of distilled water (adjusted to pH 7 before the addition of fish). 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 aquarium. The fish were held in the test aquaria for 72 h, at which time water samples were taken for bacteriological examination. The pH of the water in the aquaria was then between 6.5 and 6.8.

Antibacterial compounds. Chloramphenicol and kanamycin were purchased from Sigma Chemical Co., St. Louis, Mo., and potassium penicillin, neomycin sulfate, sodium sulfamerazine, sodium sulfamethazine, sodium sulfathiazole, streptomycin sulfate, and tetracycline hydrochloride were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. All solids were dried to a constant weight before use.

Quantitative bacteriological examination. Duplicate dilutions of the water samples were prepared in 0.1% (wt/vol) peptone water (pH 7.2), and the viable aerobic bacteria present were enumerated by the drop-plate method of Miles and Misra (3). Total counts were done on Mueller-Hinton agar (Baltimore Biological Laboratory, Cockeysville, Md.) or on this medium supplemented with one, two, or three antibacterial compounds. Counts of lactose-fermenting organisms were done on MacConkey agar or on this medium supplemented with antibacterial compounds. When added singly to Mueller-Hinton agar, the following concentrations (expressed as active ingredients) were used: neomycin, 120 μg/ml; penicillin, 300 U/ml; streptomycin, 40 μg/ml; sulfathiazole (Su), 300 μg/ml; triple sulfa (SSS), 300 μg/ml (100 μg each of sulfamerazine, sulfamethazine, and sulfathiazole per ml); and tetracycline, 30 μg/ml. Lower concentrations were generally employed in MacConkey agar due to the selective nature of this medium, and final concentrations for single antibacterials were: neomycin, 30 μg/ml; penicillin, 10 U/ml; triple sulfa, 30 μg/ml; sulfathiazole, 300 μg/ml; streptomycin, 10 μg/ml; and tetracycline, 30 μg/ml. Chloramphenicol and kanamycin were used only in combination with other antibacterials; in combination plates, a concentration of 30 μg of antibiotic per ml was used for all antibacterials, regardless of the medium employed.

Spectrum of drug resistance. Antibiotic-resistant strains were purified by subculture to antibiotic-free Mueller-Hinton agar and then grown overnight in Trypticase soy broth (BBL). Plates of Mueller-Hinton agar (BBL) were spread with 0.1 ml of overnight culture and overlaid with susceptibility disks (BBL) containing: ampicillin, 10 μg; chloramphenicol, 30 μg; kanamycin, 30 μg; neomycin, 30 μg; penicillin, 10 U; streptomycin, 10 μg; tetracycline, 30 μg; and triple sulfa, 250 μg.

Identification of isolates. All gram-negative, lactose-fermenting bacilli were identified by performing a series of 32 identification tests. Motility, lysine and ornithine decarboxylase, indole production, gluconate reduction, growth in KCN, oxidase, and O/F glucose were determined by standard methods. Carbohydrate fermentation tests, malonate utilization, urease, gelatinase, deoxyribonuclease, cephalosporinase, and phenylalanine deaminase were performed on solid media. Carbohydrate plates contained 1% (wt/vol) of the designated carbohydrate, which was sterilized separately and added to a basal medium of peptone water agar that was adjusted to pH 7.6 to 7.8 with an appropriate agar concentration to inhibit spreaders. Bromothymol blue (0.002%) was added as an indicator. The media were poured into conventional 8.5-cm, circular, disposable, plastic petri dishes and inoculated, using a replicator consisting of 37 flat-ended steel bars (3 mm in diameter and 12 mm apart; made by Biotech Co. of Canada and marketed by K.V.L. Laboratories, Cambridge, Ontario). Antibiotic susceptibility determinations were made using this same replicator, a standardized inoculum, and media into which the following concentrations of antibiotic were incorporated: penicillin, 0.06 μg; ampicillin, 8 μg; carbenicillin, 100 μg; tetracycline, 4 μg; kanamycin, 8 μg; gentamicin, 4 μg; polymyxin, 12.5 μg; nalidixic acid, 12.0 μg; nitrofurantoin, 30 μg; sulfonamide, 1 mg; trimethoprim, 10 μg; streptomycin, 16 μg; and chloramphenicol, 16 μg.

