

THE OCCURRENCE AND SIGNIFICANCE OF ORGANISMS OF THE *AEROMONAS*  
*HYDROPHILA* GROUP IN FOOD AND WATER

Samuel A. Palumbo

Eastern Regional Research Center, ARS, U.S. Department of Agriculture, 600 East Mermaid  
Lane, Philadelphia, PA 19118 (USA)

**Introduction**

*Aeromonas hydrophila* has long been recognized as a disease agent in fish and frogs and is gaining recognition as a human pathogen (1). The link between the microorganism and extra-intestinal infections (e.g., of skin and soft tissue) is firm, while that with gastro-intestinal infections awaits final confirmation (10). Members of the *A. hydrophila* group can be isolated as the sole pathogens from cases of diarrhea in humans, especially children. While these microorganisms occur widely in the environment, particularly in water and ultimately in foods exposed to these waters, the source of human isolates and thus the link between environment and human disease has not been identified. The objectives of this review are to discuss sources of the organism, behavior of the microorganism in the environment and foods, traditional phenotypic characterization of food and human isolates, and finally characterization of isolates using newer techniques which ultimately indicate that the human and food isolates are generally different. Also to be considered are the observations that culturing the microorganism at temperatures below 37°C can enhance significantly the expression of virulence associated factors. Thus, clinical microbiologists must continue seeking the source(s) of those isolates capable of causing human diarrheal disease.

### Occurrence

Microorganisms of the *Aeromonas hydrophila* group occur widely in the environment, in various foods, particularly those of animal origin (red meat, fish and seafood, poultry, raw milk), in vegetables, and in many water supplies where their numbers generally increase during the summer months. Both clinical and food isolates are capable of growth in food, water and culture media at temperatures from 0-5 to 42/43°C.

### Control

Because of the microorganism's ability to grow in foods at 0-5°C, low temperature holding by itself can not be used to restrict its growth. Work in our laboratory (13, 14, 15, 16) has defined the individual levels of NaCl, pH, and NaNO<sub>2</sub> which inhibit this organism (Table 1), and determined that combinations of factors can be more effective (Table 2). In other studies, we observed that the identity of the acidulant used to achieve acidic conditions is important, with acetic and lactic acids being particularly inhibitory, and in many instances, lethal (14). We have used the multifactoral approach to the study of inhibition of *A. hydrophila* by various combinations of temperature, NaCl, pH, NaNO<sub>2</sub>, and atmosphere. This type of study yielded a series of polynomial equations which then permits researchers to predict changes in the growth response of the organism in response to changes of culture/food conditions and formulation (15, 16).

Table 1. Control of growth of *A. hydrophila*.

---

Temperature:	5 to 42/43°C (some reports of growth at 0°)
pH:	5.3 to 8.4 acidulant important (in order of permissiveness): HCl > H <sub>2</sub> SO <sub>4</sub> > tartaric > citric > lactic > acetic
NaCl:	0 to 4.5%
NaNO <sub>2</sub> :	200 ppm
atmosphere:	facultative anaerobe (may grow in a N <sub>2</sub> atmosphere)

---

Table 2. Influence of temperature on pH and NaCl limits for *A. hydrophila* K144 (grown aerobically in BHI broth):

Temperature, °C	Limits of	
	pH	% NaCl
28	G <sup>a</sup> at 5.5 NG <sup>b</sup> at 4.5	G at 4.5 <sup>c</sup>
4	NG at 5.5	G at 3.5 NG at 4.5

<sup>a</sup> G = growth

<sup>b</sup> NG = no growth

<sup>c</sup> highest level tested

In addition to controlling the growth of *A. hydrophila* by various combinations of temperature, pH, NaCl, etc, the organism can readily be killed by either heat or irradiation (13). For example, the decimal reduction times (D-values) for five strains of the organism in raw milk at 48°C range from 3.20 to 6.23 min; in ground beef or bluefish, the irradiation decimal reduction treatment is 0.14 to 0.19 KGys. Thus, the presence of *A. hydrophila* can easily be eliminated by most of the commonly employed thermal processing operations on foods or by irradiation.

#### Characterization of Isolates

Investigators have examined clinical and environmental (food and water) isolates of the *A. hydrophila* group for the presence of potential virulence factors such as cytotoxic and cytotoxic enterotoxins, hemolysins, proteases, hemagglutinins, and endotoxins (lipopolysaccharide) (3, 4, 5, 6, 12) as well as specific biochemical reactions generally associated with the presence of individual virulence-associated factors. Using this approach of characterizing isolates, it was observed that clinical isolates are similar to environmental isolates in terms of biochemical traits and possession of virulence-associated factors (5, 6, 12). Thus, any attempt to determine the source of these organisms in human diarrheal disease has not proved fruitful. In addition, the negative human feeding study described by Morgan et al (10) utilized human isolates which produced cytotoxin (Y-1 adrenal cells), hemolysin, and enterotoxin (suckling mouse assay); the isolates also were lysine decarboxylase positive and produced acetylmethylcarbinol and DNase.

