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The importance of *Aeromonas hydrophila* in food safety

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Abstract

Aeromonas hydrophila is a widespread representative of *Aeromonas* found in water, water habitants, domestic animals and foods (fish, shellfish, poultry, and raw meat). The microorganism has the potential to be a foodborne pathogen, especially strains from hybridization group (HG1), associated with clinical cases of illness. The pathogen produces different virulence factors including exotoxins, cytotoxins and others. As a psychrotroph, *A. hydrophila* grow in foods during refrigeration. The disease spectrum associated with this microorganism includes gastroenteritis, septicemia, traumatic and aquatic wound infections, and infections after medical leech therapy. Multiple resistance of the bacterium to many antimicrobials is a fact of high significance. The potential of *A. hydrophila* to become a foodborne pathogen is a controversial issue. Many approaches are effective for control of the presence of *A. hydrophila* in food for human consumption.

Keywords: *Aeromonas hydrophila*; Foodborne pathogen; Food safety; Foodborne illness

1. Introduction

Aeromonas hydrophila is an emerging aquatic pathogen, widely distributed in the environment. Originally, *A. hydrophila* was identified as one of four *Aeromonas* species by Popoff (1984). According to Joseph and Carnahan (2000) the genus *Aeromonas* is now classified within the family *Aeromonadaceae* and consists of 14 different confirmed species, one of which is *A. hydrophila*.

Swann and White (1991), Gosling (1996) and Austin and Adams (1996) consider *A. hydrophila* as a cause of several disease conditions in cold-blooded animals (fish, reptiles, amphibians) and in warm-blooded animals (mammals and birds). An important fact, however, is that *A. hydrophila* is the cause of zoonotic diseases (i.e., diseases which can be spread from animals to humans and vice versa).

According to Adams and Moss (2000) and Kirov (2001) *Aeromonas* (principally *A. hydrophila*) currently has the status of a foodborne pathogen of emerging importance. It has attracted attention primarily because of its ability to grow at cold temperatures. *Aeromonas* spp. were first considered as possible causative agents of human gastroenteritis more than 30 years ago (Lautrop, 1961). Palumbo (1996) reported that *A. hydrophila* has been isolated from a wide range of both animal and plant food products, including raw red meat, poultry, fin fish, seafood, dairy products, vegetables and miscellaneous foods. The potential role of *A. hydrophila* in human gastrointestinal infections is noted by Kirov (2003). The majority (>85%) of gastroenteritis cases are attributed to three *Aeromonas* species, one of them is *A. hydrophila* (hybridization group HG1). The aim of this review is to describe the potential association of

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A. hydrophila with foodborne illness, its pathogenic characteristics, the distribution of the pathogen in the environment and foods, and some approaches to control the microorganism in drinking water and food.

2. Characteristics of *A. hydrophila*

Members of the genus *Aeromonas* (from the Greek aer-air/gas monas-unit hence gas-producing unit) are Gram-negative, facultatively anaerobic, non-spore-forming, rod-shaped bacteria (Roberts, Baird-Parker, & Tompkin, 1996). According to Adams and Moss (2000) and Kirov (2003) *A. hydrophila* is motile by a single polar flagellum, catalase-positive, oxidase-positive rod, which ferments glucose. It is neither salt (<5%) nor acid (min. pH ~ 6.0) tolerant and grows optimally at around 28 °C. It has the ability to grow at cold temperatures, reportedly as low as −0.1 °C for some strains. Its principal reservoir is the aquatic environment such as freshwater lakes and streams and wastewater systems. Kirov (2003) reported on its ability to form lateral flagella on solid surfaces.

