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Diversity and Virulence Profile of *Aeromonas* from potable water

B.A. Desai* and P.B. Desai
Correspondence should be addressed to B.A. Desai.

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Abstract

Aeromonas spp. are autochthonous in the aquatic ecosystem and some of them has been increasingly found, in patients with various diseases like enteritis, wound infection and even septicemia in amphibians, reptiles, frog, fish and in patients with impaired immunity. There are different virulence factors like aerolysin, hemolysins, cytotoxins, enterotoxins, proteolytic activity, lipolytic activity, gelatinase, slime production, DNases, and adhesions. These virulence factors are used as survival means, self defense mechanism and establishment of pathogenicity. They are also capable of forming biofilm and are found multiresistant, although free cells of *Aeromonas* may be relatively susceptible to disinfection; populations associated with biofilms may survive high chlorine dosing. Thus water distribution systems should be monitored to check the quality of water and for the purpose of public health.

Keywords: *Aeromonas* species, human pathogens, virulence factors.

Introduction:

Aeromonas species are ubiquitous microorganisms found in both aquatic and environmental habitats such as estuary, sediment, sea water , sea grass, sea weed, waste and used water, food and drinking water (Abbott *et al.,* 2003; Matyar *et al.,* 2007; Martinez-Mucia *et al.,* 2008). They are Gram negative, short rod shape, oxidase and catalase positive, motile, facultative anaerobes, multiple resistant and non spore forming. Like coliforms, *Aeromonas* spp. is Gamma-proteobacteria but they are taxonomically distinct from the Enterobacteriaceae in which coliform genera have been placed. (Leclerc et al. 2001).

Nineteen species of the genus have been identified till date (Alperi et *al.,* 2010; Matyar et al., 2007). Motile group includes *Aeromonas hydrophila, A. sobria* and *A. caviae*. Non-motile group mainly consist of *A. salmonicida*, mostly fish pathogen. Among these species, *A. hydrophila* is the most studied due to its presence in food (Radu et al., 2003), water (Asmat and Gires, 2002), estuary (Odeyemi et al., 2012), antibiotic resistance and it ability to cause infections in human and animals (Evangelista-Barreto et al., 2010). *A. hydrophila* has been identified as causative agent of human diseases such as septicemia, meningitis, wound infections as a result of exposure to contaminated marine environment and diarrhea (Evangelista-Barreto et al., 2010; Messi et al., 2003).

***Aeromonas* human pathogens:**

Popoff, Kluyver and Niel (2009); Carnahan and Joseph (1991) suggested that *Aeromonas* speciation based on phenotypic characteristics is difficult. The main reason for this is that current *Aeromonas* taxonomy is based on the DNA relatedness of the species within the genus. There have been reports by Abbott (1998) of mistyping *Aeromonas* isolates as *Vibrio cholera*. Janda and Abbott (1998); Gavriel (1998) reported that it has been useful to divide the genus into non-motile that are commonly fish pathogens (e.g., *A. salmonicida*) and motile mesophiles (especially *A. hydrophila*, *A. caviae*, and *A. sobria*, *A. veronii*) that are considered opportunistic human pathogens and the causative agents in gastroenteritis and wound infections.

Aeromonas hydrophila was on the U.S. EPA's Contaminant Candidate List-2 (CCL) primarily because of its potential to grow in water distribution system biofilms (Edberg et al. 2007). No point-source disease outbreaks have been attributed to *Aeromonas* spp. (U.S. EPA Office of Water 2006). Bernagozzi (1995) suggested that *Aeromonas* spp. might be indicators of nutrient loading in surface waters rather than fecal contamination. However, they are present in feces of healthy animals and humans (U.S. EPA Office of Water 2006). Percival (2004) stated that *Aeromonas* spp. may be normal fecal micro flora and concluded that "it is possible that *Aeromonas* may not even be a true enteric pathogen". Messi (2002) thought *A. hydrophila* should "not be considered a normal inhabitant of the human gastrointestinal tract". Edberg (2007) considered *Aeromonas hydrophila* a "putatively emerging enteric pathogen" but stated that "the role of drinking water consumption in *Aeromonas* infections is unclear". While some evidence for waterborne transmission has been reported by Moyer and Larew (1996) from drinking untreated water, only one case of human infection (a 3 month old infant suffering from Kwashiorkor) has been reported from drinking treated water. Pablos (2010) reported that the infectious dose is high enough that waterborne transmission is unlikely except in persons with fragile gastrointestinal tracts, e.g. small children.