Currently, there are several newer techniques available which can allow epidemiologists to compare various food and environmental isolates and determine which are the same, thus the source of the clinical isolates can be identified. These techniques include gas liquid chromatography of cell wall fatty acid methyl esters (FAMES) (4), multilocus enzyme electrophoresis (MLEE) (19), biochemical fingerprinting (7), and ribotyping (11).

In contrast to the traditional biochemical characterization and speciation which showed similarities between the environmental and clinical isolates, these newer techniques indicated differences. In their comparison of drinking water and diarrheal isolates, Havelaar et al (4) found that biotyping alone was of little value for epidemiology of the strains; however, GLC of cell wall FAMES gave distinct profiles which indicated that there was little overall similarity between *Aeromonas* isolates from the two sources. Kuhn et al (7) described the technique of biochemical fingerprinting using a commercial microtiter plate test kit in which the growth response of cultures on 48 different substrates is determined. With this approach, they observed differences between food isolates and those from water. Tonolla et al (19) studied the genetic relationship between clinical and environmental isolates of *Aeromonas* strains with multilocus enzyme electrophoresis and determined that clinical isolates were genetically distinct from those collected from the environment. Moyer et al (11) used rRNA gene restriction (rDNA) patterns (ribotyping) to compare aeromonads isolated from clinical and environmental sources. With this technique, they were able to determine that the microorganism involved in an outbreak of gastroenteritis was *A. caviae* and that water from an unchlorinated well was the source of the organism. Thus, this technique permitted a ready identification of the source of the isolates and origin of the outbreak.

The basic questions are 1) "is food and water the vehicle of microorganisms of the *A. hydrophila* group which cause gastroenteritis?" and 2) "do we know what constitutes the mechanism by which the *A. hydrophila* group causes gastroenteritis?" Two different answers can be given to the first set depending on which technique the epidemiologist uses to characterize and identify the strains. Biochemical fingerprinting, FAMES, and MLEE suggest that environmental (food and water) isolates are different from clinical isolates, and ribotyping proved useful for determining the source of disease-causing isolates of a waterborne outbreak.

The answer to the second is less straightforward. We know that both human and environmental isolates of the *A. hydrophila* group possess factors associated with virulence in

other bacteria (3, 12). As mentioned above, the feeding study described by Morgan et al (10) failed to produce gastroenteritis in volunteers even though the isolates used possessed the virulence-associated factors of cytotoxin (against Y-1 adrenal cells), hemolysin, and enterotoxin (suckling mouse assay); their strains were positive in the rabbit ileal loop, but did not produce mannose-resistant hemagglutinins. Their biotype was VP+, LDC+, and DNase+. One possible explanation for the negative results from the feeding study may lie in the observation that temperature can have an effect on the expression of pathogenicity and virulence-associated traits (Table 3). It is likely (though unstated) that Morgan et al (10) employed 37°C for culturing their isolates. As can be seen in Table 3, temperature can have a major influence on the expression of several virulence-associated factors, with increased expression at lower temperatures. Thus, the strains used in any future feeding studies should be grown at room temperature or below; for food isolates, these should be grown at 5°C, the suggested holding temperature for most refrigerated foods.

Sherlock et al (18) who found that, based on frequency of isolation, *A. hydrophila* will preferentially colonize the bowels of immunocompromised patients (neutropenic patients and bone marrow transplant recipients).

In conclusion, the *A. hydrophila* group exists widely in food and water. Conventional identification and characterization techniques (biochemical and virulence associated factors) indicate that food and water isolates are similar to clinical isolates. However, newer techniques such as FAMES, MLEE, and biochemical fingerprinting of *Aeromonas* isolates have indicated that clinical isolates appears to a unique subset of the *A. hydrophila* group and distinct from those strains isolated from food and water. In a small outbreak of gastroenteritis associated with well water, ribotyping appeared to be a useful technique to trace the origin of the clinical isolates (11). Future research in this area should include: a) identification of possible new virulence associated factors, especially the importance/relationship of pili to gastroenteritic disease, and the use of additional epidemiological tools to characterize the specific subset of microorganisms of *A.*

Two further observations should be mentioned. The first is that of Kirov et al (5) who observed that environmental isolates which produced enterotoxin possessed numerous pili. However, these structures appear to be lost after infection, since isolates from patients with diarrhea were poorly piliated. From studies of other pathogens it is known that the presence of pili often contributes to the virulence of microorganisms (5). The second observation is that of

Table 3. Effect of temperature on the expression of virulence-associated factors and pathogenicity of *A. hydrophila* cultures.