Current genomospecies (*A. hydrophila*, *A. bestiarum* and one unnamed species) and phenospecies (*A. hydrophila*, and *A. hydrophila*-like) within *A. hydrophila* by Kirov (2003) are grouped in identified by Popoff (1984) three DNA hybridization groups (HG1, isolated from clinical specimens, HG2 and HG3). According to Kirov (2003) pathogenicity and virulence of *A. hydrophila* depend on the ability to produce factors associated with gastroenteritis. These properties are exotoxins, cytotoxins, endotoxins, siderophores, invasins, adhesins, S-layers and flagella. Many authors assayed enterotoxic, cytotoxic, hemolytic activities, adhesion, and invasion of already listed virulence determinants. Jiwa (1983) studied enterotoxigenicity, hemagglutination and cell-surface hydrophobicity in 31 strains of *A. hydrophila*. The origin of *A. hydrophila* was from human stools (diarrhea) 17, hare (septicemia) 7, aquarim fish and tibifex worm (routine) 5, and gold fish/carp (septicemia) 2 strains. Of 31 *A. hydrophila* strains, 28 caused cytotoxic reactions; the 3 cytotoxic-negative strains were from routine fish isolates. Hemagglutination depends on the growth conditions of *A. hydrophila* strains; 23 of 31 strains, grown on, (TY-1) broth showed mannose resistant hemagglutination. Thirty of the 31 *A. hydrophila* strains were hemolytic on horse blood agar. Fricker and Tompsett (1989) reported cytotoxin production by 50% of the *A. hydrophila* strains isolated from retail outlets (mostly poultry and offal). Knochel (1989) examined the production of hemolysin at low (10 °C) and at body temperature (37 °C) of 97 clinical strains of *Aeromonas* spp. (74 strains of *A. hydrophila*) isolated from warm and cold sources. Enterotoxin-like activity of some strains was assessed by the suckling mouse assay. Hemolysin production of 3 from 5 strains of *A. hydrophila*, isolated from source with high temperature (>24 °C) was high (titer >128) at low (10 °C) and at body temperature (37 °C). Significantly higher numbers of *A. hydrophila* (69 strains) isolated from low-temperature sources were able to produce high hemolysin titers at 10 °C (47 strains) as compared with 37 °C (6 strains). After growth at 37 °C, regardless of the hemolytic titer, 40% (4 strains) of *A. hydrophila* were enterotoxic; at the same time 30% (3 strains) after growth at 10 °C were enterotoxigenic. Majeed, Egan, and Mac Rae (1989a) isolated enterotoxigenic aeromonads (*A. hydrophila*, *A. sobria* and *A. caviae* strains) from retail lamb meat and offal. This study showed that exotoxin production (haemolysin and enterotoxin) was more characteristic of *A. hydrophila* and *A. sobria*. Isolates of *A. hydrophila* dominated other *Aeromonas* representatives and they have no parallele between haemolysin and enterotoxin production of strains. Todd, Hardy, Stringer, and Bartholomew (1989) studied four strains of *A. hydrophila* grown at 30 and 37 °C in two laboratory media and prawn puree for toxin production. Results showed reduced cytotoxic and hemolytic activities in prawn puree compared with two media, but in most cases increased proteolytic activity. No enterotoxic activity was observed in prawn puree. Kirov and Brodribb (1993) reported high levels of exotoxin production in different foods of a strain of *A. hydrophila* isolated from goats' milk. Esteve, Amaro, Garay, Santos, and Toranzo (1995) reported that pathogenic strains of *A. hydrophila* from eel produced elastases, hemolysins and exotoxins, inactivated by heat treatment. Handfield, Simard, Coullard, and Letarte (1996) investigated pathogenicity of isolates from food and drinking water by studying its hemolysis, hemagglutination and cytotoxicity. Hemolysis was more frequently seen with water isolates (64%), hemagglutination was more frequently encountered with food isolates (92%), and cytotoxicity was frequently observed with food (92%) and water (73%) isolates. Heat treatment (56 °C for 10 min) inhibited the toxicity of some but not all toxin-producing isolates. Moro et al. (1999) isolated four strains of *A. hydrophila* from four Hereford bulls with seminal vesiculitis in South Brasil. All isolates produced enterotoxins, two cytotoxins, and 1 isolate hemolysin. Castro-Escarpulli et al. (2003) reported two strains of *A. hydrophila* from market tilapia in Mexico with putative virulence factors such as aerolysin/hemolysin, lipases including the glycerophospholipid-cholesterol acyltransferase, serine protease and DNases.

The majority of *A. hydrophila* strains produce exotoxic properties (enterotoxins, hemolysins, and cytotoxins). Adhesion to and colonization of mucosa, followed by fluid accumulation, or epithelial change, are likely events leading to human disease. Sanderson, Ghazali, and Kirov (1996) studied enteropathogenicity of *A. hydrophila* (three human diarrhoeal strains) in mice

pre-treated with streptomycin. Strains of pathogen were recovered in high numbers ($\geqslant 10^3$ cfu/g faeces). *A. hydrophila* strains localized in the large intestine and appeared not to be cell associated. Ascencio, Martinez-Arias, Romero, and Wadstrom (1998) studied adherence of *A. hydrophila* to mucosal components such as mucin. *A. hydrophila* strains had high ability to bind with various horseradish peroxidase-labeled mucins. The mucin-rich media greatly influence the expression of *A. hydrophila* mucin-binding activity. This study proved the high ability of *A. hydrophila* to colonize the gastrointesinal tract, by adhering to mucous receptors of intestinal cells.

