ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1987, p. 1281–1282
0066-4804/87/081281-02$02.00/0

Vol. 31, No. 8

Plasmids and Resistance to Antimicrobial Agents in *Aeromonas sobria* and *Aeromonas hydrophila* Clinical Isolates

BARBARA J. CHANG* AND SANDRA M. BOLTON†

Department of Microbiology, University of Western Australia, The Queen Elizabeth II Medical Centre, Nedlands, Western Australia 6009, Australia

Received 16 January 1986/Accepted 29 December 1986

The antimicrobial susceptibilities of 75 *Aeromonas* isolates were determined by agar dilution. Differences in resistance patterns were observed between strains isolated from different geographic locations and between *A. sobria* and *A. hydrophila* isolates. Multiple resistance was common; however, only one conjugative plasmid was detected. This 110-megadalton plasmid mediated resistance to eight antibiotics.

The motile aeromonads are recognized both as causes of infection in immunocompromised patients and as enteric pathogens which may cause diarrhea in healthy individuals (4, 9, 20). In view of the increasing clinical importance of these organisms, and since little is known about the genetic basis for drug resistance in the genus (1, 7, 12, 19), we wished to assess the plasmid content of hospital isolates of *Aeromonas* spp. Because previous studies on antimicrobial susceptibility and its genetic basis were performed before the recent division of the motile aeromonads into three species (16), our studies also aimed to examine any species-associated antimicrobial resistance patterns.

A total of 75 *Aeromonas* isolates were tested. Thirty-one strains were from patients in metropolitan hospitals in Australia and were collected during 1980 and 1981. A total of 29 of these were fecal isolates, including 26 from patients with diarrhea and 2 from patients with an infected wound and septicemia, respectively. Forty-four strains were collected in 1982, forty from diarrheal patients in Jakarta, Indonesia, and four from patients in Vellore, India, comprising three fecal isolates from diarrheal patients and one isolate from an infected wound. In all cases, isolates were taken from individual patients without duplication. The isolates from infected wounds or septicemias were recovered in pure culture and were considered to be the primary pathogens, whereas the fecal isolates were recovered in mixed culture and their clinical significance remains undetermined. The isolates were identified by the criteria of Popoff (16).

Antimicrobial susceptibility testing was performed using a standard agar dilution method (17). Strains were considered resistant if more than 100 colonies grew from an inoculum of approximately 10^4 CFU on Iso-Sensitest agar (Oxoid Ltd., Basingstoke, England) containing antimicrobial agents at the concentrations shown in Table 1. These concentrations were chosen on the basis of the MICs reported for *Aeromonas* spp. by Richardson et al. (17). In general, concentrations 10-fold higher than those required to kill at least 50% of the *Aeromonas* strains in that study were used.

All isolates were found to be resistant to at least one of the 17 antimicrobial agents tested. Multiple resistance to 3, 4, or 5 agents was common (76% of isolates), and resistance to as many as 11 agents was observed.

Previous studies indicated that cefamandole, trimethoprim, chloramphenicol, and tetracycline are extremely active against *Aeromonas* spp. (5, 15, 17). In this study, the frequency of resistance to some antimicrobial agents was much greater than previously reported (Table 1). In particular, 43% of isolates were resistant to cefamandole and 21% were resistant to tetracycline. This resistance was found principally among the Asian isolates. Chloramphenicol resistance, which is generally considered to be an extremely rare trait in *Aeromonas* spp., appeared in 14% of our Asian isolates but in none of the Australian strains. Similarly, resistance to mercuric chloride, nalidixic acid, and rifampin was detected only among Asian isolates, which also exhibited a high frequency of colistin resistance.

The spread of drug resistance among *Aeromonas* spp. is of some concern. Recent surveys have emphasized the emergence of *A. hydrophila* as a primary pathogen (4, 8, 20), and antibiotic therapy has now been recommended for patients with chronic diarrhea and for those at risk of developing *Aeromonas* septicemia (4, 8).

Motyl et al. (15) reported that *A. hydrophila* is more resistant than *A. sobria* to antimicrobial agents, especially the penicillins and cephalosporins. Similarly, in the present study, the frequencies of resistance to cefamandole and colistin were significantly greater among *A. hydrophila* isolates (73 and 59%) than among *A. sobria* isolates (6%) (two-tailed hypothesis test, $P < 0.005$). However, resistance to carbenicillin, ticarcillin, streptomycin, and spectinomycin occurred more frequently in *A. sobria* isolates ($P < 0.05$). Such species differences may well be related to the source of the *Aeromonas* isolates and in particular to the frequency and type of antimicrobial agent used in different geographical areas for different kinds of *Aeromonas* infections. *A. sobria* is more frequently associated with bacteremia than is *A. hydrophila* (9) and may also be more frequently the cause of invasive enteric infections (21).

