Prevalence and survival of *Aeromonas* spp. in foods – a review

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### SUMMARY

*Aeromonas* spp. is causing a broad spectrum of human and animal illnesses. *Aeromonas hydrophila* is the agent of haemorrhagic septicemia in cyprinids, whereas *Aeromonas salmonicida* – of furunculosis in salmonids. About 85% of gastrointestinal disorders in humans are attributed to *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas veronii* biovar sobria. Other human pathogens are *Aeromonas veronii* biovar veronii, *Aeromonas jandaei* and *Aeromonas schuberti*, which are known to cause wound infections, meningitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, urinary tract and ocular infections.

*Aeromonas* spp. is isolated from hydrobionts, meat, meat products, milk, dairy products and vegetables at densities of $10^2$ to $10^5$ cfu/g. A substantial risk is posed by the ability of microorganisms of genus *Aeromonas* to grow in foods stored in refrigerator. That is why the research is focused on the influence of temperature, pH, the content of salt, nitrites, phosphates, organic acids and modified atmosphere on their resistance and pathogenicity.

Keywords: *Aeromonas* spp., foods, risk.

### Characteristics of bacteria from the genus *Aeromonas*

The genus *Aeromonas* belongs to the family Aeromonadae [14]. Its members are Gram-negative nonspore-forming rods, facultative anaerobes, oxidase- and catalase positive, able to reduce nitrates to nitrites and resistant to the vibriostat O/129 (2,4-diamino-6,7-diisopropylpteridine). Motile species possess a single polar flagellum [48].

According to Bergey’s Manual of Systematic Bacteriology [48], the genus *Aeromonas* includes the following species: *Aeromonas hydrophila*, *Aeromonas bestiarum*, *Aeromonas salmonicida*, *Aeromonas caviae*, *Aeromonas media*, *Aeromonas eucrenophila*, *Aeromonas sobria*, *Aeromonas veronii* (biovars sobria and veronii), *Aeromonas jandaei*, *Aeromonas schuberti*, *Aeromonas trota*, *Aeromonas allosaccharophila*, *Aeromonas encheleia*, *Aeromonas popoffii* and two DNA homology groups: *Aeromonas* spp. (HG11) and *Aeromonas* spp. (HG13; former Enteric Group 501). During the last decade, several new species are described: *Aeromonas culicicola* [71], *Aeromonas simiae* [29], *Aeromonas mollascorum* [53], *Aeromonas bivalvium* [52], *Aeromonas aquariorum* [49], *Aeromonas piscicola* [9], *Aeromonas fluviatilis* [4], *Aeromonas taiwanensis* and *Aeromonas sanarellii* [3], *Aeromonas rivalii* [23]. *Aeromonas* spp. are not part of the natural intestinal flora. In the faeces of healthy animals and people, they could be detected only after ingestion of contaminated food or water [31].

The pathogenicity of aeromonads, as per JANDA and ABBOTT [34, 35], is due to intracellular structures (pili, flagella, capsule, lipopolysaccharides, outer membrane proteins) and extracellular products (cytotoxic, cytolytic, haemolytic and enterotoxic proteins).

Some fish diseases are caused by *Aeromonas* spp. members. Mesophilic *Aeromonas hydrophila* and *Aeromonas veronii* cause the haemorrhagic septicemia in carp [38], while *Aeromonas salmonicida* induces furunculosis in salmonids [7].

### Pathogenicity of *Aeromonas* organisms in humans

With regard to the prevalence of disease they cause, JANDA and ABBOTT [34] classify aeromonads into:

1. Aeromonads associated with human disease, based upon clinical frequency rather than disease presentation:
   - Major pathogens: *A. hydrophila* (HG 1), *A. caviae* (HG 4) and *A. veronii* bt sobria (HG 8).
   - Minor pathogens: *A. veronii* bt veronii (HG 10), *A. jandaei* (HG 9) and *A. schuberti* (HG 12).

### RéSUMÉ

**Prevalence et survie d’*Aeromonas* spp. dans les aliments – une revue**


**Mots clés : Aeromonas spp., aliments, risque.**
2. Environmental species, including species primarily recovered from water, fish, other animals, and industrial sources: *A. salmonicida* (HG 3), *A. sobria* (HG 7), *A. media* (HG 5), *A. eucrenophila* (HG 6), *A. trota*, *A. allosaccharophila*, *A. enchelaeia* (HG 11), *A. bestiarum* (HG 2) and *A. popoffii*.

*Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas veronii* biovar sobria are responsible for 85% of human gastrointestinal disorders. *Aeromonas veronii* biovar sobria and *Aeromonas caviae* provoke enterites with watery diarrhoea and are most commonly isolated from so-called traveller’s diarrhoea cases. Enteritis caused by *Aeromonas hydrophila* and *Aeromonas jandaei* is characterized by loose stools. *Aeromonas caviae* prevails in juvenile diarrhoeas cases [69]. From 100 diarrhoeic faecal samples in children, YADAV and KUMAR [95] have isolated enterotoxigenic *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae*. Five isolates (*Aeromonas sobria* and *Aeromonas hydrophila*) were obtained from children under 5 years of age. Cytotoxicogenic, enterotoxigenic and haemolytic *Aeromonas hydrophila* have been detected in clinical samples and foods (meat, milk, vegetables) [50]. ABDULLAH et al. [1] isolated and typed aeromonads from diarrhoeic children in Libya, and detected the genes coding for synthesis of haemolysin and enterotoxin in all strains.

Wound *Aeromonas* infections, according to VOSS et al. [91] and WAKABONGO [92], occur mainly by contact with water or soil containing large amounts of *Aeromonas* spp. bacteria.

Septicaemia caused by pathogenic members of the genus is reported in cancer and diabetes patients [37], patients with hepatobiliary diseases [12], liver cirrhosis [44], and infected wounds [24].

Some motile *Aeromonas* spp., as reported by GHEGHESH et al. [24] are emerging food pathogens with increasing importance. They are responsible for several foodborne epidemics and are isolated from patients with "traveler’s diarrhoea". From clinical samples, water and foods, the most commonly isolated species are *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas veronii* biovar sobria. *Aeromonas salmonicida* is frequently isolated from foods, but rarely from clinical samples and water.

Theoretically, the foodborne disease caused by aeromonads could result not only from colonisation, but also from intoxication as the organisms release endotoxins secondary to their reproduction in foods [17]. According to JANDA and ABOBIT [36] the pathogenesis of aeromonad gastroenteritis is that of a toxicoinfection (attaching to the gastrointestinal epithelium – biofilm formation – colonisation-release of virulent factors).

The infection dose of *Aeromonas hydrophila* for humans has been investigated by MORGAN et al. [55]. Out of 57 volunteers, only 2 exhibited diarrhoeic signs after intake of strain 3647 broth culture with $10^7$ CFU or strain 6Y with $10^9$ CFU.

Typical human gastroenteritis caused by *Aeromonas* spp. occur with a watery self-limiting diarrhoea. Some patients exhibit fever, abdominal pain and bloody stools. The severe cases could require hospital admission. In elderly patients, the acute aeromonad diarrhoea could lead to chronic colitis [17]. Aeromonad septicaemias, as believed by PARKER and SHAW [69], have a severe course and are accompanied by diarrhoea, fever, abdominal pain. In immunocompromised subjects with cancer or diabetes, the mortality ranges from 25% to 50%, whereas in patients with infected wounds could be over 90%.

Clinical *Aeromonas* spp. isolates are resistant to ampicillin. The species isolated from 863 patients with traveller’s diarrhoea showed a different resistance to chloramphenicol, tetracycline and co-trimoxazole. Fluoroquinolones are appropriate for treatment of aeromonad diseases. Ciprofloxacin is reported efficient against clinical *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas veronii* biovar sobria isolates [69].

The prevalence of *Aeromonas*-caused traveller’s diarrhoea in Asia ranged between 1% and 57%, in South America – from 1% to 5% and in Africa – from 0% to 9% [72].

Data about incidents of food-borne disease associated with *Aeromonas* species in Russia, Hungary, England, Scotland, Nigeria, USA (Louisiana, Florida) and Japan are summarised by KIROV [40].