Distribution of *A. hydrophila* isolates into current three DNA/DNA hybridization groups (HGs) (HG1, HG2 and HG3) also was studied. Kirov, Hudson, Hayward, and Mott (1994) assigned 182 *A. hydrophila* strains isolated from the environmental (food and water), clinical (stool) and other sources to one of three HGs on the basis of biochemical characteristics, and tested them with regard to their ability to produce virulence factors. Strains HG1 (nine, common isolates from clinical sources) were more likely to produce virulence factors, while HG3 strains formed the majority of environmental isolates. Hanninen, Oivanen, and Hirvela-Koski (1997) studied fish, fish-eggs, shrimp and freshwater samples and isolated 117 *Aeromonas* strains. The predominant HG in fish (22/37), fish-eggs (16/57) and freshwater (16/20) isolates were *A. hydrophila* HG_3. *A. hydrophila* HG_2 was isolated only from fish samples. Villari, Crispino, Montuori, and Stanzione (2000) in a survey study in Italy on ready-to-eat products found a high level of genetic heterogeneity of isolates of *A. hydrophila* (27) in 24 genomic DNA patterns analyzed with pulsed-field gel electrophoresis.

Palumbo, Moragan, and Buchanan (1985) studied the growth of clinical isolates of *A. hydrophila* at various temperatures, pH values and salt levels in BHI broth. A majority of isolates grew at 4–5 °C and 42 °C, and all grew over the range 20–35 °C. At 28 °C, most isolates could tolerate 4% NaCl, while at 4 °C only a limited number grew in 3% NaCl. Similarly, isolates could better tolerate acidic conditions when cultured at 28 °C as compared to 4 °C. These data suggest that it is likely that *A. hydrophila* associated with human gastroenteritis are capable of growing in foods at refrigeration temperatures currently considered adequate for preventing growth of foodborne pathogens. Production of virulence properties is depended on many environmental factors, such as temperature, pH values, salt levels and other. Tsai, Tsai, and Kong (1997) studied the effects of temperature, pH, salt content and dissolved oxygen on production of hemolysin and cytotoxin by one strain of *A. hydrophila*. The experiments showed that the pathogen produced toxins faster at 28 °C, and production of hemolysin and cytotoxin was apparently decreased in the presence of 1–5% NaCl or when the pH was greater or less than 7.2. The higher quantities of dissolved oxygen stimulated production of toxins. McMahon, Blair, and McDowell (1998) reported filamentation and chain formation at 5, 10 or 28 °C in the presence of 100% CO_2. No such filamentation was noted in aerobically grown cells. Cultures exhibiting filamentation were not proteolytically or hemolytically active. Reversion to normal morphology and enzymatic activity occured within 24 h of subsequent aerobic incubation. Braun, Balzer, and Fehlhaber (2001) tested the lipolitic ability of three *A. hydrophila* strains at temperatures ranging from −2 °C to +7 °C over a period of 38 days. A decrease in storage temperature was associated with a significant reduction of enzyme activity but no complete inactivation even at −2 °C; initial reactions of lipolitic ability occurred after 3 days.

3. Incidence of human illness

According to Kirov (1993, 2003), Kirov and Sanderson (1995), and Isonhood and Drake (2002), *Aeromonas* species have been recognized as pathogens which can cause a number of serious extraintestinal infections including bacteraemia, meningitis, pulmonary and wound infections. *Aeromonas* spp. may play a significant role in "summer-diarrhoea", a worldwide problem particularly in children under five years old, the elderly, and travellers. The role of these bacteria in foodborne incidences is not firmly established, but *Aeromonas* spp. have the potential to emerge as significant foodborne pathogens. *A. hydrophila* (HG1, HG2 and HG3) were reported by Janda and Duffey (1998) to predominate in cases of *Aeromonas*-associated gastroenteritis (~50% of strains). Kirov (2003) noted that the disease spectrum of *A. hydrophila* HG1 included gastroenteritis, septicemia, traumatic and aquatic wound infections, and infections after medicinal leech therapy.

