

# 12. *Aeromonas Hydrophila*: A Re-Emerging Pathogen

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## Introduction

Species of aeromonads are Gram negative, non-spore forming, rod shaped, facultatively anaerobic bacteria that occur ubiquitously and autochthonously in aquatic environments. The genus consists of psychrophiles and mesophiles from aquatic and soil environments encompassing a diverse spectrum of disease causing species/strains to many warm and cold-blooded animals.

## Taxonomy

The taxonomy of this group was not well understood until recently. Studies on the genotypic characteristics have resulted in the description of a number of closely related genotypic species. In ecological terms, however, we can still refer to the four classically described species, divided into two groups,

1. The motile mesophilic species - *A. hydrophila*, *A. caviae* and *A. sobria*.
2. The psychrophilic and non-motile *A. salmonicida*.

Although historically the genus *Aeromonas* has been placed in the family *Vibrionaceae* (Popoff, 1984), there have been proposals to place it in its own family, the *Aeromonadaceae* (Colwell et al., 1986). The aeromonads share many biochemical characteristics with members of the Enterobacteriaceae, from which they are primarily differentiated by being oxidase positive. Earlier literature focused mainly on *A. hydrophila*. But several later studies have shown that the majority of clinical isolates fall within three species (Burke et al., 1984a). *Aeromonas hydrophila* (Group 1), *A. sobria* (Group 8) and *A. caviae* (Group 4). After the theoretical conception of the genus *Aeromonas* by Kluver and van Niel (1936) and the first classification of a recognizable species i.e., *A. hydrophila* by Stanier (1934), scientific interest on this group of organisms virtually ceased. When some Scandinavian bacteriologists interested in Enterobacteriaceae isolated some strains with biochemical properties akin to Enterobacteriaceae but

with polar flagella common to *Pseudomonadaceae*. This resulted in what may be regarded as the first phase of *Aeromonas* taxonomy.

## Distribution of *Aeromonas* sp.

The distribution of mesophilic *Aeromonas* species in the aquatic environment may be related to the levels of pollution in the water. It has been reported by several authors including Schubert (1975); Araujo et al. (1991) and Stecchini and Domenis (1994) that *A. caviae* predominates waters with a high degree of faecal pollution. In less polluted waters, either fresh or marine, *A. caviae* and *A. hydrophila* were almost equally distributed. *A. sobria* can be found in unpolluted waters, brackish waters and sewage effluents. Large numbers of aeromonads, especially *A. caviae*, could therefore be considered to be indicative of nutrient-rich conditions of water. The numbers of mesophilic *Aeromonas* species in some aquatic environments have been reported to show a seasonal variation, with populations greatest in the warmer months of the year for both temperate freshwater lakes and chlorinated drinking water.

The aquatic environments for which the ecology of aeromonads has been investigated can be conveniently divided into four water types: freshwater, seawater, sewage and potable water.

## Distribution of *Aeromonas hydrophila* in marine waters

The majority of studies of *Aeromonas* in saline waters have been initiated by their importance as fish pathogens or as a potential pathogen of humans and have, therefore, tended to focus on commercial and sport fisheries and recreational waters. Mesophilic aeromonads are halotolerant and are associated with direct discharges to the sea or via rivers and streams. In their study, Hazan et al. (1978) concluded that although *A. hydrophila* was not generally considered to be a marine bacterium, it could be found naturally in marine systems which interface with fresh water,

and at all salinities except the most extreme. In general, their populations in saline waters were higher (up to  $9 \times 10^5$  cells  $100 \text{ ml}^{-1}$ ) than in freshwater habitats.

As within the freshwater, seasonal variations in abundance have been noted in the saline environs, with a rise in spring and a decrease after autumn. In the tidal estuary of the Anacosta River, Washington DC, Seidler et al. (1980) recorded a peak of  $3 \times 10^4$  CFU  $100 \text{ ml}^{-1}$  in August, declining to a minimum by February and rising again in May when the water temperature rose to  $22.5^\circ\text{C}$ . Kaper et al. (1981) noted a similar seasonal variation in planktonic *A. hydrophila* populations in Chesapeake Bay, but did not find a wide fluctuation in sediment populations associated with the seasons. Correlations with *A. hydrophila* numbers were demonstrated for temperature, total viable counts and faecal coliforms and inversely for dissolved oxygen and salinity. In a study from a coastal bay in Japan, Nakano et al. (1990) found that *A. caviae* was the most abundant of the mesophilic aeromonads, while *A. sobria* predominated in brackish waters.

