ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1985, p. 938–942
0066-4804/85/060938-05$02.00/0

Vol. 27, No. 6

Antimicrobial Susceptibility Patterns and R Plasmid-Mediated Resistance of the Fish Pathogen *Yersinia ruckeri*

STEPHANIE A. DE GRANDIS AND ROSELYNN M. W. STEVENSON*

Department of Microbiology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Received 13 November 1984/Accepted 21 March 1985

Fifty strains of *Yersinia ruckeri*, the causative agent of enteric redmouth disease of salmonid fish, were tested for susceptibility to 23 antimicrobial agents by using an agar dilution procedure. The MICs were generally uniform for all serological varieties. Two of the 50 strains carried a 36-megadalton plasmid which determined resistance to tetracyclines and sulfonamides and was transferable to both *Escherichia coli* and *Y. ruckeri* recipients. The serovars did differ in their response to polymyxin B. Strains of serovars II, III, and V were highly resistant (MICs of 128 to 512 μg/ml), whereas most serovar I strains were susceptible to ≤2.0 μg/ml. Of 33 serovar I strains, 6 were highly resistant to polymyxin B, which is a characteristic that may divide serovar I (Hagerman) strains into two distinct subgroups.

Enteric redmouth (ERM) disease, caused by the bacterium *Yersinia ruckeri*, has been a significant problem for commercial trout farming in the Hagerman valley of Idaho since the 1950s (6, 9). Outbreaks of disease initially appeared to be geographically restricted to the western United States, but have since been reported to occur over a wider area of North America (5). In the last few years, ERM disease has become a problem of increasing importance in fish farming operations in Europe (10).

Sulfamerazine and oxytetracycline are registered by the U.S. Food and Drug Administration for use in food fish and as a treatment for furunculosis and pseudomonad diseases (11). Both have been used, alone or in combined therapy, to control epizootics of ERM disease, but in some outbreaks the disease has been unresponsive to treatment with oxytetracycline and sulfamerazine and was attributed to antibiotic-resistant *Y. ruckeri* strains (9, 13). Other antimicrobial agents shown to be effective by in vivo tests include the potentiated sulfonamide, Ro5-0037 (sulfadimethoxine and ormetoprim) (4), tribrissen and tiamulin (2), and oxolinic acid (14). Although in vitro testing of *Y. ruckeri* susceptibility to antimicrobial agents often preceded these in vivo studies, there are no published studies that define precisely the in vitro responses of multiple strains of *Y. ruckeri* to a broad range of antimicrobial agents. In this study we examined the effects of 23 antimicrobial agents on 50 strains of *Y. ruckeri* using a standardized agar dilution procedure (1). Most outbreaks of ERM disease are caused by strains of the serovar I Hagerman type (12), but strains belonging to serovar II (Oregon), serovar III (Australian), serovar V (Colorado), and serovar I′ (Salmonid Blood Spot) also occur (5, 17). We included these serovars in the test group to determine whether the serovars had differences in antimicrobial susceptibility patterns that would be significant in disease treatment. We show here that strains of *Y. ruckeri* have a relatively similar pattern of susceptibility to most antimicrobial agents but that serovar I strains vary in their responses to polymyxin B. We were also able to demonstrate the presence of R plasmid-mediated resistance to sulfonamides and tetracyclines, antibiotics that are important as chemotherapeutic agents in fish culture.

* Corresponding author.

MATERIALS AND METHODS

***Y. ruckeri* strains.** The 50 strains used in the antibiotic susceptibility tests included 33 serovar I strains, 3 serovar I′ strains, 10 serovar II strains, 3 serovar V strains, and 1 untyped isolate. The strain designations and sources were listed previously (16). Three additional isolates representing serovar III (Australian), kindly provided by G. L. Bullock, National Fish Health Research Laboratory, Leetown, W.Va., were included in the study of susceptibility to polymyxin B.

Biochemical tests and serological typing were carried out as described previously (17, 18). Working cultures were maintained on slants of Trypticase soy agar (TSA; BBL Microbiology Systems) at 15°C and grown in Trypticase soy broth (BBL) at 25°C.