Incidences of *A. hydrophila*-associated illness were reported from different authors. Krovacek, Peterz, Faris, and Mansson (1989) reported on the long-term diarrhoeal case of a 1.5 year old child who consumed contaminated water. All *A. hydrophila* isolates from water samples were enterotoxin producers. The *A. hydrophila* enterotoxin was inactivated by heating at 80 °C for 30 min. They were identified by ribopattern analysis as *A. hydrophila* HG2 and HG3. Krovacek, Dumontet, Eriksson, and Baloda (1995) reported in Sweden a case of illness after eating fish and meat products, which contained high numbers of *A. hydrophila* cells (10^6–10^7 CFU/g food sample). Hanninen et al. (1997) reported isolation of three strains of *A. hydrophila* from frozen shrimp during two suspected foodborne outbreaks. From Norway Granum, O'Sullivan, Tomas, and Ormen (1998) presented the disease of three people after eating

raw fermented fish containing up to 10^7 CFU/g of *A. hydrophila* (HG1, HG2, and HG3). *A. hydrophila* usually causes diarrhoea in children; as noted by Bloom and Bottone (1990). Adults are the second group of people which can be affected by *A. hydrophila*. George, Nakata, Thopson, and White (1985) reported predisponding risk factors (antibiotic therapy), which can increase the risk of *Aeromonas*-associated gastroenteritis.

4. Antibiotic resistance

Studies of antibiotic resistance of isolates of *A. hydrophila* indicated existence of many strains of the pathogen highly resistant to some antibiotics applied in clinical practice; it may become difficult to cure disease caused by *A. hydrophila*.

Some reports showed that isolates of *A. hydrophila* from water, food, clinical specimens and other sources are not susceptible to many antimicrobials (antibiotics). Krovacek et al. (1989) noted that isolates of *A. hydrophila* were susceptible to chloramphenicol, neomycin, sulfamethoxazole, streptomycin and trimethoprim/sulfamethoxazole. Kelley, Pancorbo, Merka, and Barnhart (1998) studied antibiotic resistance of nine litter isolates of *A. hydrophila* collected from four broiler houses in the North Georgia area. All isolates were resistant to ampicillin, bacitracin, penicillin, tetracycline and streptomycin, and were susceptible to erythromycin, gentamycin, kanamycin, nalidixic acid, neomycin and sulfisoxazole. Wang and Silva (1999) tested antibiotic resistance of 80 *A. hydrophila* isolates from 238 channel catfish fillets. Most of the isolates were susceptible to chlortetracycline, oxytetracycline, tetracycline, trimethoprim/sulfamethoxazole, neomycin and chloramphenicol. Schmidt, Bruun, Dalsgaard, and Larsen (2001) reported a significant effect of aquaculture on antibiotic-resistance of motile aeromonads (including *A. hydrophila*). High levels of multiresistance (48%) indicated the horizontal spread of resistance genes. Vivekanandhan, Savithamani, Hatha, and Lakshmanaperumalsamy (2002) reported on the multiple antibiotic resistance of 319 strains of *A. hydrophila* isolated from fish and prawns. All strains were resistant to methicillin and rifampicin followed by bacitracin and novobiocin, but sensitive to chloramphenicol. Radu, Ahmad, Ling, and Reezal (2003) reported data from 87 market fish samples representing five types of fish, which were evaluated for the presence of *Aeromonas* spp. Of the samples examined, 69% harbored *Aeromonas* spp. and 11.5% were *A. hydrophila*. The results indicate that hemolytic, multiple antibiotic resistant and genetically diverse aeromonads are easily recovered from fish in Malaysia. Castro-Escarpulli et al. (2003) proved that the best antimicrobial effect against strains of *A. hydrophila* had first generation quinolones and second and third generation cephalosporins. Thayumanavan, Vivekanandhan, Savithamani, Subashkumar, and Lakshmanaperumalsamy (2003) concluded that the increasing presence of haemolysisn-producing multiple drug-resistant *A. hydrophila* in fish and prawn may become a potential human health hazard.