Experiments designed to demonstrate transmissible resistance were performed using *Escherichia coli* K-12 strain CSH 55 (F^- *lac*$^-$ *pro*$^-$ nalidixic acid resistant) and CSH 55 (F′ *lac*$^+$ *pro*$^+$ nalidixic acid resistant); the latter strains were obtained from matings of CSH 55 with CSH 23 (F′ *lac*$^+$ *pro*$^+$). CSH strains were initially obtained from Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

RESULTS

Table 1 shows that water samples from aquaria containing goldfish maintained at the University of Victoria contained 9.5×10^6 viable aerobic bacteria per ml, including 2.9×10^4 organisms able to ferment lactose during growth on MacConkey agar. Between 0.2% (Te_{30}) and 28.4% (P_{300}) of the bacteria present grew on media supplemented with a single antibiotic. Countable lactose-fermenting bacteria were not observed on the Ne_{30}-MacConkey medium, but between 3% (Sm_{10}) and 93% (P_{10}, Te_{30}) of the total lactose fermenters present in the water samples grew on MacConkey agar supplemented with a single antibiotic.

Water samples containing ornamental fishes purchased at retail outlets also contained bacteria capable of growth on media supplemented with antibiotics or chemotherapeutic agents. Table 2 shows that these water samples all contained from 10^2 to 10^4 viable aerobic bacteria per ml capable of growing on Mueller-Hinton agar supplemented with eight different combinations of two antibacterials and one medium supplemented with three antibacterials. These

TABLE 1. *Numbers of viable bacteria in aquarium water[a] capable of growth in the presence of single antibacterial compounds[b]*

Medium	Total viable aerobic counts/ml (mean)[c]	Medium	Total lactose-fermenting organisms/100 ml (mean)[c]
MH	9.5×10^6	Mc	2.9×10^6
MH + Ne_{120}	6.0×10^4	Mc + Ne_{30}	TFC
MH + P_{300}	2.7×10^6	Mc + P_{10}	2.7×10^6
MH + Su_{300}	9.1×10^5	Mc + Su_{300}	3.4×10^5
MH + SSS_{300}	1.4×10^5	Mc + SSS_{300}	3.4×10^5
MH + Sm_{40}	2.0×10^6	Mc + Sm_{10}	8.6×10^4
MH + Te_{30}	2.0×10^4	Mc + Te_{30}	2.7×10^6

[a] Aquaria maintained at the University of Victoria.

[b] Abbreviations: MH, Mueller-Hinton agar; Mc, MacConkey agar; TFC, too few organisms to count.

[c] Mean value from four samples.

values represent from 0.16 to 21.3% of the total aerobes present. Countable numbers of lactose fermenters were not found growing on MacConkey agar supplemented with $Cm_{30}Te_{30}$, $K_{30}Su_{30}$, $K_{30}Te_{30}$, or $Ne_{30}Te_{30}$, but between 4.7 and 53.1% of the total lactose-fermenting organisms present were able to grow on MacConkey agar supplemented with $Su_{30}Te_{30}$, $Sm_{30}Te_{30}$, or $Sm_{30}Te_{30}Su_{30}$.

The patterns of antibiotic resistance of strains isolated from the various antibacterial-containing media are shown in Table 3. Of the 415 strains tested, 198 were found to be non-lactose fermenters, which included 57 isolates identified as species of *Aeromonas*, 57 *Pseudomonas* isolates, and 51 *o*-nitrophenyl-β-D-galactopyranoside-positive strains of *Citrobacter*. Other strains included species of *Acinetobacter*, *Flavobacterium*, *Proteus*, *Providencia*, *Serratia*, *Staphylococcus*, and *Vibrio*. A total of 70 different patterns of resistance were demonstrated, with 47% of the isolates being resistant

TABLE 2. *Numbers of multidrug-resistant bacteria in water containing ornamental fishes purchased from retail outlets*[a]

No. of samples	Medium[b]	Total viable aerobic counts/ml (mean)	Medium[b]	Total lactose-fermenting organisms/100 ml (mean)
40	MH	3.8×10^5	Mc	1.6×10^5
13	MH + Cm Te	1.7×10^4	Mc + Cm Te	TFC
6	MH + K Su	6.4×10^2	Mc + K Su	TFC
13	MH + K Te	3.8×10^4	Mc + K Te	TFC
13	MH + Ne Te	4.8×10^4	Mc + Ne Te	TFC
13	MH + Su Te	8.1×10^4	Mc + Su Te	8.5×10^4
34	MH + Sm Te	3.5×10^3	Mc + Sm Te	7.5×10^3
13	MH + Sm Te Su	9.9×10^3	Mc + Sm Te Su	1.4×10^4

[a] Abbreviations: MH, Mueller-Hinton agar; Mc, MacConkey agar; TFC, too few organisms to count.
[b] Medium contained 30 μg of each antibacterial per ml.

