

A Review on Occurrence and Characterization of the *Aeromonas* Species from Marine Fishes

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Abstract: *Aeromonas* are rod shaped bacteria that can be divided into eight to nine distinct species, including *A. caviae*, *A. eucrenophila*, *A. schubertii*, *A. sobria*, *A. veronii* and *A. hydrophila*. Diseases due to *Aeromonas* spp. also occur in amphibians, reptiles and fish and these animals may act as carriers of the bacteria. Of the various *Aeromonas* species most is known about *Aeromonas hydrophila*-a species of bacterium present in freshwater and brackish environments that is frequently isolated from raw and processed seafood products. *A. hydrophila* has frequently been found in fish and shellfish. In a retail survey of seafoods, motile *Aeromonas* were found in 66% of shellfish and 34% of finfish. Seafoods probably become contaminated by *Aeromonas* spp. through the growing waters and the animals themselves, with many fish species containing *Aeromonas* spp. in their gut. Although the production of processed seafood products incorporates steps lethal to *Aeromonas* spp., these products have been found to be contaminated, which suggests that the processing facility also acts as a source of contamination.

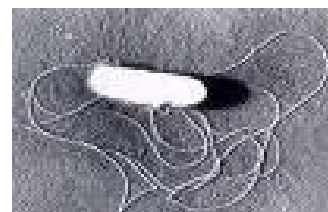
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INTRODUCTION

Aeromonas species (Figure 1) are facultative anaerobic Gram-negative bacteria that belong to the family *Aeromonadaceae*. These bacteria have a broad host spectrum, with both cold- and warm-blooded animals, including humans and are known as psychrophilic and mesophilic [1]. In fish, these bacteria cause hemorrhagic septicemia, fin rot, soft tissue rot and furunculosis. It was recently reported that epizootic ulcerative syndrome (EUS) caused by *Aeromonas sobria* resulted in great damage to fish farms in parts of southeast Asia such as Bangladesh and India [2]. *A. sobria* was also the causative agent of fish disease in a farm of perch, *Perca fluviatilis* L, in Switzerland [3]. In humans, *Aeromonas* causes diarrhea, gastroenteritis and extraenteric conditions such as septicemia, wound infection, endocarditis, meningitis and pneumonia [4]. *Aeromonas* species secretes many extracellular proteins, including amylase, chitinase, elastase, aerolysin, nuclease, gelatinase, lecithinase, lipase and protease. These proteins are known as virulence factors that cause disease



Aeromonas Bakterien



Aeromonas hydrophila

Fig. 1: *Aeromonas* species

in fish and humans. Aerolysin is a representative virulence factor of *Aeromonas* and was reported to function as hemolysins and cytolytic enterotoxins [4,5]. The detection method of *aerA* was recently proposed as a reliable approach by which to identify a potential pathogenic *Aeromonas* strain. Glycerophospholipid cholesterol acyltransferase (GCAT) is a lipase that is secreted by *A. salmonicida* and causes furunculosis in fish [6]. Serine protease is known to activate toxins such as aerolysin and GCAT, which were controlled by virulence mechanisms; this process is referred to as

quorum sensing [7]. Aerolysin also forms channels by heptamerization to the host cell membrane after proaerolysin is activated by the proteolytic activity of furin [8-11]. The virulence of aerolysin was confirmed in *A. hydrophila* when Tn-induced protease-deficient mutants were used [12]. Nuclease has not yet been confirmed in terms of its association with pathogenicity, but reports have indicated that it participates in the development of host infection.

The detection of *Aeromonas* is a key component in the determination of potential pathogenicity, because more than two virulence factors act multifunctionally and multifactorially. A multiplex-PCR (m-PCR) assay has been used to identify viruses and bacteria in clinical samples [13].

Aeromonas species are widely distributed in the aquatic environment, including raw and processed drinking water [14] and have been frequently isolated from various food products such as fish and shellfish, raw meat, vegetables and raw milk. Motile *Aeromonas* are considered as emerging food-borne pathogens because it was shown that some *Aeromonas* food isolates can produce different virulence factors, not only at optimal growth temperature, but also at refrigeration temperatures [15]. This may be of importance for refrigerated food products that usually have an extended shelf-life at this temperature. Although the exact role of these virulence factors in the pathogenesis of motile *Aeromonas* species is still not fully elucidated, representatives of these taxa have been incriminated in cases of human gastroenteritis, particularly in children younger than 2 years, the elderly and immunocompromised patients [16]. Further, *Aeromonas* species are frequently associated with travellers' diarrhoea [17-18]. Consequently, *Aeromonas* should be carefully monitored in foodstuffs as a possible source of food-borne infections. Phenotypic characteristics of *Aeromonas* spp. have been used to differentiate between environmental strains and those strains causing gastroenteritis. Historically, motile *Aeromonas* were phenotypically classified into the species *Aer. hydrophila*, *Aer. sobria* and *Aer. caviae*, according to the criteria of Popoff [19]. With the introduction of DNA±DNA hybridization techniques [19], the taxonomy of the genus *Aeromonas* has become much more refined and consequently, the use of biochemical characteristics alone for the reliable identification of unknown *Aeromonas* isolates is no longer adequate. Currently, the genus *Aeromonas*