5. Distribution of *A. hydrophila*

A. hydrophila is widely spread in waters, water habitants, and many food products (seafood, shellfish, raw foods of animal origin like poultry, ground meat, raw milk, and raw vegetables). Some authors (Fricker & Tompsett, 1989; Gobat & Jemmi, 1993; Krovacek et al., 1992; Nishikawa & Kishi, 1988) noted that strains belonging to *A. hydrophila* are frequently isolated from meat, fish and poultry. The pathogen is a common inhabitant of water recourses. Drinking or mineral water can be a possible source of contamination for humans. Warburton, Harrison, Crawford, Foster, and Fox (1998) conducted a survey (1992–1997) of the microbiological quality of bottled water in Canada. From a total of 2703 samples 3 were found contaminated with high numbers of *A. hydrophila* ($>10^3$). Croci, Di Pasquale, Cozzi, and Toti (2001) studied the growth and survival of *A. hydrophila* in three types of natural mineral waters with different levels of mineral content (low, medium and high), which were experimentally contaminated. The greatest number of cells was observed in water with a low mineral content stored in PET bottles at 10 °C. Mary, Defives, and Hornez (2001) investigated the ability of *A. hydrophila* ATCC 7966 (HG1, clinical isolate) and other three *Aeromonas* species to survive and grow in tap water microcosms; *A. hydrophila* was more susceptible than the three other species tested. Other authors Kersters and Verstraete (1996) and Kersters et al. (1996) reported that *A. hydrophila* survives very poorly in drinking waters, which is of utmost importance for public health.

A. hydrophila is frequently found in seafoods. Wang and Silva (1999) found that from 238 channel catfish fillets, 36.1% were contaminated with *A. hydrophila*. The incidence of this pathogen contamination was higher in the summer than other seasons. Fifty two isolates from catfish fillets had (α-49 and β-3 isolates) hemolytic activity. Davies, Cappel, Jahanno, Nychas, and Kirby (2001) reported results of a study on the incidence of foodborne pathogens on European fish (fresh fish from commercial outlets in France, Great Britain, Greece and Portugal). *A. hydrophila* was detected from all sites, with an overall incidence of 40%. The results of Fricker and Tompsett (1989), Hudson, Mott, Delacy, and Edridge (1992), Gobat and Jemmi (1993) and Tsai and Chen (1996) showed incidences of *A. hydrophila* of 19%, 28%, 90% and 22% in fish samples from UK, New Zealand, Switzerland and Taiwan, respectively. Abeyta

et al. (1989) found that *A. hydrophila* in shellfish growing waters ranged from 3 to 4600 cells/100 g in oysters and from 3 to 2400 cells/100 ml in water. Colburn et al. (1989) studied the microbiological quality of oysters (*Crassostrea gigas*) and water of live holding tanks at five different Seattle area retail markets. *A. hydrophila* was the most frequently isolated potential pathogen in this study with a higher incidence in oysters (78%) compared to water (53%). Wang and Silva (1999) tested 238 channel catfish fillets and found that 36.1% of the samples were positive for *A. hydrophila*. The incidence of this pathogen was higher in the summer than other seasons. Ramnarine (2001) reported presence of *A. hydrophila* in hatcheries of commersially cultured armoured catfish (*Hoplosternum littorale*). Castro-Escarpulli et al. (2003) isolated 82 strains of *Aeromonas* spp. from 250 samples of frozen fish (Tilapia, *Oreochromis niloticus niloticus*) purchased in local markets in Mexico City. Molecular identification demonstrated prevalence of *Aeromonas salmonicida* (67.5%) and *A. hydrophila* (2.6%; 2 strains). Thayumanavan et al. (2003)studied the incidence of toxigenic, multiple antibiotic-resistant *A. hydrophila* from freshly caught finfish and prawns from coastal South India. It was found that 37.3% of finfish and 35.6% of prawn samples were contaminated with the pathogen. Of the total isolates (225), about 78.4% of them were producers of haemolysin, and all were resistant to bacitracin. Radu et al. (2003) reported data from 87 market fish samples representing five types of fish, which were evaluated for the presence of *Aeromonas* spp. Of the samples examined, 69% harbored *Aeromonas* spp., and 11.5% *A. hydrophila*.