Virulence factors:

Several studies by Albert (2000) have identified toxins or other virulence factors in *Aeromonas* strains. The virulence factors include hemolysins, cytotoxins, enterotoxins, proteases, elastase, lipases, DNases, and adhesins (type IV pili, lateral and polar flagella) (Agarwal et al. 1998; Cascon et al. 2000; Rabaan et al. 2001; Sen et al. 2007). Chopra and Houston (1991) provided a detailed review of *Aeromonas* toxins. Additional virulence factors and regulatory genes have been reported recently, and they include enolase, glucose-inhibited divison A (*gidA*), virulence-associated protein B (*vacB*), DNA adenine methyltransferase (*dam*), T3SS and T6SS effectors, and ToxR regulated lipprotein (*tagA*) (Khajanchi et al. 2010). It is likely that pathogenicity of *Aeromonas* strains is multifactorial, but the presence of a particular array of virulence genes probably distinguishes pathogenic from non-pathogenic strains (Percival et al. 2004; von Graevenitz 2007). Several of the virulence factors have been identified in strains isolated from water (Kuhn et al. 1997a; Handfield et al. 1996; Fernandez et al. 2000; Sen and Rodgers 2004; Pablos et al. 2009; Bhowmik et al. 2009). Aguilera-Arreola (2005) reported a high degree of genetic diversity within the species *A. hydrophila*, based on the distribution of virulence factors in environmental and clinical isolates.

Virulence factors such as aerolysin, haemolysin, cytosine, enterotoxin, proteolytic activity, lipolytic activity, gelatinase, slime production and antimicrobial peptides have been identified in *A. hydrophila*. (Asmat and Gires, 2002; Castro - Escarpulli et al., 2003; Martins et al., 2002; Illanchezian et al., 2010). These virulence factors are used as survival means, self defense mechanism and establishment of pathogenicity. These are mostly found in bacteria including *Aeromonas* spp. (Singh et al., 2010). Aeromonads have been attributed to human infections like *gastroenteritis*, septicemia and wound infections (Illanchezian et al., 2010). In 2004, Subashkumar and colleagues, stated protease, aerolysin, hemolysin, enterotoxins, lipases, gelatinase and biofilm formation as virulence factors in *Aeromonas* spp. Biofilm is an irreversible growth of aggregated bacterial micro-colonies on surfaces embedded in extracellular polysaccharide matrix. Biofilm formation results into resistance of bacteria to conventional antibiotics and persistent infections (Rodney 2008). Anne and Elizabeth (2003) attributed increase of antibiotics resistance in *Aeromonas* spp., hence posing threat to human health and environment. In the study made by

Chacon (2003), the detection of virulence factors of *Aeromonas hydrophila* is a key component in determining potential pathogenicity because these factors act multifunctionally and multifactorially. For rapid detection of two virulence factors of isolated *Aeromonas hydrophila*, a polymerase chain reaction assay was used. The detected virulence factors include aerolysin (aer A) and haemolysin (hyl H).

Gonza´lez (2001) reported that because of *Aeromonas hydrophila's* structure, it is very toxic to many organisms. When it enters the body of its victim, it travels through the bloodstream to the first available organ. It produces Aerolysin Cytotoxic Enterotoxin (ACT), a toxin that can cause tissue damage. *Aeromonas hydrophila, Aeromonas caviae,* and *Aeromonas sobria* are all considered to be opportunistic pathogens, meaning they rarely infect healthy individuals. Neyts (2000) studied that *Aeromonas hydrophila* is widely considered a major fish and amphibian pathogen, and its pathogenicity in humans has been recognised for decades. It was proved by Yogananth (2009) that the pathogenicity of *Aeromonas* spp. is mediated by a number of extracellular proteins such as aerolysin, lipase, chitinase, amylase, gelatinase, hemolysins and enterotoxins. However the pathogenic mechanisms of *Aeromonas* spp. are unknown. The recently proposed type III secretion system (TTSS) has been linked to *Aeromonas* pathogenesis. The TTSS is specialized protein secretion machinery that exports virulence factors directly to host cells.