### Aeromonas spp. in foods

Members of the *Aeromonas* genus are widely prevalent in the aquatic environment and are frequently isolated from various foods, mainly seafood (fish, clams, shrimps etc), meat, milk and vegetables [25, 43, 50, 57, 60]. The number of motile mesophilic *Aeromonas* spp. organisms in foods varies from $<10^2$ cfu/g to $10^5$ cfu/g [57]. This is also confirmed by PA-LUMBO et al. [64], demonstrating that *Aeromonas* spp. counts in lamb, veal, pork and minced beef ranged from $10^2$ to $10^5$ cfu/g. PENCHEV et al. [70] enumerated the counts of *Aeromonas* spp. in foods and found variations from $1\times10^2$ to $5\times10^5$ cfu/g. By the 7th day of storage at 5°C, the counts of aeromonads increased by 1–3 log [64] or 10–1000 times [70]. SCHUMAN et al. [76] isolated *Aeromonas hydrophila* from a variety of foods (red meat, poultry meat, eggs and raw milk) stored in the fridge.

From foods and water in Australia, KIROV [40] isolated virulent strains of *Aeromonas veronii* biovar sobria and *Aeromonas hydrophila*. Of all aeromonads in foods, the prevalence of *Aeromonas veronii* biovar sobria strains in Japan was 24%, in the UK – 42% and in the USA – 22%. The commonest species isolated from sea fish, vegetables and their products in Japan was *Aeromonas caviae* (60%), whereas *Aeromonas hydrophila* predominated in vegetables in the USA. In Denmark, about 5% of foods were contaminated with *Aeromonas veronii* biovar sobria, but this species were rarely isolated in New Zealand (incidence lower than 5%, except for poultry products).

### Aeromonas spp. in fish and fish products

The research has shown that fish is most frequently and most extensively contaminated with bacteria from the *Aeromonas* genus (positive samples ranging from 37% to 93%, Table 1).

The identification of *Aeromonas* spp. isolated from fish demonstrated the preponderance of *Aeromonas hydrophila*, followed by *Aeromonas sobria* and *Aeromonas caviae* (Table 2) [77, 95].
Among freshwater fish species, *Aeromonas hydrophila* is the most prevalent. In channel catfish fillets, WANG and SILVA [93] detected *Aeromonas hydrophila* in 36.1%, *Aeromonas sobria* in 35.7% and *Aeromonas caviae* in 10.9% of samples. RUZICA et al. [74] isolated 8 motile *Aeromonas* spp. strains from freshwater fish, 6 of them being identified as *Aeromonas hydrophila* and two – as *Aeromonas sobria*. From freshwater fish, ERDEM et al. [21] isolated 78 strains as followed: 36 *Aeromonas hydrophila*, 22 *Aeromonas caviae* and 20 *Aeromonas veronii* biovar sobria. From the skin and the intestinal tract of catfish and tilapia, ASHIRU et al. [6] recovered *Aeromonas caviae*, *Aeromonas hydrophila* and *Aeromonas sobria*. *Aeromonas hydrophila* and *Aeromonas sobria* were the predominant species in catfish, while *Aeromonas caviae* – in tilapia. YECEL et al. [97] affirmed that among freshwater fish species, *Aeromonas caviae* was the most prevalent species (66%), followed by *Aeromonas hydrophila* (22.6%) and *Aeromonas veronii* biovar sobria (11.6%).

In sea fish, RUZICA et al. [74] isolated one strain, identified as *Aeromonas sobria*. *Aeromonas veronii* biovar sobria was the most prevalent aeromonad in sea fish species (41.5%) followed by *Aeromonas hydrophila* (30.1%) and *Aeromonas caviae* (28.3%) [97]. In sea fish fillets, 62% of samples were *Aeromonas* positive [30] with the following prevalence of the strains from different species: *Aeromonas hydrophila* (9), *Aeromonas veronii* biovar veronii (8), *Aeromonas bestiarum* (6), *Aeromonas caviae* (4), *Aeromonas veronii* biovar sobria (2) and *Aeromonas eucrenophila* (1). In fresh mullet samples, the overall incidence of *Aeromonas* spp. was 85%, and among the 34 isolates, there were 30 *Aeromonas hydrophila*, 3 *Aeromonas caviae* and 1 *Aeromonas sobria* strains [75]. The *Aeromonas* spp. counts were from 2.29 to 7.20 log cfu/g in sea fish fillet [30], and 4.91 log cfu/g in fresh mullet fillet [75].