Concerning warm-blooded animals, Majeed et al. (1989a) isolated enterotoxigenic aeromonads (*A. hydrophila*, *A. sobria* and *A. caviae* strains) from retail lamb meat and offal. This study showed that exotoxin production (haemolysin and enterotoxin) was more characteristic of *A. hydrophila* and *A. sorbia*. Isolates of *A. hydrophila* dominated over other *Aeromonas* representatives. Majeed, Egan, and Mac Rae (1989b) reported about 20% incidence of *A. hydrophila* in carcasses from an abattoir processing lambs. Kelley et al. (1998), in a study found *A. hydrophila* in four broiler houses in the North Georgia area; the pathogen dominated to other *Aeromonas* spp. representatives. Melas, Papageorgiou, and Mantis (1999) examined raw milk and other milk products in Northern Greece and found that *A. hydrophila* dominated compared to other *Aeromonas* spp. Villari et al. (2000) in a survey study in Italy carried out on ready-to-eat foods (vegetables, cheese, meat products, and ice cream) found that *A. hydrophila* was the most common isolate from foods of animal origin. The conclusions of this study were that consumers are regularly exposed to many genetically distinct strains of *A. hydrophila*, without evident sign of malaise, and therefore, few of these strains, if any, are likely to be pathogenic.

The pathogen can cause disease in pets or animals used for breeding. Pasquale, Baloda, Dumontet, and Krovacek (1994) reported an outbreak of *A. hydrophila* infection (beta-hemolytic isolates) with a high rate of mortality in turtles (*Pseudemis scripta*) in a pet shop in Naples, Italy. The study indicated that pet turtles can act as reservoirs of this pathogen and may play an important role in the etiology of *Aeromonas*-associated human infections. Garcia, Domenesh, Domingues, Ramiro, and Fernandez-Garayzabal (1992) isolated *A. hydrophila* from a case of bilateral conjunctivitis in a pet parrot (*Amazona versicolor*). Moro et al. (1999) isolated *A. hydrophila* from Hereford bulls with seminal vesiculitis in South Brasil. Forga-Martel, Gonzalez-Valle, and Weinzlerl (2000) reported a case of infectious abortion associated with *A. hydrophila* in a mare. Austin and Adams (1996) reported that *A. hydrophila* is associated with several disease conditions in fishes, including tail and fin rot and haemorrhagic septicaemias.

6. Prevention and control

Many scientific studies are done to assess the influence of different factors on survival of *A. hydrophila*. The ability of the pathogen to grow at refrigeration temperatures may have great impact on refrigerator-stored foods. Many factors for control of growth of *A. hydrophila* have been studied.

Some of them are elements of hurdle technology.

Hurdle technology (temperature, pH, NaCl, $NaNO_2$). Palumbo, Williams, Buchanan, and Phillips (1991) studied the combined effects of temperature (5–42 °C), NaCl (0.5–4.5%), pH (5.3–7.3) and $NaNO_2$ (to 200 μg/ml) on the aerobic growth of *A. hydrophila* K144. The data indicated that low pH, salt and nitrite can decrease growth of the pathogen when combined with low temperature incubation. Gram (1991) studied simple fish preservation techniques by use of NaCl, potassium sorbate and liquid smoke, applicable in the tropical zone. Growth of *Aeromonas* spp. was no detected in 5% salt or temperatures below 5 °C. The combination of 5% salt and 1000 ppm sorbate inhibited growth at 25–37 °C. Liquid smoke inhibited growth at 37 °C only when an initial inoculum of 10^2 CFU/ml was used. Gill, Greer, and Dilts (1997) proved in a study of aerobic growth of *A. hydrophila* that numbers of the microorganism declined on muscle tissue of low pH 5.6 ± 0.2 at any temperature (0–25 °C).

Washing. Barnhart, Pancorbo, Dreesen, and Shotts (1989) reported that waterchilling and washing of broiler carcasses resulted in a significant reduction in *A. hydrophila*, while refrigeration at 1.1 °C for 48 h resulted in a significant increase.

Oxidizing. Kersters and Verstraete (1996) reported rapid decreases of 2–3 log units of *A. hydrophila* in

oxidizing raw ground waters, containing high concentrations of Fe2+ (460–1.070 μmol).

Smoking. Boyle, Sofos, and Maga (1988) showed that several strains of *A. hydrophila* were sensitive to smoke concentrate from a variety of wood smokes. Cold smoking is a traditional method to preserve fish. Sunen, Aristimuno, and Fernandez-Galian (2003) tested the effect of four wood smoke condensates against *A. hydrophila* in vacuum-packed cold-smoked rainbow trout, stored at 4 °C for 21 days. All smoke extracts showed activity against *A. hydrophila*.