Albert (2000) found that Aeromonads are causative agents of a number of human infections. Even though aeromonads have been isolated from patients suffering from diarrhea, their etiological role in gastroenteritis is unclear. In spite of a number of virulence factors produced by *Aeromonas* species, their association with diarrhea has not been clearly linked. Recently, he has characterized a heat-labile cytotonic enterotoxin (Alt), a heat-stable cytotonic enterotoxin (Ast), and a cytotoxic enterotoxin (Act) from a diarrheal isolate of*Aeromonas hydrophila*. Alt and Ast are novel enterotoxins which are not related to cholera toxin; Act is aerolysin related and has hemolytic, cytotoxic, and enterotoxic activities. For the first time, *Aeromonas eucrenophila* was isolated from two children, one with diarrhea and another without diarrhea.

Prevalence in Drinking Water:

Prevalence and abundance in Drinking Water, the occurrence of *Aeromonas* spp. in a municipal drinking water system was monitored for 1 year in Leon, Spain (Pablos et al. 2009). Sen and Rodgers (2004) used PCR to determine the distribution of six virulence genes in these isolates. There was a variety of combinations of the genes among different strains of the same species. About 50 % of 171 strains were hemolytic against human erythrocytes (Ghenghesh et al. 2001). Of the 21 environmental strains isolated from these waters that were regularly used for domestic purposes, 81% showed cytotoxicity, 71 % produced 27 hemolysin, 90 % demonstrated human serum resistance and all were multiple drug resistant. Some of the isolates were able to induce fluid accumulation (enterotoxic) (Bhowmik et al. 2009). A PCR assay detected the *aer* gene (a marker for aerolysin) in 80 % of the isolates (n=445) (Ormen et al 2001). *Aeromonas* spp. were cultured from 30 % of environmental samples (n=2120) in Bangladesh, including surface waters, sediments, and aquatic plants. Colony blots from isolates were hybridized with probes for three toxin genes (*act*, *alt*, *ast*). The assortment of these genes among isolates of different species was very diverse. Only two *A. hydrophila* isolates (n=18) were positive for all three genes. Two of the genes, *alt* and *ast*, were considered reliable markers for strains responsible for diarrheal infections in children (Albert et al. 2000).

Antibiotic susceptibility of Aeromonas:

Hazen (1978); Janda and Abbott (1996) reported the *in vitro* susceptibility of the isolates was studied by disk diffusion method using discs (Oxoid) containing the following antibiotics were used: penicillin G (10 U), tetracycline (30 µg), gentamycin (30 µg), azitromycin (15 µg), trimethoprim-sulfamethoxazole (25 µg), naladixic acid (30 µg) and norfloxacin (10 µg). All bacterial strains studied in the research showed high degree of resistance to antibiotics. Resistance rate to tetracycline and penicillin was more than other antibiotics. Multiple drug resistance was also observed in all *Aeromonas hydrophila* isolates. The development of resistant or even multidrug resistant pathogens in recent years has become a major problem in Iran and many countries. Antimicrobial resistance of *Aeromonas* has been studied by many authors

(Thayumanavan et al., 2003). Some of the authors Poobalane (2008) indicated that *A. hydrophila* isolated from water, food and clinical samples was not susceptible to many antimicrobial agents. However, 30 antibiotics resistance was found in 91.6% of studied isolates and in all *A. hydrophila* strains. The results also show that resistance to penicillin and tetracycline was observed in 79 and 83% of the isolates. In addition, all *A. hydrophila* strains were multiresistant, what may be the result of the spread of resistance genes among the isolated bacteria. Castro-Escarpulli (2003) reported that the best antimicrobial effect on *Aeromonas* is obtained by applying the first-generation quinolone and the second and third generation cephalosprins. Although, Stojanov (2010) indicated that a high percentage of the *Aeromonas* strains were resistant to Flumequine (over 35%) and Olaquindox (around 20%), as a representative of Quinolone. Resistance profile of antibiotics observed in decreasing order of resistance of the isolates was tetracycline, penicillin, gentamycin, norfloxacin, azitromycin, trimethoprim-sulfamethoxazole and nalidixic acid.