TABLE 3. *Drug resistance patterns of resistant strains of bacteria isolated from water containing ornamental fishes*

Resistance pattern[a]	No. of lactose fermenters	No. of other species[b]	Resistance pattern	No. of lactose fermenters	No. of other species	Resistance pattern	No. of lactose fermenters	No. of other species
P Ne Cm Te K SSS Sm Am	7	6	Ne Te K SSS Sm		1	P Te K	1	
P Ne Cm Te K SSS Sm	21	2	P Cm Te Sm Am		1	P Te SSS	4	8
P Ne Cm Te SSS Sm Am	4	3	P Cm Te K SSS		1	Te SSS Sm	10	3
P Cm Te K SSS Sm Am		1	P Te K Sm	1		P Ne Am		1
P Ne Cm Te SSS Sm	25	4	Cm Te SSS Sm	4	3	Cm SSS Sm		1
P Ne Te K SSS Sm	13	1	P Te SSS Sm	16	10	K SSS Sm	7	2
P Ne Cm Te K SSS		1	P Cm K SSS	1	1	P Cm Sm		2
			P Ne Te K	1				
P Ne Te SSS Sm Am	3	1	P Ne Te SSS	4	1	P Cm SSS		1
P Ne K SSS Sm Am		1	P Cm SSS Sm	2	1	P K SSS		1
P Cm Te K SSS Sm	2		P Cm Te SSS	2	5	Cm Te SSS		2
P Cm Te K Sm Am		1	P Ne K SSS	1		Cm SSS Sm	3	3
P Cm Te SSS Sm Am	1	1	Ne Te SSS Sm	2	1	P SSS	1	5
P Cm Te SSS Am	2		P Te K SSS	1				
P Ne Cm Te SSS		2	P Te SSS Am	1		P Ne	1	
P Ne Te SSS Sm	26	1	P Cm Te Am		1	Te SSS		
P Ne Te Sm Am		1	P Ne Te Am		1	P Te	1	2
P Ne Te K SSS		1	P Ne SSS Sm		2	Te K		1
			P K SSS Sm	1				
P Ne K SSS Sm		1	Ne K SSS Sm		1	Cm Te		1
			Te K SSS Sm	1				
P Cm K SSS Sm	4	1	P K SSS Sm		1	P Sm		2
P Cm Te SSS Sm	9	14	P Te Sm	11		P Cm		1
P Te SSS Sm Am	6	2	P SSS Sm	6	22	SSS Sm	10	4
Ne Cm K SSS Sm	1	1	P Ne K	1		P K		1

[a] Penicillin, 10 U; neomycin, 30 μg; chloramphenicol, 30 μg; tetracycline, 30 μg; kanamycin, 30 μg; triple sulfa, 250 μg; streptomycin, 10 μg; ampicillin, 10 μg.
[b] Other species include *Acinetobacter* (2), *Aeromonas* (50), *Citrobacter* (45), *Flavobacterium* (12), *Proteus morganii* (2), *Providencia* (2), *P. fluorescens* (16), *Serratia* (3), *Staphylococcus* (2), and *Vibrio* (6).

to five or more of the antibacterials employed; the most frequent patterns of resistance common to both lactose-fermenting and non-lactose-fermenting isolates were P Te SSS Sm and P Cm Te SSS Sm. The most frequent patterns of resistance displayed by lactose-fermenting isolates were P Ne Te SSS Sm, P Ne Cm Te SSS Sm, and P Ne Cm Te K SSS Sm, whereas P SSS Sm was the most frequent pattern displayed by non-lactose-fermenting isolates.

The multidrug-resistant, lactose-fermenting isolates were subjected to an intensive biochemical characterization, and their resistance to a wide variety of antibacterials was confirmed by the agar dilution method. The biochemical tests employed and the number of strains positive in each test are shown in Table 4. All 218 strains were identified as *Citrobacter* species, and since none fermented adonitol they were considered to be *Citrobacter freundii*. The antibiotic resistances of the 218 *C. freundii* strains are given in Table 5. The majority of strains were resistant to penicillin, tetracycline, streptomycin, ampicillin, cephaloridine, sulfonamide, and kanamycin, but resistance to chloramphenicol, furadantin, and nalidixic acid was not uncommon. Resistance to carbenicillin and trimethoprim was rare, and resistance to gentamicin and polymyxin was not observed.