Laboratory studies on survival of *A. hydrophila* and *A. sobria* in saline waters by Araujo et al. (1991); Monfort and Balcux (1991) and Lowcock and Edwards (1994) have shown that when *Aeromonas* were inoculated into sterile seawater, there was at first an acute reduction in the numbers of culturable cells, followed by an increase, the extent of which was dependent on the concentration of organic matter present.

## Association with seafood

Seafood products are among the ideal substrates for proliferation of *Aeromonas* (Freij, 1987; Janda, 1991). In tropical countries like India, fishery products are contaminated by various food-borne pathogens. Pollution and cross contamination levels are very high in many developing tropical countries due to lack of infrastructure. It is important that fishery products should be maintained free from pathogens before consumption.

Variations in the incidence level of *A. hydrophila*, the most common aeromonad in seafood can be attributed by secondary contaminations such as handling, usage of polluted/non-hygenic water, storage of seafoods in inadequate facilities etc. Also, aeromonads may be introduced from water, animal faeces, or food-handlers. The ubiquity of this organism means it has the potential to be food-borne (Buchanan and Palumbo, 1985; Morgan and Wood, 1988; Wadstrom and Ljungh, 1991).

In United States food poisoning is responsible for a major loss of economic resources, being second to the common cold in causing time lost from work (Madden et al., 1986). About 35 - 40% of the reported food-borne outbreaks, which occur in the US each year, are of unknown etiology (Buchanan, 1984). It has been generally assumed that a large numbers of these unidentified food poisoning cases are caused by species not routinely tested for during investigations of outbreaks. In the last few years, motile aeromonads have been included in the list of bacterial species that are considered enteric pathogens (Gracey et al., 1982; Janda et al., 1983; Holmberg et al., 1986; Janda, 1987). There have also been several reports of aeromonad-contaminated meat, poultry and raw milk (Eddy and Kitchell, 1959; Kielwein et al., 1969; Kleeberger, 1975; Toule and Murphy, 1978; Abeyta et al., 1986) but there have been few systematic studies on the incidence of *Aeromonas* sp. in foods although one report suggested that these organisms were very common contaminants of food (Palumbo et al., 1985). Hudson et al., (1992) examined the presence of *A. hydrophila* from ready to eat seafood. Among the ready-to eat seafood about 40% of the shellfish were contaminated by *A. hydrophila* whereas in finfish it was found to be 28%. During the past several years there has been increasing interest concerning the possible role of species of *A. hydrophila* as a cause of human infections. During monsoon season, increased incidence of *A. hydrophila* was reported (Vivekanandhan et al., 2004).

The much higher incidence of *Aeromonas* sp. in foods purchased at retail outlets suggests that the source of contamination may not be faeces (Burke et al., 1984a; Slade et al., 1986) and there is an increasing evidence that some strains of *Aeromonas* sp. cause diarrhoea in human beings and it is likely that uncooked or cross contaminated foods act as a source of infection.

This organism has been isolated from all over the world, and its source is wild fish, and pond cultured edible and ornamental fish. *A. hydrophila* was first reported as associated bacteria exclusively with diseased fish (Snieszko and Bullock, 1976). The organism is now known to be part of the intestinal flora and tissue of healthy fish (Trust and Sparrow, 1974; Wolke, 1975; Shotts et al., 1976; Boulanger et al., 1977; Heuschmann - Brunner, 1978; Nieto et al., 1984) and for this reason *Aeromonas* is easily isolated from retail market finfish. Another known reservoir is shellfish, particularly, oysters (Abeyta et al., 1986). Thayumanavan et al., (2003) reported 37 % of the freshly caught seafood (finfish and prawn) collected from east coast (Rameshwaram, Tuticorin and Porto Novo) and west coast (Kochi) of South India were

contaminated with *A. hydrophila*. They also reported the maximum incidence (about 42 %.) of *A. hydrophila* was recorded at Kochi, followed by Rameshwaram, Tuticorin and Porto Novo.