Biofilm formation and chlorine sensitivity:

Various types of drinking-water demonstrated large differences in biofilm formation rates. *Aeromonas* in drinking-water in distribution systems has been controlled by increased disinfection, and it appears that free cells of *Aeromonas* are relatively susceptible to the common chlorine-based disinfectants. Knochel (1991) found that strains of *A. hydrophila*, *A. sobria*, *A. caviae*, and *A. veronii* were generally more susceptible to chlorine and monochloramine, and Edge et al, 1987 found that laboratory-grown and environmental *Aeromonas* were also susceptible to chlorine dioxide. Despite this relative susceptibility to chlorine-based disinfectants, controlling the numbers of aeromonads in a distribution system may require some considerable time and chlorine concentrations in excess of 0.2 mg/l (Edge et al, 1987). This is probably due to association of the organisms with biofilms. Langsrud (2003) proved that there was evidence that the biofilm-associated *A. hydrophila* would also survive 0.6 mg/l monochloramine, which was sufficient to eradicate biofilm-associated *E. coli*. These data indicate that, although free cells of *Aeromonas* may be relatively susceptible to disinfection, populations associated with biofilms may survive high chlorine dosing.

Conclusion:

Thus it is suggested, that the presence of *Aeromonas species* in drinking water needs public health appraisal and further work should be undertaken to permit revaluation of standards for the quality of drinking water. Even some solution should be made to solve the problem of Biofilm formation in different water distribution systems. Thus it serves to check the quality of water and for the purpose of public health.

References:

1. Abbott S L, L S Seli, M Catino, M A Hartley, and J M Janda. (1998). Misidentification of unusual Aeromonas species as members of the genus Vibrio: a continuing problem. J Clin Microbiol. 36: 1103-1104.
2. Albert M J, M Ansaruzzaman, K A Talukder, A K Chopra, I Kuhn, M Rahman, A S G Faruque, M S Islam, R B Sack, and R Mollby. (2000). Prevalence of enterotoxin genes in Aeromonas spp. isolated from children with diarrhea, healthy controls, and the environment. J Clin Microbiol. 38: 3785-3790.
3. Alperi, A. – Martínez-Murcia, A. J. - Wenchien, K. O. – Arturo, M. –Maria, J. S. – Maria, J. F. (2010). Aeromonas taiwanensis spp. and Aeromonas sanarellii spp., Clinical species from Taiwan. in international journal of systematic and evolutional microbiology,60: 2048 – 2055.
4. Bernagozzi M, F Bianucci, E Scerre, and R Sacchetti. (1994). Assessment of some selective media for the recovery of Aeromonas hydrophila from surface waters. Zentralblatt for Hygiene und Umweltmedizin. 195: 1221-134.
5. Bhowmik P, P K Bag, T K Hajra, R De, P Sarkar, and T Ramamurthy. (2009). Pathogenic potential of Aeromonas hydrophila isolated from surface waters in Kolkata, India. J Med Microbiol. 58: 1549-1558.
6. Carnahan A M and S W Joseph. (1991). Aeromonas update - new species and global distribution. Experentia. 47: 402-403.