Modified atmosphere. Ingham and Potter (1988) studied survival of *A. hydrophila* in mince, salt-added surimi and low-salt surimi prepared from Atlantic pollock. High salt-added (2.55%) surimi and modified atmosphere (51% N_2, 13% O_2, and 36% CO_2) reduced significantly growth of *A. hydrophila*. Gill and Reichel (1989) found that *A. hydrophila* could grow on high-pH (>6.0) vacuum packaged beef at all storage temperatures (−2, 0, 2, 5 or 10 °C). In carbon dioxide packs, *A. hydrophila* grew only at 10 °C. Hudson, Mott, and Penney (1994) proved that *A. hydrophila* on sliced roast beef declined in controlled atmosphere (give gas composition) packs at 1.5 °C, but were able to grow under vacuum packaging. Doherty et al. (1996) studied the growth of *A. hydrophila* on normal pH (5.5–5.8) and high pH (>6.0) lamb stored under modified atmospheres. On lamb of normal pH, pathogen numbers decreased during storage at 5 and 0 °C under all packaging conditions (in air, vacuum pack, 80%O_2/20%CO_2; 50%CO_2/50%N_2 or 100% CO_2). In the case of high pH only, 100% CO_2 was effective at 5 °C. Bell, Penney, and Moorhead (1995) studied the growth of *A. hydrophila* on smoked blue cod (*Parapercis colias*) packed under vacuum or carbon dioxide and stored at 3 °C or −1.5 °C. In vacuum-packs the pathogen was able to grow during storage at 3 °C. Reduction of the storage temperature to −1.5 °C retarded but did not prevent pathogen proliferation. Under carbon dioxide, *A. hydrophila* was able to grow at 3 °C and then only after a 21-day lag period, but it did not grow at −1.5 °C. Carbon dioxide (100%) controlled atmosphere can be used to extend product life at or below 0 °C. A study by Davies and Slade (1995) examined growth/survival of *A. hydrophila* on modified-atmosphere-packaged (MAP) cod and trout. MAP fish had less growth in higher carbon dioxide-containing atmosphere and at the lower temperature (0 and 5 °C). Mano, Ordonez, and de Fernando (2000) studied the growth/survival of *A. hydrophila* on refrigerated (at 1 and 7 °C) normal/low (pork) and high (turkey) pH meats packaged in modified atmospheres (100%N_2, 20/80 and 40/60 CO_2/O_2) or in air in plastic bags. Packaging in modified atmosphere resulted in a strong inhibition of bacterial growth at 1 °C, particularly in samples stored in CO_2/O_2 enriched atmospheres. *A. hydrophila* grew on turkey and pork meat stored in 100% N_2 at 1 and 7 °C. Likewise, growth of this bacterium was detected on turkey stored in 20/80 CO_2/O_2 at 7 °C. No growth was observed in any meat at both temperatures assayed. Berrang, Brackett, and Beuchat (1989) Garcia-Gimeno, Sanchez-Pozo, Amaro-Lopez, and Zurera-Cosano (1996) reported that controlled-atmosphere storage did not significantly affect populations of *A. hydrophila* on fresh vegetables. Commercial mixed vegetable salads packed under modified atmosphere and stored at 4 and 15 °C showed no growth of *A. hydrophila* at 4 °C or growth in the first 24 h, with a subsequent decline after that time.

Probiotics. Lewus, Kaiser, and Montville (1991) reported on the opportunity for inhibition of two *A. hydrophila* strains by bacteriocin-producing lactic acid bacteria, isolated from retail cuts of meat. Vescoto, Scolari, Orsi, Sinigaglia, and Torriani (1997) reported that combinations of carbon dioxide, *Lactobacillus casei* and low storage temperature can reduce *A. hydrophila* survival in ready-to-use mixed salad vegetables. Santos, Lopez-Diaz, Garcia-Fernandez, Garcia-Lopez, and Otero (1996) proved that *Lactococcus lactis* subsp. *lactis* strain 388 had inhibitory activity against three strains of *A. hydrophila*.