Considering the psychrotrophic nature of this organism and the obvious implication of food poisoning strains that can grow readily at refrigeration temperatures, it seems imperative that the information needed to assess their food safety significance be obtained at the earliest possible date. Apart from the psychrotrophic nature of this organism, during the transportation, the periodical dampening of seafood with contaminated water, besides sprinkling with contaminated wet-sand and packaging with cheap quality of ice are customary practices to decelerate the tropical heat. Cumulative effect of such conventional practices, coupled with unhygienic handling during transportation results in secondary contamination and explains the high level of *A. hydrophila* in marketed seafood.

## Association with human illness

*A. hydrophila* has received particular attention because of its association with human diseases. It has been isolated from both polluted and unpolluted bodies of water through out the world (Schubert, 1975). A study conducted by Fricker and Tompsett (1989) showed that *Aeromonas* sp. was extremely common contaminants of human foodstuffs and that some of the strains appear to be virulent. In their study most of the toxigenic strains were isolated from seafood and they suggested that seafoods were potential sources of virulent aeromonads. Therefore, in cases of foodborne bacterial illnesses in which oysters are implicated, *Aeromonas* sp. should be included in the general screening for causative microorganisms (Abeyta et al., 1986).

A common source of *A. hydrophila* in outbreaks of gastroenteritis is from water supplies such as mineral springs, seawater environments, chlorinated and unchlorinated domestic supplies and watersheds polluted by sewage effluents. Sources other than water from which this organism can be readily isolated include seafood, foods of terrestrial animal origin such as meats, dairy products and poultry and vegetables. Asymptomatic and immunocompromised human carriers involved with handling of foods are another potential source. The presence of *Aeromonas* in the food chain should not be ignored. *Aeromonas* species are regarded as controversial primary pathogen but several worldwide investigations clearly now indicate that at least some strains are clearly enteropathogens and few cases are linked to gastroenteritis (Altwegg

and Geiss, 1989; Janda, 1991). Although the incidence of *Aeromonas* in foods is high, they vary between countries and also among the type of strains.

Two factors appear to be affecting the significance of *Aeromonas* spp. as a re-emerging pathogen. The first is the consumer driven demand for less processed and more natural foods containing fewer additives, there in growing emphasis on, refrigeration as the primary means for controlling microbial growth in food. The second point is that during the warm season there is an increase in water associated *Aeromonas* spp. strains (also in some cases associated with an increase in gastroenteritic patients; Burke et al., 1984). Gastroenteritis caused by *Aeromonas* spp. has been documented, and the incidence of gastroenteritis tends to be higher in summer than other seasons (Burke et al., 1984).

While *Aeromonas* is undoubtedly more commonly isolated from patients with gastroenteritis, reports of *Aeromonas* sepsis, wound and ocular infections, peritonitis and meningitis, as well as a number of miscellaneous infections have appeared increasingly in the literature. Unlike gastroenteritis these infections are often reported to have fatal or serious debilitating outcomes, such as amputation. Various diseases caused by the species of *Aeromonas* sp. are:

1. Septicaemia,
2. Wound infections,
3. Ocular infections,
4. Respiratory infections,
5. Bone and joint infections,
6. Intra-abdominal infections,
7. Miscellaneous infections.

## Drug resistance in *Aeromonas*

The spread of drug resistance among *Aeromonas* spp., is of concern because recent surveys indicate the emergence of these organisms as primary human pathogens (Holmberg and Farmer, 1984; Burke and Gracey, 1986). The presence of antibiotic resistant *A. hydrophila* in natural habitats can pose a public health risk. Occurrence of multiple antibiotic resistant *A. hydrophila* in shoreline sediments, marine waters and shellfishes has been recognised as an important public health hazard. The prevalence of such multiple antibiotic resistant organisms in food and water is of considerable significance in relation to public health.