Antibiotic susceptibility testing. Antimicrobial susceptibility tests were done by an agar dilution method described previously (1). Diagnostic sensitivity testing agar (DST; Oxoid Ltd.) was used as the base medium. A supplement of 5% lysed horse blood was added for tests of susceptibility to trimethoprim, sulfamethoxazole, sulfamerazine, and potentiated sulfonamide. Before being tested, strains were streaked onto TSA plates and grown at 25°C for 24 h. Growth from 24-h plate cultures was used to inoculate tubes of Penassay broth (Antibiotic Medium no. 3; Difco Laboratories), and the cultures were grown at 25°C for 24 h. Subsequently, subcultures into Penassay broth were made using 1/100 dilutions of the 24-h broth cultures. The subcultures were incubated at 25°C to a density approximating a McFarland no. 2 turbidity standard and distributed into the wells of a Steers replicator apparatus (15). Antibiotic plates were inoculated with stainless steel inoculation pins with 3-mm flat ends, which transfer 5-μl volumes of each culture, corresponding to a final inoculum size of 10^6 viable organisms. For trimethoprim, sulfamethoxazole, sulfamerazine, and potentiated sulfonamide, 10^4 and 10^6 organisms were inoculated. Plates were incubated at 25°C, and the results were read after 24 h. The MIC was defined the lowest concentration of antibiotic which produced complete inhibition of growth. All strains grew satisfactorily on control plates containing no antibiotics. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and a non-penicillinase-producing *Staphylococcus aureus* strain (Oxford), kindly provided by P. C. Fleming, Hospital for Sick Chil-

dren, Toronto, Ontario, Canada, were included as controls on each plate. The MICs reported are the results from three replications of testing.

Antibiotics. The antibiotics tested were as follows: erythromycin grade 1, chloramphenicol, tetracycline, oxytetracycline, nalidixic acid, furazolidone, rifampin, streptomycin sulfate, kanamycin sulfate, novobiocin, sulfamethoxazole, sulfamerazine, and oxolinic acid (Sigma Chemical Co.); gentamicin sulfate (Schering Corp.); vancomycin hydrochloride (Eli Lilly & Co.); trimethoprim and polymyxin B sulfate (Burroughs Wellcome Co.); penicillin G sodium, ampicillin sodium, and carbenicillin disodium (Ayerst Laboratories); and cephaloridine (Glaxo Pharmaceuticals, Ltd.). Tiamulin was a gift from E. R. Squibb & Sons, and the potentiated sulfonamide Ro5-0037 was kindly provided by Hoffmann-La Roche Inc.

Conjugal-transfer experiments. The *Y. ruckeri* plasmid-less recipient strains, RS604 and RS605, were rifampin-resistant variants of RS7 (serovar I) and RS3 (serovar II), respectively, and were obtained by plating a culture (10^8 cells per ml) onto TSA plates containing rifampin (500 μg/ml). The plasmid-less *E. coli* recipient strain RG176, a nalidixic acid-resistant and restriction-deficient mutant of *E. coli* K-12, was obtained from D. E. Taylor, Department of Medical Bacteriology, University of Alberta, Edmonton, Alberta, Canada.

Transfer experiments between the *Y. ruckeri* strains resistant to tetracycline and sulfonamides (RS33 and RS51) and the *E. coli* and *Y. ruckeri* recipients were carried out by a plate mating method (19). Equal volumes (0.5 ml) of 24-h broth cultures of the donor and recipient strains were mixed, spread onto TSA plates, and incubated for 48 h at 25°C. To select for *Y. ruckeri* transconjugants, cells were washed from the plates with saline, diluted, and spread on TSA containing rifampin (250 μg/ml) and tetracycline (16 μg/ml). The plates were incubated at 25°C for 48 h. When *E. coli* RG176 was used as the recipient, the cells were spread on MacConkey agar containing nalidixic acid (50 μg/ml) and tetracycline (16 μg/ml), and the plates were incubated at 37°C for 24 h. Ten transconjugants derived from each mating were screened for sulfonamide and tetracycline resistance on TSA plates containing sulfamethoxazole (1,000 μg/ml) or tetracycline (16 μg/ml).

Plasmid DNA isolation. Plasmid DNA was obtained and examined by agarose gel electrophoresis as previously described (7), except that plasmid DNA was purified in cesium chloride-ethidium bromide density gradients centrifuged in a Beckman 65 Ti rotor at 50,000 rpm for 18 h.