Plasmid DNA was found in 20 of the 75 wild-type *Aeromonas* isolates, using the procedure of Birnboim and Doly (2). This is a low plasmid-carriage frequency compared with that found in populations of antibiotic-resistant strains of members of the family *Enterobacteriaceae* (11, 18). Molecular sizes ranged from approximately 2 to 110 megadaltons; 15 of the 20 isolates harbored plasmids of less than 25 megadaltons. There were 12 *A. sobria* and 8 *A. hydrophila* plasmid-containing isolates. Eleven of the stains were Australian isolates, and nine were from Asian sources.

* Corresponding author.
† Present address: School of Medical Technology, Western Australian Institute of Technology, Bentley, Western Australia 6012, Australia.

TABLE 1. Frequency of drug resistance among *Aeromonas* clinical isolates

Antibacterial agent (μg/ml)	% Resistant isolates				
	All	Source		Species	
	(n = 75)	Australia (n = 31)	Asia (n = 44)	*A. sobria* (n = 32)	*A. hydrophila* (n = 41)
Ampicillin (100)	95	94	95	94	95
Carbenicillin (50)	35	48	25	56	20
Cefamandole (10)	43	9	66	6	73
Chloramphenicol (10)	8	0	14	6	10
Colistin (10)	34	6	54	6	59
Gentamicin (10)	0	0	0	0	0
Mercuric chloride (27)	1	0	2	0	2
Kanamycin (25)	4	3	5	3	5
Nalidixic acid (50)	1	0	2	0	2
Rifampin (300)	1	0	2	3	0
Streptomycin (10)	41	45	39	66	24
Spectinomycin (10)	76	84	70	88	66
Sulfisoxazole (250)	11	9	12	13	7
Tetracycline (6)	21	16	25	25	20
Ticarcillin (50)	8	6	9	16	2
Tobramycin (5)	4	3	5	9	0
Trimethoprim (20)	7	9	5	6	5

Strains were selected for genetic analysis on the basis of drug resistance pattern as well as plasmid content; 39 strains were screened for transfer of antibiotic resistance markers in conjugation experiments using *Escherichia coli* JP990 nalidixic acid-resistant (13) and *A. hydrophila* 2688 rifampin-resistant (10) as plasmid-free recipients. Matings were performed on solid media at 37 and 30°C for 4 and 18 h.

The only isolate in which a conjugative plasmid was detected was *A. sobria* 236 from Vellore. The plasmid, pSOB1, had a molecular size of approximately 110 megadaltons and transferred resistance to ampicillin, kanamycin, streptomycin, spectinomycin, sulfisoxazole, ticarcillin, tobramycin, and trimethoprim. It was transferable to *E. coli*, *A. hydrophila*, and *Salmonella typhimurium* LT2. The plasmid exhibited entry exclusion with the two IncC plasmids R40a and RA1 (3, 6), suggesting that it is a member of the IncC group. IncC plasmids were previously reported in *Aeromonas* spp. (6, 7, 14), and pSOB1 is a typical member of the group, with its high molecular weight and wide host range. It is interesting that pSOB1 confers resistance to ampicillin, since this is generally considered to be an intrinsic or chromosomal resistance of *A. hydrophila* and *A. sobria* (1).

Preliminary mobilization experiments for the detection of nonconjugative R plasmids have been performed with conjugative reference plasmids from Inc groups FII, I, P, W, and C (13). No mobilization of *Aeromonas* plasmids has been observed, although results suggestive of the transposition of a tetracycline resistance marker have been obtained. Curing experiments have also failed to demonstrate the existence of R plasmids in the isolates. Thus, it appears that the observed high frequency of antibiotic resistance is not due primarily to the presence of R plasmids in this group of *Aeromonas* isolates.

We thank V. Burke and J. Robinson for kindly supplying and biotyping *Aeromonas* strains. We are grateful to B. J. Mee and T. V. Riley for valuable discussions and advice.