Multiple antibiotic resistance of *A. hydrophila* strains from organs of infected catfish, *Clarius batrachus* has been reported (Pathak et al., 1993). In their studies, frequency of resistance was exhibited against ampicillin followed by streptomycin, chloromphenicol and nalidixic acid. But all isolates exhibited susceptibility to tetracycline. Strains of *Aeromonas*

sp. also showed high frequency of resistance to vancomycin, novobiocin, rifampicin and methicillin (Pettibone et al., 1996). Son Radu et al. (1997) have reported resistance towards chloramphenicol, erythromycin, kanamycin, nalidixic acid, streptomycin, sulphamethoxazole - trimethoprim and tetracycline among *A. hydrophila* isolates from cultured fish. Pettibone et al. (1996) reported the susceptibility of *Aeromonas* sp. to chloramphenicol, erythromycin, kanamycin, polymyxin-B, streptomycin and trimethoprim. These antibiotics are generally active agents against strains of *Aeromonas* (Khardori and Fainstein, 1988). Vivekanandhan et al. (2002) reported that 99 % of the fish isolates and 100 % of the prawn isolates were resistant to bacitracin, while none of the prawn isolates and 4.4 % of the fish isolates isolated from retail seafood outlets of Coimbatore, South India were sensitive to chloramphenicol. Thayumanavan et al. reported that all the *A. hydrophila* isolates isolated from freshly caught seafood of coastal South India were sensitive to chloramphenicol and resistant to bacitracin.

## Virulence factors

Aerolysin is a major factor in the pathogenicity of *A. hydrophila*. The protein crosses the inner and outer membranes of the exporting bacteria as well as the plasma membrane of eucaryotic cells, which it punctures by forming discrete channels.

Our understanding of the role of *Aeromonas* extracellular toxins in the pathogenesis of disease has largely been based upon the moieties resulting from previous attempts to isolate and purify *Aeromonas* haemolysin and enterotoxin. Multiple biological activities of cell-free culture filtrates (CFCF) of strains of *A. hydrophila*, including the ability to cause haemolysis of erythrocytes, were reported by Caselitz and Gtinther (1960). The haemolytic activity was heat-labile, inactivated by trypsin and shown to be cytotoxic to HeLa cells and diploid lung fibroblasts, dermonecrotic in rabbit skin and lethal to mice. Thayumanavan et al., (2003) reported 78.4 % of the *A. hydrophila* strains isolated from freshly caught seafood collected from coastal South India were the producers of haemolysin.

Partial purification of an extracellular heat-labile haemolysin produced by a strain of *A. hydrophila* was also reported by Bernheimer and Avigad (1974). The resulting toxigenic fractions of partially purified haemolysin contained several different proteins, shown as faintly staining bands by SDS-PAGE, in addition to the haemolysin. The toxin appeared extracellularly during the logarithmic growth phase and its molecular weight was estimated to be 49-53 k Da.

*A. hydrophila* has been associated with several disease conditions, including tail and fin rot and haemorrhagic septicaemia. Fish pathogenic isolates produced various toxins of largely undetermined functions. Interestingly, the presence of lateral flagella has been considered to be related to the presence of toxigenic properties. Surface structures of *A. hydrophila* have been associated with autoaggregation, hydrophobicity and haemagglutination. The surface of such strains has been shown to be completely covered by a regularly arrayed surface layer (S-layer) that may provide protection for microorganisms in their natural environment, composed of tetragonally arranged subunits. S-layers (S-Layer protein) are present on a number of human pathogenic bacteria, including *Campylobacter* spp., as well as on many non-pathogenic bacteria. Fish pathogenic isolates produce extra cellular proteins (ECP) with considerable enzymatic activity.

## Outer membrane proteins (OMP)

The OMP of *A. hydrophila* are rather heterogeneous, although most strains produce a 36 k Da protein. In addition, iron-starved *A. hydrophila* cells synthesize new OMP of 68 – 93 k Da. Mittal et al., (1980) reported that a group of *A. hydrophila*, virulent fish pathogens, exhibited a number of unique phenotypic properties that were thought to be cell-surface associated, including autoaggregation during growth in static broth culture and resistance to the bactericidal activity of normal serum.

## Lipopolysaccharide (LPS) endotoxin

The O-antigen structure of virulent strains of *A. hydrophila* has been shown to have many points of similarity with that of the O-antigen of *A. salmonicida*. LPS endotoxin is an important component of the outer membrane of *A. hydrophila*, which has been shown to enhance red-leg disease in frogs (Rigney et al., 1978). However, the importance of this toxin in human infections has not yet been elucidated. Tsai and Chen (1995) reported the toxigenicities of *A. hydrophila* isolates from seafood in relation to their growth at various temperatures, pH and salinities. Presence of *A. hydrophila* in a refrigerated food, which is undergoing spoilage, suggests the capability of competitive growth at refrigeration temperatures.

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