RESULTS

Susceptibility patterns. Fifty strains of *Y. ruckeri* were tested for levels of inhibition by 23 antimicrobial drugs. For all antimicrobial agents, the concentrations required to inhibit 50 and 90% of the strains tested are given in Table 1. Although different serological varieties were represented, the strains were initially treated as a uniform population. The distribution patterns for cumulative percentages of strains inhibited at different concentrations showed that all strains shared a uniform pattern of susceptibility to many of the antibiotics tested (data not shown). The most active antibiotics were carbenicillin, gentamicin, tetracycline, oxytetracycline, chloramphenicol, nalidixic acid, oxolinic acid, and trimethoprim, with more than 90% of the isolates inhibited by concentrations of 4 μg/ml or less of these compounds (Table 1). Strains of all serovars were susceptible to Ro5-0037, a 1:5 combination of ormetoprim and sulfamethoxine. At a concentration ratio of 0.1 μg of ormetoprim to 0.5 μg of sulfamethoxine per ml, 90% of the 50 strains were inhibited, and at a ratio of 0.4 to 2.0 μg 100% of the strains were inhibited. Ampicillin, kanamycin, streptomycin, rifampin, cephaloridine, and furadolizone were moderately effective, with 90% of the isolates inhibited by concentrations of 16 μg/ml or less. Penicillin, novobiocin, erythromycin, and vancomycin showed poor activity against all *Y. ruckeri* strains (Table 1).

Tiamulin appeared to be only moderately to poorly effective, with MICs ranging from 16 to 128 μg/ml. Since tiamulin derivatives are most effective at a high pH and show optimum activity at pH 8.0 (8), we adjusted the pH of the DST from 7.5 to 8.0 with 1 M sodium hydroxide. At pH 8.0 the MICs of tiamulin for 10 strains of *Y. ruckeri* and for *E. coli* ATCC 25922 decreased two- to threefold (data not shown).

Sulfamethoxazole and sulfamerazine showed only moderate to poor activity. The inoculum size affected susceptibility to both sulfonamides, but it did not significantly affect the susceptibility of isolates to trimethoprim or the potentiated sulfonamide, Ro5-0037 (Table 1). At an inoculum of 10^4 CFU, 2 strains were highly resistant to sulfamethoxazole, having MICs of 256 μg/ml compared to the MIC range (2 to 32 μg/ml) of the other 48 strains.

Tetracycline and sulfonamide resistance. Two of the 50 isolates showed high-level resistance to tetracycline derivatives and sulfonamides. Both of these strains, RS33 and

TABLE 1. Antimicrobial drug resistance concentration required to inhibit 50 *Y. ruckeri* strain[a]

Antimicrobial agents	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)
Ampicillin	4.0	8.0	2.0–8.0
Penicillin	32.0	32.0	16.0–64.0
Carbenicillin	1.0	2.0	≤0.5–2.0
Chloramphenicol	4.0	4.0	2.0–4.0
Kanamycin	4.0	8.0	4.0–8.0
Streptomycin	8.0	8.0	4.0–16.0
Gentamicin	1.0	1.0	≤0.5–2.0
Tetracycline	2.0	2.0	1.0–128.0
Oxytetracycline	2.0	2.0	1.0–256.0
Novobiocin	64.0	256.0	16.0–≥512.0
Erythromycin	32.0	64.0	16.0–64.0
Rifampin	16.0	16.0	8.0–16.0
Nalidixic acid	≤0.5	1.0	≤0.5–1.0
Oxolinic acid	≤0.5	≤0.5	≤0.5
Cephaloridine	4.0	8.0	2.0–16.0
Polymyxin B	2.0	256.0	1.0–≥512.0
Furadolizone	16.0	16.0	2.0–16.0
Vancomycin	≥512.0	≥512.0	≥512.0
Tiamulin	64.0	128.0	16.0–128.0
Trimethoprim	≤0.5	≤0.5	≤0.5–1.0
Potentiated Sulfa[b]	0.1:0.5	0.1:0.5	0.5:0.25–0.4:2.0
Sulfamethoxazole			
10^4 CFU	8.0	16.0	2.0–256.0
10^6 CFU	512.0	512.0	64.0–≥512.0
Sulfamerazine			
10^4 CFU	4.0	32.0	2.0–≥512.0
10^6 CFU	64.0	256.0	64.0–≥512.0

[a] MICs required to inhibit 50 and 90% of strains (MIC_{50} and MIC_{90}, respectively) were determined by the agar dilution method using DST and inocula of approximately 10^4 CFU. No differences in MICs were observed with 10^6 CFU inocula except where specifically indicated.