7. Cascon A, J Yugueros, A Temprano, M Sanchez, C Hernanz, J M Luengo, and G Naharro. (2000). A major secreted elastase is essential for pathogenicity of Aeromonas hydrophila. Infect Immun. 68: 3233-3241.
8. Castro-Escarpulli G, M.J Figueras, G Aguilera-Arreola, L Soler, E Fernandez-Rendon, G.O Aparicio, J Guarro and M.R Chacon. (2003). Characterisation of Aeromonas spp. isolated from frozen fish intended for human consumption in Mexico. International Journal of Food Microbiology. 84 (1): 41-49.
9. Chacon M.R., M.J. Figueras, G. Castro-Escarpulli, L. Soler and J. Guarro, (2003). Distribution of virulencegenes in clinical and environmental isolates of of Aeromonas spp.
10. Edberg S C, F A Browne, and M J Allen. (2007). Issues for microbial regulation: Aeromonas as a model. Crit Rev Microbiol. 33: 89-100.
11. Edge JC and Finch PE (1987). Observations on bacterial aftergrowth in water supply distribution systems: implications for disinfection strategies. Journal of the Institute for Water and Environmental Management. 1, 104–110.
12. Fernandez M C, B N Giampaolo, S B Ibanez, M V Guagliardo, M M Esnaola, L Conca, P Valdivia, S M Stagnaro, C Chiale, and H Frade. (2000). Aeromonas hydrophila and its relation with drinking water indicators of microbiological quality in Argentina. Genetica. 108: 35-40.
13. Gavriel A A, J P B Landre, and A J Lamb. (1998). Incidence of mesophilic Aeromonas within a public drinking water supply in northeast Scotland. J Appl Microbiol. 84: 383-392.
14. Ghenghesh K S, A El Ghodban, R Dkakni, S Abeid, A Altomi, A Tarhuni, and K Marialigeti. (2001). Prevalence, species differentiation, haemolytic activity, and antibiotic susceptibility of aeromonads in untreated well water. Memorias do Instituto Oswaldo Cruz. 96: 169-173.
15. Gonzalez C.J., J.A. Santos, M.L. Garcia-Lopez, N. Gonzalez and A. Otero, (2001). Mesophilic Aeromonads in wild and aquacultured freshwater fish. J. Food Prot. 64: 687-691.
16. Handfield M, P Simard, and R Letarte. (1996). Differential media for quantitative recovery of waterborne Aeromonas hydrophila. Appl Environ Microbiol. 62: 3544-3547.
17. Hazen T C, C B Fliermans, R P Hirsch and G W Esch. (1978). Prevalence and distribution of Aeromonas hydrophila in the United States. Appl. Environ. Microbiol. 36 (5): 731-738.
18. Illanchezian, S. – Sathishkumar, J. – Muthu, S. M. – Saritha, V. 2010. Virulence and cytotoxicity of seafood borne Aeromonas hydrophila. In Brazilian Journal of Microbiology, 41, 2010, p. 978 - 983.
19. Janda J. M. and P. S. Duffey. (1988). Mesophilic aeromonads in human disease: current taxonomy, laboratory identification, and infectious disease spectrum. Rev. Infect. Dis. 10, 980-997.
20. Janda J. M. and S. L. Abbott. (1998). Evolving concepts regarding the genus Aeromonas: an expanding panorama of species, disease presentations, and unanswered questions. Clin. Infect. Dis. 27: 332-344.
21. Khajanchi B K, A A Fadl, M A Borchardt, R L Berg, A J Horneman, M E Stemper, S W Joseph, N P Moyer, J Sha, and A K Chopra. (2010). Distribution of virulence factors and molecular fingerprinting of Aeromonas species isolates from water and clinical samples: suggestive evidence of water-to-human transmission. Appl Environ Microbiol. 76: 2313-2325.
22. Knochel Susanne. (1991). Chlorine Resistance of Motile Aeromonas spp. Water Science & Technology. 24(2): 327-330.
23. Kuhn I, G Allestam, G Huys, P Janssen, K Kersters, K Krovacek, and T A Stenstrom. (1997a). Diversity, persistence, and virulence of Aeromonas strains isolated from drinking water distribution systems in Sweden. Appl Environ Microbiol. 63: 2708-2715.
24. Langsrud S, M Sidhu, E Heir and A Holck. (2003). Bacterial disinfectant resistance - a

challenge for the food industry. International Biodeterioration & Biodegradation. 51 (4): 283-290.