Since aquaria form a common adjunct to the domestic environment and are also commonly found as a decoration in public places, offices, and even pediatric wards in hospitals, it seemed important to attempt to determine whether the presence of plasmid-mediated resistance transfer factor could be detected in these highly antibiotic-resistant strains. For this purpose individual direct matings between 10 penicillin-, ampicillin-, cephalothin-, tetracycline-, streptomycin-, and kanamycin-resistant *C. freundii* strains representing five biotypes and a nalidixic acid-resistant strain of *E. coli* K-12 were attempted. In no experiment were any recombinants found. Attempts were also made to mobilize a citrobacter plasmid by an F plasmid using an F′ *lac*$^+$ *pro*$^+$ plasmid of *E. coli* K-12. The F′ *lac*$^+$ *pro*$^+$ *E. coli* K-12 was mixed with cells from the same 10 citrobacter strains, and after 9 min the F$^-$ Nal^r *E. coli* K-12 was added. Again no recombinants were demonstrated, but it was not possible to prove that F had been transmitted to the *C. freundii* strains since the latter were all *lac*$^+$. In this respect the experiments were inconclusive, but they failed to demonstrate the presence of transferable resistance plasmids in the *C. freundii* strains. If transfer had occurred, it would have been detected at any frequency greater than 1 in 10^7 bacterial cells.

TABLE 4. *Biochemical characteristics of multidrug-resistant strains of C. freundii isolated from aquaria*

Test on substrate	No. of positive strains[a]	Positive (%)	Positive Ewing and Davis (1) series (%)
Glucose fermentation	218	100	100
H_2S production in TSI[b]	179	82.1	81.6
Indole	20	9.2	6.7
Motility	209	96.5	95.7
Gluconate	1	0.5	
Malonate	39	17.9	21.8
Citrate	194	89.0	90.4
Phenylalanine deaminase	0	0	
Urease	0	0	69.4
Gelatin	0	0	0
Deoxyribonuclease	0	0	
Oxidase	0	0	
Cephalosporinase	212	97.2	
Lysine decarboxylase	0	0	0
Ornithine decarboxylase	43	19.7	17.2
Lactose	217	99.5	39.4[c]
Sucrose	214	98.2	15.3
Mannitol	218	100	99.8
Dulcitol	65	29.8	59.8
Salicin	35	16.0	4.1
Adonitol	0	0	0
Inositol	0	0	3.3
Sorbitol	218	100	98.0
Arabinose	217	99.5	100
Raffinose	204	93.6	14.2
Rhamnose	218	100	99.4
Maltose	217	99.5	98.5
Xylose	213	97.7	99.8
Trehalose	218	100	100
Cellobiose	102	46.8	60.8
Glycerol	204	93.6	97.9
Esculin	35	16.0	0.9

[a] A total of 218 strains were tested.
[b] TSI, Triple sugar iron agar.
[c] An additional 58% were late lactose fermenters.

TABLE 5. *Antibiotic resistance of C. freundii strains isolated from aquaria*

Antibiotic	No. of strains resistant[a]	Resistant (%)
Penicillin	218	100
Ampicillin	155	71.1
Carbenicillin	10	4.5
Cephaloridine	140	64.2
Tetracycline	215	98.6
Kanamycin	123	56.4
Gentamicin	0	0
Polymyxin	0	0
Nalidixic acid	29	13.3
Nitrofurantoin	52	23.8
Sulfonamide	162	74.3
Trimethoprim	5	2.3
Streptomycin	172	78.9
Chloramphenicol	102	46.8

[a] A total of 218 strains were tested.

DISCUSSION

These data indicate that water containing ornamental fishes frequently contains bacteria that are resistant to more than one antibiotic or chemotherapeutic agent. The multidrug-resistant strains most commonly isolated from aquarium water in this study were identified as lactose-fermenting *C. freundii*. It is interesting to note that these drug-resistant strains of *Citrobacter* were isolated on the assumption that they were *E. coli*, but biochemical tests revealed that no isolations of *E. coli* were in fact obtained.

There is fairly close accord with the behavior of these strains of *C. freundii* and those reported by Ewing and Davis (1), with a few exceptions. Some differences require comment; the multiple-inoculation method we use for urease determination is only suitable for reading within 24 h of inoculation. *C. freundii* strains are not rapid urease producers and are almost always negative when tested by this method. The strains selected for examination had already been found to be lactose fermenters; therefore, comparison with Ewing and Davis is inappropriate for lactose fermentation. There is a notable difference in the high frequency of fermentation of sucrose and raffinose by our strains and of dulcitol by the "strains" of Ewing and Davis, which may reflect their different origins. Our strains were all isolated from aquaria, but 399 of the 616 isolates reported by Ewing and Davis were from human sources, whereas only 20 were from water and 37 were from foods and feeds.