[b] Hoffmann-La Roche Inc., Ro5-0037. MICs are expressed as the concentration ratio of ormetoprim to sulfadimethoxine.

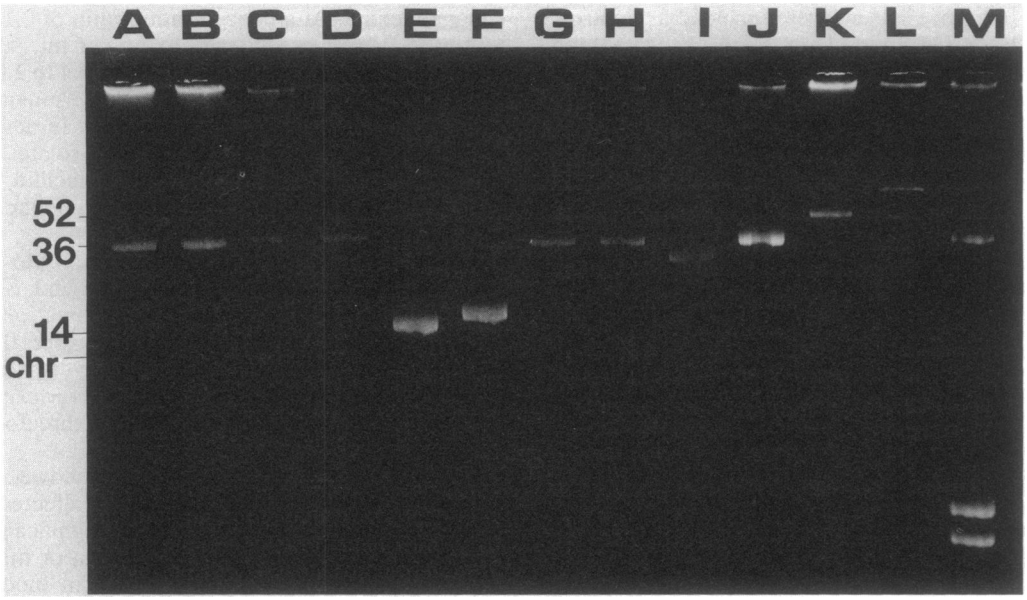

FIG. 1. Agarose gel electrophoresis of plasmids of *Y. ruckeri*. Plasmid DNA from *Y. ruckeri* donor strains and *Y. ruckeri* and *E. coli* transconjugants were subjected to electrophoresis in 0.8% agarose gels as previously described (7), and the molecular sizes were calculated from the relative positions of reference plasmids (lanes I through M) with known molecular sizes. Lanes: A, *Y. ruckeri* donor strain RS33; B, *Y. ruckeri* donor strain RS51; C, *Y. ruckeri* RS604 transconjugant harboring plasmid pRS33; D, *Y. ruckeri* RS604 transconjugant harboring plasmid pTC1; E, *Y. ruckeri* RS605 transconjugant harboring plasmid derivatives of pRS33; F, *Y. ruckeri* RS605 transconjugant harboring plasmid derivatives of pTC1; G, *E. coli* transconjugant carrying plasmid pRS33; H, *E. coli* transconjugant carrying pTC1; I, plasmid S-a (25 MDa); J, plasmid RP4 (36 MDa); K, plasmid R1P69 (47 MDa); L, plasmid RA-1 (86 MDa); M, strain V517 (plasmids; 36, 4.8, 3.7, and 3.4 MDa). Plasmids of size 2.6, 2.0, and 1.8 MDa are also present but are not indicated.

RS51, belong to serovar I. Plasmid DNA profiles of these two isolates showed that they carried the 52-megadalton (MDa) plasmid commonly found in serovar I strains (7) and also a 36-MDa plasmid (Fig. 1). The presence of the 36-MDa plasmid in both strains suggested that this plasmid carried determinants of tetracycline and sulfonamide resistance. Conjugal transfer studies were carried out to test this hypothesis.

The determinants of tetracycline and sulfonamide resistance were successfully transferred to *E. coli* RG176 and *Y. ruckeri* recipients (Table 2). The frequency of transfer of tetracycline and sulfonamide resistance determinants was similar when either a *Y. ruckeri* serovar I (RS604) or serovar II (RS605) strain was used as a recipient. The transfer frequency to *E. coli* RG176 was generally 10^2- to 10^3-fold higher than with the *Y. ruckeri* recipients.