25. Leclerc H, D A A Mossel, S C Edberg, and C B Struijk. (2001). Advances in the bacteriology of the Coliform Group: their suitability as markers of microbial water safety. Ann Rev Microbiol. 55: 201-234.
26. Martins, L. M. – Marquez, R. F. – Yano, T. 2002. Incidence of toxic Aeromonas isolated from food and human infestion. In FEMS Immunology and Medical Microbiology, 32, 2002, p. 237- 242.
27. Messi P, E Guerrieri, and M Bondi. (2002). Survival of an Aeromonas hydrophila in an artificial mineral water microcosm. Water Res. 36: 3410-3415.
28. Neyts K., G. Huys, M. Uyttendaele, J. Swings and J. Debevere, (2000). Incidence and identification of mesophilic Aeromonas spp. from retail foods. Lett. Appl. Microbiol. 31: 359-363.
29. Odeyemi, A. O. – Asmat, A. – Usup, G. (2012). In - vitro antimicrobial activity of Aeromonas spp. isolated from estuary using different screening protocols. In International Journal of Pharmaceutical Sciences and Research, 3: 428 - 433.
30. Ormen O. and O. Ostensvik. (2001). The occurrence of aerolysin-positive Aeromonas spp. and their cytotoxicity in Norwegian water sources. J. Appl. Microbiolol. 90: 797–802.
31. Pablos M, M A Remacha, J M Rodriguez-Calleja, J A Santos, A Otero, and M L Lopez-Garcia. (2010). Identity, virulence genes, and clonal relatedness of Aeromonas isolates from patients with diarrhea and drinking water. Eur. J. Clin Microbiol. Infect Dis. 29: 1163-1172.
32. Pablos M, J M Rodriguez-Calleja, J A Santos, A Otero, and M L Garci-Lopez. (2009). Occurrence of motile Aeromonas in municipal drinking water and distribution of genes encoding virulence factors. Int J Food Microbiol. 135: 158-164.
33. Percival SL, RM Chalmers, M Embrey, PR Hunter, J Sellwodd, and P Wyn-Jones. (2004). Microbiology of waterborne diseases.
34. Popoff M., Kluyver and Van Niel. (2009). Genus III. Aeromonas. In Bergey's Manual of Systematic Bacteriology.
35. Rabaan A A, I Gryllos, J M Tomas, and J G Shaw. (2001). Motility and the polar flagellum are required for Aeromonas caviae adherence to HEp-2 cells. Infection Immunity. 69: 4257-4267.
36. Radu, S. – Ahmad, N. – Ling, F. H, – Reezal, A. (2003). Prevalence and resistance to antibiotics for Aeromonas species from retail fish in Malaysia. In International Journal of Food Microbiology, 81: 261 – 266.
37. Sen K, and D Lye. (2007). Importance of flagella and enterotoxins for Aeromonas virulence in a mouse model. Can J Microbiol. 53: 261-269.
38. Sen K, and M Rodgers. (2004). Distribution of six virulence factors in Aeromonas species isolated from U.S. drinking water utilities: PCR identification. J Appl Microbiol. 97: 1077-1086.
39. Singh, V. – Dharmendra, K. C. V. – Indra, M. – Pallavi, S. - Gaurav, R. -Neeraj, S. (2010). Molecular identification and codon optimization analysis of major virulence encoding genes of Aeromonas hydrophila. In African Journal of Microbiology Research, 4: 952 - 957.
40. Stojanov I., Nada Plavsa, Dragica Stojanovic, R. Ratajac, Jasna Prodanov Radulovic, I. Pusic And M. Kapetanov, (2010). Susceptibility of Aeromonas Hydrophila Isolates To Antimicrobial Drugs. Lucrari Stiintifice Medicina Veterinara. XLIII (1): 132-136.
41. Thayumanavan Tha, G Vivekanandhan , K Savithamani, R Subashkumar and P Lakshmanaperumalsamy. (2006). Incidence of haemolysin-positive and drug-resistant Aeromonas hydrophila in freshly caught finfish and prawn collected from major commercial fishes of coastal South India. FEMS Immunology & Medical Microbiology. 36 (1-2): 41-45.
42. U.S. EPA Office of Water. (2006). Aeromonas: Human Health Criteria Document. U.S. EPA. Washington DC.

43. Yogananth N. R. Bhakyaraj, A. Chanthuru, T. Anbalagan and K. Mullai Nila. (2009). Detection of Virulence Gene in Aeromonas hydrophila Isolated from Fish Samples Using PCR Technique. Global Journal of Biotechnology & Biochemistry 4 (1): 51-53.