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Isolation of Enterotoxigenic, Hemolytic, and Antibiotic-Resistant *Aeromonas hydrophila* Strains from Infected Fish in Bangladesh

ZEAUR RAHIM,[1] S. C. SANYAL,[1]* K. M. S. AZIZ,[1] M. I. HUQ,[1] AND A. A. CHOWDHURY[2]

International Centre for Diarrhoeal Disease Research, Bangladesh,[1] and Department of Microbiology, University of Dhaka, Dhaka, Bangladesh[2]

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Strains of *Aeromonas hydrophila* isolated from skin infections of common freshwater fish in Bangladesh were tested for enterotoxin production, hemolysin production, and any correlation between these two activities. We also tested the resistance patterns of *A. hydrophila* to different drugs, especially in relation to ampicillin. The *A. hydrophila* strains produced an enterotoxin that was related to their beta-hemolytic activities. Production of beta-hemolysin may thus be an indicator of enterotoxicity. As 50% of the strains of *A. hydrophila* were found to be susceptible to 12.5 μg of ampicillin per ml, media containing this antibiotic may not be suitable for their isolation.

Aeromonas hydrophila has been implicated in the etiology of a variety of systemic and localized diseases in different mammals, reptiles, fish (5, 13, 15), and humans (7). During the past decade, this organism has been confirmed as a diarrheal pathogen in humans (11; S. Goings, R. L. Anderson, J. V. Bennett, and R. E. Dixon, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C88, p. 324). Sanyal and co-workers (21) first demonstrated the enterotoxicity of *A. hydrophila* strains isolated from diarrheal stool samples and environmental sources. They also demonstrated that culture supernatants of the organism can cause fluid accumulation in rabbit ileal loops (2, 3) and an increase in permeability of the skin of rabbits (9, 10). In addition, Sanyal and co-workers have purified the enterotoxin to electrophoretic homogeneity (11), cloned the enterotoxin gene (23), and showed the mediation of cyclic AMP in its enterotoxic activity (8). Elaboration of other extracellular toxic factors such as hemolysin and cytotoxin has also been identified in human and fish isolates (1, 6, 24). There are a few reports on the isolation of drug-resistant *A. hydrophila* strains from humans (12) and the environment (16), and a selective medium has been developed for the isolation of the organism based on resistance to ampicillin (18). In the present study, eight strains of *A. hydrophila* were isolated from 15 swab specimens of superficial skin ulcers of the common freshwater fish *Platosus anguillaris* (Kain), *Lates calcarifer* (Vetki), *Epinephalus megachir* (Koiful), and *Telapia nilotica* (Telapia). The present study was undertaken to examine the enterotoxicity, hemolytic activity, and correlation, if any, between these two properties. In addition, we tested the resistance patterns to different drugs, especially in relation to ampicillin.

Live cells and culture filtrates of *A. hydrophila* were tested in adult rabbit ileal loops by the method described earlier (2); seven strains gave positive reactions (Table 1). However, strain T52 required two consecutive passages (3) to cause fluid accumulation. Strain V21 did not cause fluid accumulation in the first set of experiments done with three rabbits or after consecutive passages in loops of another three rabbits. These data indicated that *A. hydrophila* strains isolated from skin infections of different genera of commonly consumed riverine fish in Bangladesh produced an enterotoxin as determined by fluid accumulation in ileal loops. Similar observations have been made by other workers with certain *A. hydrophila* strains isolated from healthy and diseased fish (5, 17). The accumulation of fluid observed only after two passages through rabbit guts by strain T52 was probably caused by preferential selection of an enterotoxic bacterial population during passages through a susceptible host. Similar observations have also been made with human and environmental isolates of *A. hydrophila* (3), *Plesiomonas shigelloides* (20), *Vibrio cholerae* non-O1 (22), and *Vibrio fluvialis* (19). Strain-to-strain and rabbit loop-to-rabbit loop variations in fluid accumulations were probably caused by quantitative differences in toxin production and biological variations in rabbits (20).