The 36-MDa plasmid from strain RS33 was designated pRS33; the plasmid from RS51 had been previously designated pTC1 (T. Cook and P. Gemski, Abstr. XIII Int. Congr. Microbiol. 1982, P26:5, p. 97). Plasmids pRS33 and pTC1 were transferred to the *Y. ruckeri* serovar I recipient, RS604, and to *E. coli* RG176 (Fig. 1). However, transconjugants derived from matings of either RS33 or RS51 with the *Y. ruckeri* serovar II recipient, RS605, instead carried a 14- to 16-MDa plasmid and a 28-MDa plasmid (Fig. 1) or either one of these two smaller plasmids (data not shown). The transfer of antibiotic resistance determinants was not bacteriophage mediated since cell-free filtrates of broth cultures of tetracycline- and sulfonamide-resistant *Y. ruckeri* donor strains did not promote the acquisition of resistance determinants. Transformation was also eliminated as the transfer of antibiotic resistance determinants to recipient strains was not affected by the addition of DNase (100 μg/ml) to the agar in the plate mating experiments. Thus, the transfer of antibiotic resistance determinants appears to occur by conjugation.

Polymyxin B resistance. *Y. ruckeri* strains showed a broad range of susceptibility to polymyxin B (Table 1). When this variation was analyzed with respect to serological groups, a

TABLE 2. Conjugal transfer frequencies of R plasmids to *Y. ruckeri* and *E. coli* recipients

Donor strain	Selecting drug[a]	Transfer frequencies[b]		
		Y. ruckeri recipients		*E. coli* RG176
		RS604 (serovar I)	RS605 (serovar II)	
RS33	Tc	3.0×10^{-4}	4.2×10^{-3}	8.4×10^{-1}
RS51	Tc	5.0×10^{-3}	1.2×10^{-4}	1.9×10^{-2}

[a] Conjugal transfer was performed by a plate mating method previously described (19). Selective agar contained tetracycline (Tc; 16 μg/ml) and either nalidixic acid (50 μg/ml) for *E. coli* or rifampin (250 μg/ml) for *Y. ruckeri*.

[b] Transfer frequencies were obtained by dividing the number of transconjugants obtained by the number of recipients used in each mating. Values are the averages of three experiments.

TABLE 3. Polymyxin B concentrations required to inhibit different serological varieties of *Y. ruckeri*[a]

Serovar	No. of strains	No. of strains inhibited by polymyxin B at conc (μg/ml):									
		1.0	2.0	4.0	8.0	16	32	64	128	256	≥512
I	33	20	27	27	27	27	27	27	29	32	33
I′	3		2	3							
II	10								3	9	10
III	3									3	
V	3								2	2	3
UT[b]	1								1		

[a] MICs were determined by the agar dilution method using DST. None of these strains were inhibited at concentrations of polymyxin B of ≤0.5 at μg/ml.

[b] UT, Untyped strain (RS63).

definite pattern appeared (Table 3). All serovar II, III, and V strains were highly resistant, with MICs ranging from 128 to 512 μg/ml. Of the serovar I strains, 81% were susceptible to polymyxin B at ≤2.0 μg/ml, whereas serovar I′ strains had slightly higher MICs of 2 to 4 μg/ml. Six serovar I isolates were as resistant to polymyxin B as the serovar II, III, and V strains.

DISCUSSION

The different serological varieties of *Y. ruckeri* do not appear to differ significantly in their in vitro response to most antimicrobial agents, with the exception of polymyxin B. These results suggest that the same chemotherapeutic approach can be used to treat ERM disease outbreaks regardless of the serological variety of *Y. ruckeri* involved.