Frozen tilapia (*Oreochromis niloticus niloticua*) was demonstrated to contain *Aeromonas* spp., and 88.3% of isolates belonged to two species: *Aeromonas salmonicida* (67.5%) and *Aeromonas bestiarum* (20.8%) [11]. The other 11.7% of strains included *Aeromonas veronii* (5.2%), *Aeromonas encheleia* (3.9%) and *Aeromonas hydrophila* (2.6%). SALAH EL-DIEN et al. [75] demonstrated *Aeromonas* spp. at 4.38 log cfu/g in 30% of frozen tilapia fillet samples. Among the isolated 12 strains, 10 were identified as *Aeromonas hydrophila*, 1 – as *Aeromonas caviae* and another 1 – as *Aeromonas sobria*.

### Table I: Prevalence of *Aeromonas* spp. in fish samples.

<table>
<thead>
<tr>
<th>Percentage of <em>Aeromonas</em> positive fish samples</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.3</td>
<td>[84]</td>
</tr>
<tr>
<td>50</td>
<td>[15]</td>
</tr>
<tr>
<td>65</td>
<td>[2]</td>
</tr>
<tr>
<td>69</td>
<td>[73]</td>
</tr>
<tr>
<td>80.3</td>
<td>[97]</td>
</tr>
<tr>
<td>93</td>
<td>[28]</td>
</tr>
</tbody>
</table>

### Table II: Percentage of different *Aeromonas* spp. in fish.

<table>
<thead>
<tr>
<th><em>A. hydrophila</em></th>
<th><em>A. sobria</em></th>
<th><em>A. caviae</em></th>
<th><em>A. veronii</em> biovar sobria</th>
<th><em>A. allosaccharophila</em></th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>63</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[28]</td>
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<tr>
<td>60</td>
<td>-</td>
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<td>-</td>
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<td>[89]</td>
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<tr>
<td>59</td>
<td>-</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>[57]</td>
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<tr>
<td>51.52</td>
<td>39.39</td>
<td>9.09</td>
<td>-</td>
<td>-</td>
<td>[43]</td>
</tr>
<tr>
<td>28</td>
<td>12</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>[2]</td>
</tr>
<tr>
<td>11.5</td>
<td>2.3</td>
<td>55</td>
<td>-</td>
<td>-</td>
<td>[73]</td>
</tr>
<tr>
<td>3</td>
<td>7.6</td>
<td>7.6</td>
<td>18.2</td>
<td>-</td>
<td>[15]</td>
</tr>
</tbody>
</table>

*Aeromonas hydrophila* was found in 26.6% of salted and smoked *Trichurus lepturus* fish samples [86].

Some *Aeromonas* spp. isolates from fish are pathogenic. According to the data of KUMAR et al. [43] 70.59% of *Aeromonas hydrophila*, 69.23% of *Aeromonas sobria* and 33.33% of *Aeromonas caviae* strains are enterotoxigenic. Out of 45 *Aeromonas* hydrophila, *Aeromonas sobria* and *Aeromonas caviae* strains isolated by YADA V and KUMAR [95], 26 (58%) produced enterotoxin, whereas all *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae* strains in the investigation of SHARMA and KUMAR [77] were enterotoxigenic. Out of 255 *Aeromonas hydrophila* strains, 78.4% produced haemolysin [84] whereas RADU et al. [73] reported a haemolytic activity in over 90% of *Aeromonas veronii* biovar sobria, *Aeromonas hydrophila* and *Aeromonas caviae* isolates. In fish fillets, 14 out of 16 *Aeromonas hydrophila* isolates were shown to produce haemolysin and cytotoxin [85].

WANG and SILVA [93] observed a haemolytic activity in 86% of *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae* isolates, which was also confirmed in another research [97] where more than 80% of isolates were *Aeromonas veronii* biovar sobria and *Aeromonas hydrophila*. The *Aeromonas hydrophila* and *Aeromonas veronii* biovar sobria strains isolated by ERDEM et al. [21] were found to possess a strong haemolytic activity, but not *Aeromonas caviae* isolates. Protease activity was established in all *Aeromonas hydrophila* and *Aeromonas veronii* biovar sobria isolates and in 81% of *Aeromonas caviae* strains. Of all strains, 92% of *Aeromonas hydrophila*, 91% of *Aeromonas caviae* and 5% of *Aeromonas veronii* biovar sobria isolates were able to hydrolyze pyrazinamide.
Over 80% of *Aeromonas veronii* biovar sobria and *Aeromonas hydrophila* isolates [97] possessed a haemolytic activity.