All of the strains of *A. hydrophila* were grown in T1N1 broth (1% Trypticase [BBL Microbiology Systems] plus 1% NaCl, pH 7.4) for 4 to 5 h, plated on 7% sheep blood agar, and incubated at 37°C for 18 to 24 h. The presence of hemolysin was determined by the formation of zones of alpha- or beta-hemolysins around the colonies (5). The seven strains that caused fluid accumulation in rabbit gut loops produced zones of beta-hemolysin around their colonies. However, colonies of strain V21 that gave negative loop reactions had zones of alpha-hemolysis around them (Table 1). Elucidation of this correlation between enterotoxic and beta-hemolytic activities is significant because it may be useful for identification of enterotoxigenic strains by a simple hemolysis test. Some investigators (5, 17) in earlier studies with fish isolates observed the production of two hemolysins by some strains, but made no effort to correlate this property with enterotoxic activity.

Susceptibility of the organisms to different antibiotics was tested by the agar diffusion method of Bauer et al. (4), using disks purchased from Biomerieux. The antibiotic and chemotherapeutic agents and their concentrations per disk were (in micrograms, except where indicated otherwise): ampicillin, 10; cefamandole, 30; chloramphenicol, 30; colistin, 10; erythromycin, 15; gentamicin, 10; neomycin, 30; novobiocin, 30; polymyxin B, 300 U; streptomycin, 10; tetracycline, 30; vancomycin, 30; and trimethoprim-sulfamethoxazole, 1.25 plus 23.75. All eight strains were susceptible to streptomycin, neomycin, gentamicin, and polymyxin B (Table 2). Two of the strains were resistant to chloramphenicol, trimethoprim-sulfamethoxazole, and cefamandole, but the others were susceptible. Three strains were resistant to tetracycline and erythromycin, and five were resistant to vancomycin. Strain V21 was susceptible to novobiocin and

* Corresponding author.

TABLE 1. Hemolytic and enterotoxic activities of *A. hydrophila* strains

Strain	Hemolysis	Rabbit ileal loop test (ml/cm)[a]	
		Live cells	Culture filtrate
K4	Beta	0.9	1.7
K5	Beta	0.8	1.73
K13	Beta	1.5	0.88
V20	Beta	1.6	1.3
V21	Alpha	0	0
C27	Beta	0.9	0.6
T46	Beta	1.6	1.86
T52	Beta	1.0	1.2

[a] Mean of fluid accumulations in loops of three rabbits.

resistant to colistin, whereas all the other strains were resistant to the former and susceptible to the latter antibiotic. Four of the strains were susceptible to ampicillin, and the remaining four were resistant to it. The susceptibility patterns of the strains to certain common antibiotics differed from those determined by some other workers (12, 14), who observed that strains of *A. hydrophila* were highly susceptible to tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole. This study showed that a significant proportion of the strains were not susceptible to these antibiotics, which are commonly used in Bangladesh. Many workers have observed that almost all strains of *A. hydrophila* are resistant to ampicillin, and a selective medium containing this antibiotic in a concentration of 30 μg/ml has been recommended for isolation of *A. hydrophila* (18).

However, in our study, four of the eight strains were susceptible to ampicillin disks containing 10 μg of the antibiotic; the MIC for these strains was 12.5 μg/ml by the plate dilution technique. The MIC for the four resistant strains was 200 μg/ml. These observations suggest that many strains of *A. hydrophila* may go undetected if a selective medium containing 30 μg of ampicillin per ml is used with the aim of eliminating other enterobacteria and preferentially isolating this organism.

This study indicated that *A. hydrophila* strains isolated from infected fish were enterotoxigenic and may be responsible for outbreaks of diarrhea if the fish are consumed without proper cooking; beta-hemolytic activity of the strains may be used as an indicator of enterotoxicity, and media containing ampicillin may not be suitable for isolation of *A. hydrophila*.

TABLE 2. Antibiotic susceptibilities of *A. hydrophila* strains

Antibiotic	Resistance (R) or susceptibility (S) of strain:							
	K4	K5	K13	V20	V21	C27	T46	T52
Ampicillin	R	R	S	S	S	R	S	R
Cefamandole	R	R	S	S	S	S	S	S
Chloramphenicol	R	R	S	S	S	S	S	S
Colistin	S	S	S	S	R	S	S	S
Erythromycin	R	R	S	S	S	S	S	R
Gentamicin	S	S	S	S	S	S	S	S
Neomycin	S	S	S	S	S	S	S	S
Novobiocin	R	R	R	R	S	R	R	R
Polymyxin B	S	S	S	S	S	S	S	S
Streptomycin	S	S	S	S	S	S	S	S
Tetracycline	R	R	S	S	S	S	S	R
Trimethoprim -sulfamethoxazole	R	R	S	S	S	S	S	S
Vancomycin	R	R	S	S	S	R	R	R

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