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. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **Bradley, D. E.** 1980. Determination of pili by conjugative bacterial drug resistance plasmids of incompatibility groups B, C, H, J, K, M, V, and X. J. Bacteriol. **141**:828–837.
4. **Burke, V., and M. Gracey.** 1986. *Aeromonas* species in human diarrhoeal disease. J. Gastroenterol. Hepatol. **1**:237–249.
5. **Fainstein, V., S. Weaver, and G. P. Bodey.** 1982. In vitro susceptibilities of *Aeromonas hydrophila* against new antibiotics. Antimicrob. Agents Chemother. **22**:513–514.
6. **Hedges, R. W., and N. Datta.** 1971. fi⁻ R factors giving chloramphenicol resistance. Nature (London) **234**:220–221.
7. **Hedges, R. W., P. Smith, and G. Brazil.** 1985. Resistance plasmids of aeromonads. J. Gen. Microbiol. **131**:2091–2095.
8. **Holmberg, S. D., and J. J. Farmer III.** 1984. *Aeromonas hydrophila* and *Plesiomonas shigelloides* as causes of intestinal infections. Rev. Infect. Dis. **6**:633–639.
9. **Janda, J. M., R. B. Clark, and R. Brenden.** 1985. Virulence of *Aeromonas* species as assessed through mouse lethality studies. Curr. Microbiol. **12**:163–168.
10. **Lawson, M. A., V. Burke, and B. J. Chang.** 1985. Invasion of HEp-2 cells by fecal isolates of *Aeromonas hydrophila*. Infect. Immun. **47**:680–683.
11. **Levy, S. B., R. W. Hedges, F. Sullivan, A. A. Medeiros, and H. Sosroseputro.** 1985. Multiple antibiotic resistance plasmids in Enterobacteriaceae isolated from diarrhoeal specimens of hospitalized children in Indonesia. J. Antimicrob. Chemother. **16**:7–16.
12. **McNicol, L. A., K. M. S. Aziz, I. Huq, J. B. Kaper, H. A. Lockman, E. F. Remmers, W. M. Spira, M. J. Voll, and R. R. Colwell.** 1980. Isolation of drug-resistant *Aeromonas hydrophila* from aquatic environments. Antimicrob. Agents Chemother. **17**:477–483.
13. **Mee, B. J., and S. M. Nikoletti.** 1983. Plasmids encoding trimethoprim resistance in bacterial isolates from man and pigs. J. Appl. Bacteriol. **54**:225–235.
14. **Mizon, F. M., G. R. Gerbaud, H. Leclerc, and Y. A. Chabbert.** 1978. Presence de plasmides de resistance appartenant au groupe d'incompatibilite C chez *Aeromonas hydrophilia* isole d'eaux residuairies. Ann. Microbiol. (Paris) **129B**:19–26.
15. **Motyl, M. R., G. McKinley, and J. M. Janda.** 1985. In vitro susceptibilities of *Aeromonas hydrophila*, *Aeromonas sobria*, and *Aeromonas caviae* to 22 antimicrobial agents. Antimicrob. Agents Chemother. **28**:151–153.
16. **Popoff, M.** 1984. Genus III: *Aeromonas* Kluyver and Van Niel 1936, 398^{AL}, p. 545–548. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
17. **Richardson, C. J. L., J. O. Robinson, L. B. Wagener, and V. Burke.** 1982. *In vitro* susceptibility of *Aeromonas* spp. to antimicrobial agents. J. Antimicrob. Chemother. **9**:267–274.
18. **Taylor, D. E., J. G. Levine, and K. L. Kouvelos.** 1982. Incidence of plasmid DNA in *Salmonella* strains isolated from clinical sources in Ontario, Canada, during 1979 and 1980. Can. J. Microbiol. **28**:1150–1157.
19. **Toranzo, A. E., P. Combarro, M. L. Lemos, and J. L. Barja.** 1984. Plasmid coding for transferable drug resistance in bacteria isolated from cultured rainbow trout. Appl. Environ. Microbiol. **48**:872–877.
20. **Trust, T. J., and D. C. Chipman.** 1979. Clinical involvement of *Aeromonas hydrophila*. Can. Med. Assoc. J. **120**:942–946.
21. **Watson, I. M., J. O. Robinson, V. Burke, and M. Gracey.** 1985. Invasiveness of *Aeromonas* spp. in relation to biotype, virulence factors, and clinical features. J. Clin. Microbiol. **22**:48–51.