**AEROMONAS SPP. IN NON-FISH HYDROBIONT SPECIES**

The bacterium was found to be prevalent among oysters and mussels. In oysters, TSAI and CHEN [85] detected *Aeromonas hydrophila*. The research conducted by EVANGELISTA-BARRETO et al. [22] with *Crassostrea rhizophore* oysters established *Aeromonas* spp. in 67% of samples. Of all positive samples, the predominant species were *Aeromonas veronii* biovar sobria and *A. veronii* (43%), followed by *Aeromonas media* (37%) and *Aeromonas caviae* (23%). Other species isolated were *Aeromonas sobria*, *Aeromonas trota*, *Aeromonas eucrenophila*. In another study, 29.1% of mussel samples were shown to contain *Aeromonas hydrophila* [13]. In *Mytilus galloponti* mussels, OTAVIANI et al. [61] isolated 32 *Aeromonas* spp. strains – 22 *Aeromonas hydrophila*, 8 - *Aeromonas hydrophila* HG 2 and 2 - *Aeromonas caviae*. Twelve isolates showed virulence and enteropathogenicity when tested on mice.

Shrimps were also contaminated with *Aeromonas* spp. organisms. HANNINEN et al. [28] detected the bacterium in 16% of tested samples. In shrimps, TSAI and CHEN [85] and VIVEKANANDHAN et al. [89] isolated *Aeromonas hydrophila*. It was detected in 29.1% [13] to 35.6% [84] of samples, and 78.4% of strains were haemolysin-producing [84]. In cooled shrimps, *Aeromonas* spp. was established in 70% of samples at 4.44 log cfu/g. Among the isolated 28 strains, 20 were identified as *Aeromonas hydrophila*, 5 – as *Aeromonas caviae* and another 3 – as *Aeromonas sobria* [75].

**AEROMONAS SPP. IN MEAT AND MEAT PRODUCTS**

According to reported data, different *Aeromonas* spp. were isolated from meat of mammalian species. In lamb meat and byproducts, MAJEED et al. [46] isolated 73 *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae* strains. In raw meat HANDFIELD et al. [27] established 66 *Aeromonas hydrophila* strains. SHARMA and KUMAR [77] found out more *Aeromonas* spp. positive samples in pork as compared to goat meat. In 12.3% of fresh buffalo meat and 6.5% of fresh mutton samples, OSMAN et al. [59] isolated motile aeromonads, but they were not present in fresh beef or camel meat. In frozen buffalo meat motile aeromonads were not discovered, while 14% of frozen beef samples were positive. The prevailing species in meat according to SHARMA and KUMAR [77] was *Aeromonas hydrophila*, followed by *Aeromonas sobria* and *Aeromonas caviae*. The data of OSMAN et al. [59] affirmed that *Aeromonas hydrophila* was also most commonly isolated (from 6.8% of samples), but succeeded by *Aeromonas caviae* (2.7% of samples) and *Aeromonas veronii* biovars sobria (2.1% of samples). *Aeromonas hydrophila* was proved also in different red meats stored in the fridge [76].

*Aeromonas* spp. was detected in all minced pork and 79% of minced beef samples [78]. *Aeromonas hydrophila* was more frequently isolated from minced pork (97% of isolates) than from minced beef (87% of isolates). Motile aeromonads were present in 68% of minced meat samples investigated by YUCEL and CITAK [96] with predominance of highly haemolytic *Aeromonas hydrophila* and *Aeromonas sobria* strains.

All samples of pork and beef sausages were positive for *Aeromonas* spp. [78]. ENCINAS et al. [20] established the organism in 78.5% of mixture samples for production of the Spanish fermented sausages Chorizo and Longaniza at >1 to 4.47 log10 cfu/g. The species *Aeromonas hydrophila* was the commonest (24 isolates) followed by *Aeromonas veronii* biovar sobria (10 strains) and *Aeromonas caviae* (2 strains). All isolates were haemolytic and capable to grow at 5 °C.