comprises at least 14 genomic species or DNA hybridization groups (HG), of which some have not yet been named [20]. Interestingly, most pathogenic *Aeromonas* strains group predominantly in three of these genomic species, *Aer. hydrophila* HG1, *Aer. caviae* HG 4 and *Aer. veronii* biovar *sobria* HG 8 [21]. As most surveys used mainly phenotypic techniques for the identification of *Aeromonas* food isolates, few reliable data are available on the prevalence of these *Aeromonas* taxa in foods[22].

The Biology of the Organism: The *Aeromonas* are aquatic bacteria that are often associated with diarrhoea. However, their precise role in the aetiology of gastrointestinal illness is unclear. Most species are motile due to the presence of flagella. *Aeromonas* spp. are widely distributed in stagnant and flowing fresh water, in salt water that interfaces with fresh water, in fish tanks, water supplies and in sewage, with densities ranging from <1 to >1000/ml. Strains within each *Aeromonas* species, including *A. hydrophila*, are commonly divided into two groups based on their temperature requirements for growth. Psychrophilic strains have an optimum growth temperature of between 15 and 20°C and may grow at temperatures as low as 0-5°C. Mesophilic strains have an optimum growth temperature of around 35°C and can grow at temperatures as high as 40-45°C, but generally will not grow below 10°C. There is evidence to suggest that those strains best adapted to grow at low temperatures are more commonly pathogens of fish and not humans[23].

Diseases associated with *Aeromonas* spp.: Human infections due to *Aeromonas* species occur predominantly during warm weather. The strains probably originate from water, soil, food or the human gastrointestinal tract. Four disease categories are known:

- C Wound infection or cellulitis, related to exposure to water or soil,
- C A general infection in which the organisms spread throughout the body (septicaemia) in immunocompromised individuals or individuals with various other significant illnesses,
- C Gastroenteritis (diarrhoeal disease),
- C extraintestinal infections such as meningitis, peritonitis, or otitis, or of sites such as the eye or urinary tract [23].

Associated Foods: *Aeromonas* spp., often referred to as *Aeromonas*, are environmental bacteria that are detected in numerous gastrointestinal infections. They have been implicated in a number of cases of food-borne disease. While information on *Aeromonas* spp. is limited it appears that many strains may not be pathogenic and that the risk of poisoning from *Aeromonas* contaminated foods is low. Some strains of *A. hydrophila* can cause illness in humans. Infections may occur through open wounds or by ingesting enough of the organisms in food or water [22-23].

A total of 19 mussel samples were examined. None of the nine smoked samples tested contained motile *Aeromonas*, while four out of the five fresh samples tested were positive. Three samples of cooked mussels were positive, but both samples of marinated mussels were negative when tested. On the basis of these findings and the international data available it appears that fresh shellfish commonly contain motile *Aeromonas*. While smoking and cooking may destroy these organisms, subsequent recontamination can occur.

In the finfish, of the 32 samples tested, 34% contained motile *Aeromonas* with 28% containing *A. hydrophila* and/or *A. sobria*. As in shellfish, the presence of *Aeromonas* in fish must be considered to be a common event. In this same study 19 samples of shrimps and prawns were examined for motile *Aeromonas*. Seven out of nine cooked samples were positive for *A. hydrophila* and/or *A. sobria*, while nine out of the ten uncooked samples were positive for these species [24].