Polyphosphates/NaCl. Palumbo, Call, Cooke, and Williams (1995) proved that in BHI broth, a combination of 2% of any of polyphosphates (sodium pyrophosphate, sodium tripolyphosphate, Hexaphos, or Sodaphos) and 3.5% NaCl inactivated *A. hydrophila*; this inactivation was temperature-dependent. In ground pork, the polyphosphate-NaCl combination limited growth of the bacterium during refrigerated storage. Velazquez, Escudero, and de Guzman (2001) assessed the antibacterial effects of four phosphates (tetrasodium pyrophosphate, sodium acid pyrophosphate, trisodium phosphate and sodium tripolyphosphate) on growth of *A. hydrophila*; the growth of *A. hydrophila* was totally inhibited by concentrations between 0.5% and 3.0% in modified complete defined synthetic medium (mCDS) and cooked ground meat medium (CM). Sodium acid pyrophosphate (0.5%) had greater inhibitory effect (bactericidal and bacteriolytic effects).

Heating. Sheldon and Schuman (1996) determined D-values (1.5, 0.10 and 0.03 min) at 51, 57 and 60 °C, indicating that such thermal processes can provide a large safety factor with regard to the inactivation of *A. hydrophila* in liquid egg.

Plant extracts. Hao, Brackett, and Doyle (1998a) Hao, Brackett, and Doyle (1998b) reported that plant extracts (eugenol and pimento extracts) were most effective in inhibiting growth of *A. hydrophila*. *A. hydrophila* was more sensitive than *L. monocytogenes* to the two treatments (low 10 cfu g^{-1} and high 10^5 cfu g^{-1}), with 4 $\log_{10}$ cfu g^{-1} less growth occuring at 14 days at 5 °C on eugenol-treated breast than on control samples. Similar results were observed by use of the same plant extracts in refrigerated cooked beef.

High hydrostatic pressure. Ellenberg and Hoover (1999) studied the response of *A. hydrophila* to high hydrostatic pressure (from 51 to 304 megaPascals; MPa) for 15 min. The results showed that the pathogen had the ability to repair or grow following pressure treatment in pork.

Cooling/chilling. Papageorgiou, Melas, Abrahim, and Koutsoumanis (2003) reported that maximum populations of *A. hydrophila* were reached after 22 days at 4 °C and after 6–9 days at 12 °C in rice pudding.

Chlorine treatment. Velazquez, Escudero, DiGenaro, DeCortinez, and de Guzman (1998) proved that tomatoes should be kept at low temperatures (6 °C) during storage, shipping and retail stocking, and that chlorine at a concentration of 50 ppm should be used to reduce the levels of *A. hydrophila*.

Alcohol treatment. Birkenhauer and Oliver (2002) reported that refrigeration for 7 days at 5 °C or alcohol treatment (5 ml vodka for 10 min.) were not sufficient to reduce loads of *A. hydrophila* in or on oysters.

Predictive models. Devlieghere, Lefevere, Magnin, and Debevere (2000) developed a predictive model of growth of *A. hydrophila* in modified-atmosphere-packed cooked meat products. The pathogen was shown to multiply very rapidly at refrigerated temperatures. The developed models demonstrated, however, that proliferation of *A. hydrophila* could be prevented by use of carbon dioxide in the package atmosphere in combination with decreased water activity (<0.985). Gas-packed cured cooked meat products shall not sustain the growth of the pathogen when kept at refrigerated temperatures (<7 °C). Jeyamkondan, Jayas, and Holley (2001) reported an alternative technique "artificial neural networks (ANN)" for modeling microbial growth. One of the subjects for modeling growth was *A. hydrophila*. The conclusion of the authors was that ANN can become a vehicle whereby predictive microbiology can be applied in food safety risk assessment.

7. Conclusions

A. hydrophila is a widespread, emerging food pathogen. Some strains of this microorganism tend to cause illness in humans. It can play a significant role in intestinal disorders in children under five years old, the elderly, and immunosupressed people. Most cases of illness are associated with aquaculture products or long-term refrigerated ready-to-eat foods. *A. hydrophila* is a psychrotrophic bacterium, as it grows at refrigeration temperatures. This ability of the pathogen may play an important role in food safety of foods for human consumption. *A. hydrophila* has a number of putative virulence properties, which are associated with enterotoxic, cytotoxic and hemolytic activities. Multiple resistance to some antibiotics has occurred in many strains of the pathogen, and it may become a problem to cure intestinal disorders in human. *A. hydrophila* is quite sensitive to many factors such as temperature (heating), pH, NaCl, oxygen, phosphates, etc. Most of modern approaches to control levels of contamination with microorganisms are effective against *A. hydrophila*.

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