Factor	Observation	Reference
mouse LD <sub>50</sub>	lower with cells grown at 10° vs. 30°C.	17
serum sensitivity/resistance	20°C grown cells resistant; 37°C grown cells sensitive.	9
LPS	20°C grown cells smooth; 37°C grown cells rough.	9
titer of extracellular products --protease --hemolysin --cytotoxin	increased in 20°C grown cells compared to 37°C grown cells	9
mouse and fish LD <sub>50</sub>	lower for cells grown at 20°C compared to 37°C	9
human isolates:		
protease	decreased activity at 37°C more at 37°C than at 28°C	8
hemolysins and cytotoxins		8
environmental isolates:		
protease, cytotoxin, hemolysin	production decreased at 37°C vs. 28°C	8
fish LD <sub>50</sub> :		
human isolates	lower at 7° vs. 25°C	2
food isolates	lower at 25° vs. 7°C	

*hydrophila* group capable of causing gastroenteritis in humans; and b) the use of temperatures below 37°C to characterize and describe the activities of isolates since lower temperatures of incubation have been shown to increase the expression of virulence associated factors such as hemolysin, proteases, and cytotoxins.

## References

1. Altwegg M, Geiss HK. *Aeromonas* as a human pathogen. *CRC Crit Rev Microbiol* 1989;16:253-86.
2. Buchanan RL, Bencivengo MM, Palumbo SA. Comparison of lethality in mouse versus goldfish for clinical and food isolates of *Aeromonas hydrophila*. *J Ind Microbiol* 1989; 4:189-94.
3. Cahill MM. Virulence factors in motile *Aeromonas* species. *J Appl Bacteriol* 1990;69:1-16.
4. Havelaar AH, Schets FM, van Silfhout A, Jansen WH, van der Kooij D. Typing of *Aeromonas* strains from patients with diarrhoea and from drinking water. *J Appl Bacteriol* 1992;72:435-44.
5. Kirov SM, Rees B, Wellock RC, Golsmid JM, Van Galen AD. Virulence characteristics of *Aeromonas* spp. in relation to source and biotype. *J. Clin Microbiol* 1986; 24:827-34.
6. Krovacek K, Faris A, Baloda SB, Lindberg T, Peterz M, Mansson I. Isolation and virulence profiles of *Aeromonas* spp. from different drinking water supplies in Sweden. *Food Microbiol* 1992;9:215-22.
7. Kuhn I, Lindberg T, Olsson K, Stenstrom TA. Biochemical fingerprinting for typing of *Aeromonas* strains from food and water. *Lett Appl Microbiol* 1992;15:261-5.
8. Mateos D, Anguita J, Naharro G, Paniagua C. Influence of growth temperature on the production of extracellular virulence factors and pathogenicity of environmental and human strains of *Aeromonas hydrophila*. *J Appl Bacteriol* 1993;74:111-8.
9. Merino S, Camprubi S, Tomas JM. Effect of growth temperature on outer membrane components and virulence of *Aeromonas hydrophila* strains of serotype O:34. *Infect Immun* 1992;60:4343-9.
10. Morgan DR, Johnson PC, Dupont HL, Satterwhite TK, Wood LV. Lack of correlation between known virulence properties of *Aeromonas hydrophila* and enterotoxigenicity in humans. *Infect Immun* 1985;50:62-5.

11. Moyer N, Martinetti G, Luthy-Hottenstein J, Altwegg M. Value of rRNA gene restriction patterns of *Aeromonas* spp. for epidemiological investigations. *Curr Microbiol* 1992;24:15-21.
12. Palumbo SA, Bencivengo MM, Del Corral F, Williams AC, Buchanan RL. Characterization of the *Aeromonas hydrophila* group isolated from retail foods of animal origin. *J Clin Microbiol* 1989;27:854-9.
13. Palumbo SA, Buchanan RL. Factors affecting the growth or survival of *Aeromonas hydrophila* in foods. *J Food Safety* 1988; 9:37-51.
14. Palumbo SA, Williams AC. Growth of *Aeromonas hydrophila* K144 as affected by organic acids. *J Food Sci* 1992; 57:233-5.
15. Palumbo SA, Williams AC, Buchanan RL, Phillips JG. Model for the aerobic growth of *Aeromonas hydrophila* K144. *J Food Protect* 1991; 54:429-35.
16. Palumbo SA, Williams AC, Buchanan RL, Phillips JG. Model for the anaerobic growth of *Aeromonas hydrophila* K144. *J Food Protect* 1991; 55:260-5.
17. Schubert RHW, Matzinou D. Temperature as an environmental factor influencing the pathogenicity of *Aeromonas hydrophila*. *Zbl Bakt* 1990;273:327-31.
18. Sherlock, CH, Burdge, DR, Smith JA. Does *Aeromonas hydrophila* preferentially colonize the bowels of patients with hematologic malignancies? *Diagn Microbiol Infect Dis* 1987; 7:63-8.
19. Tonolla M, Demarta A, Peduzzi R. Multilocus genetic relationships between clinical and environmental *Aeromonas* strains. *FEMS Microbiol Lett* 1991;81:193-200.