It is difficult to relate the in vitro susceptibility of bacteria to antimicrobial agents to the in vivo effectiveness of these drugs in the treatment of fish diseases. Post (13) and Horner (11) suggest appropriate therapeutic doses for drugs that are commonly used on food fish, such as sulfamerazine and oxytetracycline. However, these agents are most often administered to fish orally (in food), making it difficult to monitor levels of the drug in tissue, particularly when some members of a population may be feeding poorly. However, the MICs determined by in vitro assay methods do appear to have some value in identifying antimicrobial agents that may be effective in in vivo tests in fish. Specifically, potentiated sulfonamides and oxolinic acid inhibited *Y. ruckeri* in vitro, paralleling their effectiveness in the disease treatment studies reported by Bullock et al. (4), Bosse and Post (2), and Rodgers and Austin (14). The potentiated sulfonamide, Ro5-0037, was highly active in vitro against all *Y. ruckeri* strains tested, whereas sulfonamides alone showed only moderate to poor activity. Strains RS33 and RS51, carrying plasmid-determined sulfonamide resistance, were inhibited by Ro5-0037. The poor-to-moderate effect in vitro of tiamulin contrasts with the results of Bosse and Post (2), who showed it to be effective in vitro and in vivo against a single strain of *Y. ruckeri*. This discrepancy may be related to the influence of pH on the activity of tiamulin, since *Y. ruckeri* strains were two- to threefold more susceptible to tiamulin in in vitro tests when the pH of the DST was increased from 7.5 to 8.0. There is no information to suggest what pH conditions could be expected in vivo in the fish system. Carbenicillin and gentamicin inhibited *Y. ruckeri* in vitro, although there are no published reports of the success of their use against ERM disease. Tetracyclines and chloramphenicol, frequently used for treating furunculosis and other bacterial diseases of fish (13), also inhibited *Y. ruckeri* strains in vitro.

Extensive use of antibiotics in fish culture has increased the frequency of resistant strains of many bacterial pathogens (20). Problems with resistant strains of *Y. ruckeri* have already occurred in epizootics (13), and of the 50 isolates studied here, 2 were found to be highly resistant to both tetracyclines and sulfonamides. We demonstrated here that determinants for both tetracycline and sulfonamide resistance are carried by a 36-MDa plasmid and are transferable to *Y. ruckeri* strains of two serological varieties and also to *E. coli*. The linkage of resistance determinants to two antibiotics on a transferable plasmid is a matter of concern, particularly when the two antibiotics are very commonly used in aquaculture. More strains from areas where antibiotics have been used to treat outbreaks of ERM disease need to be examined to assess the extent of the problem of antibiotic resistance in *Y. ruckeri*.

The frequency of transfer of the R plasmid from *Y. ruckeri* to plasmid-less *Y. ruckeri* strains was approximately 10^2- to 10^3-fold less than when the recipient was *E. coli* RG176, which may reflect the restriction-modification system deficiency in RG176 or other factors such as cell pairing. Transconjugants obtained from matings of recipient serovar II strain RS605 and *Y. ruckeri* donors RS33 and RS51 showed unexpected plasmid profiles, carrying either a 14- to 16-MDa plasmid, a 28-MDa plasmid, or both instead of a 36-MDa plasmid. These results suggest that the 36-MDa plasmid could be a cointegrate plasmid in the donor that cannot be maintained in serovar II recipients. Cook and Gemski (Abstr. XIII Int. Congr. Microbiol. 1982) previously demonstrated that strain RS51 carried the genes for tetracycline resistance on a 36-MDa plasmid which could be transferred to *E. coli* by conjugation. They suggested a cointegrate plasmid to explain similar plasmid size changes in one of their 29 transconjugants.

The greatest variation in antibiotic sensitivity among *Y. ruckeri* strains was in their responses to polymyxin B. Strains of serovars II, III, and V were highly resistant, whereas most serovar I strains were susceptible. The serovar III isolates recently received from G. Bullock showed serological similarity to our serovar I′ (Salmonid Blood Spot disease) isolates (R. Stevenson, unpublished data). However, the three serovar I′ strains were susceptible to polymyxin B in contrast to the high level of resistance seen for the three serovar III strains, suggesting that a difference does exist between these two groups.

Although 81% of the serovar I strains were highly susceptible to polymyxin B (MICs of ≤2 μg/ml), six serovar I strains showed as high a level of resistance as serovar II, III, and V strains. All of the resistant strains were also able to grow at 37°C as well as at 25°C, when tested on the basis of colony-forming ability as previously described (7). Susceptibility to polymyxin B does not correlate with the presence of the 50-MDa plasmid of *Y. ruckeri* (data not shown). However, since resistance to polymyxin B correlates with both growth at 37°C and the bacteriophage sensitivity of *Y. ruckeri* (16), it is possible that these characteristics may reflect variations in cell surface characteristics of serovar I strains. Since some evidence indicates that serovar I strains are more virulent for rainbow trout than are other serovars (3, 12), we are now attempting to determine whether there is a significant difference in virulence between the two subgroups of serovar I.

ACKNOWLEDGMENTS

This work was supported by a grant from the Science Subvention Program of the Department of Fisheries and Oceans, Canada.