All poultry meat samples in the study of SINGH [78] were *Aeromonas* spp. positive. Poultry isolates consisted of 30% *Aeromonas sobria* and 20% *Aeromonas caviae*, whereas those from turkey meat – 8% *Aeromonas sobria* and 16% *Aeromonas caviae*. In 87% of poultry meat samples YUCEL and CITAK [96] detected motile aeromonads with predominance of highly haemolytic *Aeromonas hydrophila* and *Aeromonas sobria* strains. From poultry carcasses, ABDULLAH et al. [1] isolated 32 strains out of which 30 were typed as *Aeromonas veronii*. Compared to pork and goat meat, poultry meat exhibited more *Aeromonas* spp. positive samples [77] with predominance of *Aeromonas hydrophila*, followed by *Aeromonas sobria* and *Aeromonas caviae*.

SCHUMAN et al. [76] isolated *Aeromonas hydrophila* from fridge-stored poultry and eggs. KOCA and SARIMEHMETOGLU [41] detected *Aeromonas* spp. in 53.75% of packed turkey meat from different retail stores. *Aeromonas hydrophila* – a hazard for consumer’s health, was isolated from all positive samples.

MAJEED et al. [46] deem that *Aeromonas sobria* strains produced more intensively haemolysin and enterotoxin than *Aeromonas hydrophila*, whereas *Aeromonas caviae* isolates were not haemolytic and enterotoxigenic. Data reported by HANDFIELD et al. [27] evidence that 48% of isolated *Aeromonas hydrophila* strains were haemolytic and 92% - cytotoxic. Strains isolated from meat [50] were mainly cytotoxic, then came enterotoxigenic and haemolytic *Aeromonas hydrophila*. All *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae* strains detected by SHARMA and KUMAR [77] were enterotoxigenic. The aerA gene coding for aerolysin was present only in 3 *Aeromonas hydrophila* isolates but was not present in any of *Aeromonas caviae* and *Aeromonas veronii* biovars sobria strains. None of 17 strains in another study possessed the haemolysin coding gene [54].

All strains isolated by ABDULLAH et al. [1] possessed virulence genes, coding for haemolysin and enterotoxin, while the isolates of SHARMA and KUMAR [77] were enterotoxigenic.

The contamination of meat with *Aeromonas* spp., according to GHENGHESH et al. [24], is due to washing of carcasses with water containing *Aeromonas* spp. and poor hygiene during processing and realisation of meat (cutting and mincing). ENCINAS et al. [20] speculate that the causes for meat products contamination with *Aeromonas* spp. were the neglected control of raw meat and fat, and inadequate sanitary measures during their handling and processing.
AEROMONAS SPP. IN MILK AND MILK PRODUCTS

Raw milk was also shown to be contaminated with Aeromonas spp. SUBASHKUMAR et al. [82] detected Aeromonas hydrophila in 17.14%, while YUCEL and CITAK [96] in 47.7% of studied raw milk samples. Apart Aeromonas hydrophila, milk was shown also to harbour Aeromonas sobria [95]. In raw cow milk [51], the microbrial density level of Aeromonas hydrophila was $3 \times 10^2$ to $5 \times 10^3$ cfu/ml in 15.9% of samples, of Aeromonas caviae – from $2 \times 10^2$ to $3 \times 10^3$ cfu/ml in 13% of samples, of Aeromonas sobria – $2.5 \times 10^3$ to $5 \times 10^3$ cfu/ml in 3.6% of samples. The Aeromonas spp. isolates from the other samples were not typed. In raw sheep milk, Aeromonas hydrophila were prevalent at $5 \times 10^2$ to $5 \times 10^3$ cfu/ml in 14% of samples, Aeromonas caviae at $1.5 \times 10^2$ to $1 \times 10^3$ cfu/ml in 10.5% of samples, Aeromonas sobria at $5 \times 10^2$ to $1 \times 10^3$ cfu/ml in 3.5% of samples. The other isolated aeromonads were not classified. Aeromonas hydrophila was isolated from raw milk stored in a fridge [76].

In pasteurized cow milk, MELAS et al. [51] did not detect Aeromonas spp., while YUCEL and CITAK [96] discovered a strongly haemolytic Aeromonas hydrophila strain in 16% of samples.