According to Ewing and Davis, malonate utilization is a characteristic possessed by a minority of *C. freundii* strains. Our series contained 39 strains that were malonate positive, and of these 37 were ornithine decarboxylase positive, 33 were esculin positive, 33 were salicin positive, and 32 were glycerol negative. A positive result in malonate and ornithine decarboxylase tests would be typical of *C. diversus*, but the strains all failed to ferment adonitol and were indole negative. The KCN test was performed on all malonate-positive strains, and 18/39 were found to be positive, with some positive examples from all the observed biotypes. The KCN test does not distinguish between *C. freundii* and *Enterobacter cloacae*, and on the tests so far outlined the strains might have been gluconate-negative examples of *E. cloacae*, more particularly since the strains were also H_2S negative and esculin positive. The methyl red test was found to be positive for all 39 strains; thus it is likely that the 39 strains were examples of less usual biotypes of *C. freundii* and were not examples of *E. cloacae*.

Many of the multidrug-resistant species isolated in this study, such as *Pseudomonas fluorescens*, are probably naturally resistant to many of the antibiotic agents tested. *Citrobacter* strains from clinical isolates are not necessarily highly antibiotic resistant, and the overall resistance of these aquaria strains was greater than, for instance, that described by Lund et al. (2) for H_2S-negative strains of *C. freundii* and by Slifkin and Engwall (6) for *C. intermedium*. In particular, our strains were much more resistant to tetracycline, streptomycin, kanamycin, sulfonamides, and chloramphenicol, which are all antibiotics commonly used in the cultivation of ornamental fish. In contrast, no strains were found to be resistant to gentamicin or polymyxin, and few strains displayed resistance to carbenicillin or trimethoprim. These antibacterials are not used by fish growers and are not available to the public as aquarium medications. This may explain the low resistance of the *Citrobacter* strains to these antibacterials.

It seemed likely that some of this multidrug resistance would be mediated by resistance transfer factors since R-factors are considered to be the most important elements responsible for resistance to tetracycline, chloramphenicol, streptomycin, sulfanilamide, ampicillin, and kanamycin among most genera of *Enterobacteriaceae* (4). It is not clear at this time why the strains examined appeared to lack R-factors since other studies have shown that multidrug-resistant *Citrobacter* strains carry R-factors (4). The result may only indicate that the experimental conditions used or the recipient strains selected were not appropriate for the *Citrobacter* strains examined.

Regardless of the mechanism by which this multidrug resistance is mediated, this pool of resistant bacteria may have considerable public health significance since people can be exposed to aquarium-borne, drug-resistant bacteria in numerous situations inside and outside the home. This exposure can be by either direct contact with the water or indirect contact mediated by the spread of a bacterial aerosol formed by the action of the aquarium aerator. This exposure could present considerable clinical significance to individuals at risk, such as young children, persons with impaired immunological responses, persons undergoing antibiotic therapy, and debilitated individuals. It is in these persons that normally innocuous bacteria such as *C. freundii*, *P. fluorescens* and *Aeromonas hydrophila* can cause severe infec-

tions. Interestingly, the opportunity for acquiring infections often exists since aquaria are often used as ornamentation in areas for such people.

In addition to those multidrug-resistant strains that have been selected by the use of antibacterial agents during the culture and shipping of ornamental fishes, populations of drug-resistant bacteria may also be induced in home aquaria. The aquarium contains a mixed bacterial culture growing in a liquid menstruum, and it is in these conditions in which microorganisms of different species and strains coexist and multiply simultaneously that there is ample opportunity for the transfer of genetic information, including that concerned with drug resistance. The use of antibacterial products at subtherapeutic levels will certainly provide the selection pressures necessary to increase the numbers of these drug-resistant strains in aquaria and hence in the environment. Since the efficacy of these products in protecting fish health is not proven and since the incidence of human infections by drug-resistant bacteria is on the increase, the unrestricted availability of aquarium products containing antibacterials also used in human medicine is not warranted.

Compounds used in the therapy of fish diseases should not include antibiotics used in human medicine or those known to induce R-factors. Efforts should be made to find other drugs whose application can be restricted to fish culture. These measures should lead to a decrease in the numbers of multidrug-resistant bacteria in the environment, since experience has shown that withdrawal of an antibiotic commonly leads to a decrease in the number of resistant strains encountered.

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