We thank P. Krell and J. G. Daly for helpful comments on the manuscript.

LITERATURE CITED

1. **Barry, L. A.** 1980. Procedure for testing antibiotics in agar media: theoretical considerations, p. 1–23. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore.
2. **Bosse, M. P., and G. Post.** 1983. Tribrissen and tiamulin for control of enteric redmouth disease. J. Fish Dis. **6**:27–32.
3. **Bullock, G. L., and D. P. Anderson.** 1984. Immunization against *Y. ruckeri*, cause of enteric redmouth disease, p. 151–166. *In* P. de Kinkelin (ed.), Symposium on fish vaccination. Office International des Epizooties, Paris.
4. **Bullock, G. L., G. Maestrone, C. Starliper, and B. Schill.** 1983. Potentiated sulfonamide therapy of enteric redmouth disease. Can. J. Fish. Aquat. Sci. **40**:101–102.

5. **Bullock, G. L., H. M. Stuckey, and E. B. Shotts, Jr.** 1978. Enteric redmouth bacterium: comparison of isolates from different geographic areas. J. Fish Dis. **1**:351–356.
6. **Busch, R. A.** 1982. Enteric redmouth disease (*Yersinia ruckeri*), p. 201–223. *In* D. P. Anderson, M. Dorson, and P. Dubourget (ed.), Antigens of fish pathogens. Collection Fondation Marcel Merieux, Lyon, France.
7. **De Grandis, S. A., and R. M. W. Stevenson.** 1982. Variations in plasmid profiles and growth characteristics of *Yersinia ruckeri* strains. FEMS Microbiol. Lett. **15**:199–202.
8. **Drews, J., A. Georgopoulos, G. Laber, E. Schultze, and J. Unger.** 1975. Antimicrobial activities of 81.723 hfu, a new pleureomutilin derivative. Antimicrob. Agents Chemother. **7**:507–516.
9. **Dulin, M. P., T. Huddleston, R. E. Larson, and G. W. Klontz.** 1976. Enteric redmouth disease, p. 15. Forest Wildlife, and Range Experiment Station bulletin no. 8. University of Idaho, Moscow, Idaho.
10. **Horne, M. T., M. F. Tatner, and R. J. Roberts.** 1984. Enteric redmouth: a threat or a promise? Fish Farming Int. **2**:12.
11. **Horner, R. W.** 1983. Chemotherapy, p. 99–109. *In* F. P. Meyer, J. W. Warren, and T. G. Carey (ed.), A guide to integrated fish health management in the Great Lakes basin. Special publication no. 83-2. Great Lakes Fishery Commission, Ann Arbor, Mich.
12. **McCarthy, D. J., and K. A. Johnson.** 1982. A serotypic survey and cross-protection test of North American field isolates of *Yersinia ruckeri*. J. Fish Dis. **5**:323–328.
13. **Post, G. W.** 1983. Textbook of fish health, p. 44–47. T.F.H. Publications, Inc., Neptune City, N.J.
14. **Rodgers, C. J., and B. Austin.** 1983. Oxolinic acid for control of enteric redmouth disease in rainbow trout. Vet. Rec. **112**:83.
15. **Steers, E., E. L. Foltz, and G. Graves.** 1959. An inocula-replicating apparatus for routine resting of bacterial susceptibility to antibiotics. Antibiot. Chemother. **9**:307–311.
16. **Stevenson, R. M. W., and D. W. Airdrie.** 1984. Isolation of *Yersinia ruckeri* bacteriophages. Appl. Environ. Microbiol. **47**:1201–1205.
17. **Stevenson, R. M. W., and D. W. Airdrie.** 1984. Serological variation among *Yersinia ruckeri* strains. J. Fish Dis. **7**:247–254.
18. **Stevenson, R. M. W., and J. G. Daly.** 1982. Biochemical and serological characteristics of Ontario isolates of *Yersinia ruckeri*. Can. J. Fish. Aquat. Sci. **39**:870–876.
19. **Taylor, D. E., S. A. De Grandis, M. A. Karmali, and P. C. Fleming.** 1981. Transmissible plasmids from *Campylobacter jejuni*. Antimicrob. Agents Chemother. **19**:831–835.
20. **Watanabe, T., T. Aoki, Y. Ogata, and S. Egusa.** 1971. R factors related to fish culturing. Ann. N.Y. Acad. Sci. **182**:383–410.