Aeromonas were found in fish and fresh salads [25], freshly dressed lamb carcasses [26]. It has been reported mesophilic *Aeromonas* were isolated from 72% of the fish and shrimps. Because of the obvious differences in sampling period, geographical location, the origin of the samples and methodology for analysis, it is difficult to compare the level of *Aeromonas* incidence published by different authors. However, the present data clearly confirm the widespread distribution of motile *Aeromonas* in retail foods and also reveal a large variation in the level of contamination. Numbers of motile *Aeromonas* present in the food samples varied from $<102 \text{ cfu g}^{-1}$ to $>105 \text{ cfu g}^{-1}$. The latter might be a reflection of product-specific properties that can significantly influence the survival rate and growth characteristics of *Aeromonas*, i.e., initial contamination levels, type of processing, method of packaging and preservation [27]. The biochemical classification of *Aeromonas* isolates into one of the three historically-defined fenospecies, i.e., *Aer. hydrophila*, *Aer. caviae* and *Aer. sobria*, is applied in the majority of

surveys of motile *Aeromonas* in foods. On the other hand, very few studies have reported identification at the genomic species level. Because of the significant lack of data for the different taxa, rapid miniaturized or automated systems using a panel of biochemical tests are inappropriate for identification of motile *Aeromonas* to genomic species level. As the majority of the virulent *Aeromonas* strains belong to *Aer. hydrophila* HG1, *Aer. caviae* HG4, or *Aer. veronii* biovar *sobria* HG8 [28], a reliable identification of food isolates is necessary to establish the risk associated with their prevalence in foods. In the current study, GLC analysis of FAMES was used to identify a selection of presumptive *Aeromonas* colonies to fenospecies or genomic species level. *Aeromonas* strains belonging to the *Aer. caviae* complex, which also includes the potentially pathogenic genospecies HG4, were mostly isolated from vegetables but were also found in meat, poultry and fish. In addition, three strains of HG8 were isolated from poultry and minced meat. No representatives of HG1 were found among the selected colonies as all members of the *Aer. hydrophila* complex were classified in the non-virulent taxon HG3. The isolation of *Aeromonas* HG4 and HG8 strains from a variety of retail foods may indicate that these products can act as possible vehicles for the dissemination of food-borne *Aeromonas* gastroenteritis. However, it is clear that additional data on the production of virulence factors, such as haemolysins and cytolytic enterotoxins, are required to support this hypothesis. For example, Although some results indicate that strains identified as *Aer. hydrophila* should be monitored in the epidemiology of *Aeromonas*-associated human gastroenteritis, it is unfortunate that the identification methods used in both studies did not allow discrimination among HG1, HG2 or HG3 of the *Aer. hydrophila* complex. Despite the fact that potentially pathogenic *Aeromonas* are present in food and food products, infection and the onset of gastroenteritis will only occur if the level of contamination exceeds the infective dose. However, there are currently insufficient clinical data to determine the infective dose. Moreover, it has been shown that the level of contamination at the moment of consumption depends upon the initial contamination and the opportunities for growth and/or survival during processing, preservation and preparation of the food [28]. Further research is also needed to establish contamination routes. So far, the significance of *Aeromonas* in foods remains undefined, although there is a growing concern about the consumption of *Aeromonas*-contaminated food by young children, the elderly and the immunocompromised.

All the currently recognized 13 hybridization groups (HGs) of *Aeromonas*, except *Aeromonas eucrenophila* and *A. sobria* (HG 7) [29], have been described as a pathogen of fish and human. These organisms have also been implicated in extra-intestinal infection and diarrhoea in man--the strains often originating from water. Several of these genospecies such as *A. hydrophila* (HG 1), *A. veronii* bv *sobria* (HG 8), but rarely *A. caviae* (HG 4), produced enterotoxin and haemolysin and also showed resistance to normal human serum (NHS). Moreover, enterotoxigenic diarrhoeal isolates of *A. hydrophila* showed haemagglutination (HA) which was not sensitive to D-mannose and L-fucose. But *Aeromonas* strains showing HA sensitive to D-mannose and L-fucose or no haemagglutination (NHA) were non-toxigenic strains of *A. caviae*, commonly from non diarrhoeal infection or environment. Although the production of haemolysin and enterotoxin by *A. eucrenophila* has been demonstrated in this laboratory, the link between fish and water isolates of *A. eucrenophila* and human illness has not yet been established. Therefore, this study examined the possible virulence factors, such as haemagglutination, serum resistance and chitinase production, associated with *A. eucrenophila* and their correlation with enterotoxicity[30].

In conclusion *Aeromonas* spp. are common contaminants of fish and seafood, since they are ubiquitous in the water environment. It is important to point out that the stream that drains into the estuary mentioned in this study bathes several shantytowns which have no basic sewage systems, thus increasing environmental contamination.

Control Procedures:

- C Reduce the potential for growth by keeping the product chilled
- C Maintain good hygiene in the processing plant
- C Reduce the risk of cross contamination by keeping raw and processed products separated
- C Implement handling and packaging practices that will limit the possibility of processed products becoming contaminated.

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