In Nigeria, NWAMAKA and CHIKE [58] succeeded to isolate aeromonads in 25% of yogurt samples.

Different types of cheese also were established to be contaminated with Aeromonas spp. Aeromonas hydrophila was detected in 10.2% of Anthotyros, 8.3% of Manouri, but not in Feta cheese samples [51]. ARAUJO et al. [5] recovered Aeromonas hydrophila and Aeromonas caviae in 17.7% of examined soft cheese samples. Urfa cheese analysis showed that 27% of samples were contaminated by Aeromonas spp. (18.1% with Aeromonas salmonicida and 9.02% with Aeromonas sobria) [87].

Aeromonas spp. is discovered also in ice cream. Among studied samples [19] 19% of samples were Aeromonas spp. positive. Other studies with ice cream reported a prevalence of Aeromonas caviae in 5% of samples [32].

Raw milk was reported to be contaminated with Aeromonas spp. hazardous for human health. In milk MARTINS et al. [50] have isolated mainly cytotoxic and less frequently enterotoxigenic and haemolytic Aeromonas hydrophila. Enterotoxin was produced by 58% Aeromonas hydrophila and Aeromonas sobria isolates [95]. YUCEL and CITAK [96] discovered a strongly haemolytic Aeromonas hydrophila strain in raw milk, whereas SUBASHKUMAR et al. [82] – proteolytic Aeromonas hydrophila isolates, 94.4% of them possessing also haemolytic activity.

AEROMONAS SPP. IN VEGETABLES AND SPICES

In all examined samples of parsley, spinach, celery, broccoli and lettuce, CALLISTER and AGGER [10] isolated Aeromonas hydrophila and Aeromonas caviae. MONGE et al. [54] detected Aeromonas spp. in 30% of coriander, 52% of lettuce and 46% of celery samples. Aeromonas hydrophila was also found in vegetables [50].

Cytotoxic aeromonads were present in 92% of studied vegetable samples [10]. Such properties were identified in all Aeromonas hydrophila and 6% of Aeromonas caviae strains, whereas 90% of cytotoxic strains were haemolysin-producing. Forty-five percent of aeromonads isolated by MONGE et al. [54] were cytotoxic. Aeromonas hydrophila isolates from vegetables [50] were cytotoxic, enterotoxigenic or haemolytic.

After storage of vegetables (parsley, spinach, celery, broccoli and lettuce) for 2 weeks at 5°C [10, 70], the counts of Aeromonas spp. increased.

AEROMONAS SPP. READY-TO-EAT FOODS

From ready-to-eat foods, HANDFIELD et al. [27] recovered 48% haemolytic and 92% cytotoxic Aeromonas hydrophila strains. Rice pudding [51] was free from Aeromonas spp. The bacterium was isolated from 61.5% of ready to serve vegetable salads [94]. The isolated strains were identified by SDS–PAGE as 33 belonging to the Aeromonas hydrophila complex, 12 – to the Aeromonas caviae complex and 1 – to the Aeromonas sobria complex.

All studied 17 caviar samples were Aeromonas spp. positive [28]. Of them, 57 strains with dominant hybridization group Aeromonas hydrophila HG 3 (16 or 28% strains) were isolated. Aeromonas hydrophila was detected only in one (2%) out of 48 mullet (Mugil cephalus) caviar samples [90].

Factors influencing the resistance of Aeromonas spp.

A number of factors – temperature, pH, salt, nitrite, phosphate and organic acids, as well as the modified atmosphere could influence the resistance of Aeromonas spp.

According to available reports, low temperatures do not inhibit the growth of aeromonads. All clinical Aeromonas spp. isolates were shown to grow equally within 20-35°C, and most of them – also at 4.5°C or 42°C [62]. PAPAGEORGIU et al. [68] established that rice pudding contaminated with $2.5 \times 10^2$–$4 \times 10^2$ cfu/g Aeromonas hydrophila, reached peak counts of 8 to 9.23 log$_{10}$ cfu/g after 6-9 days at 12°C, and after 22 days at 4°C. Our studies [81] in carps infected via immersion with two clinical Aeromonas hydrophila strains showed that the organism was isolated from all fish stored at 4°C after 15 days as well, and from those frozen at –18°C: by the 20th day. GRASSI et al. [25] provided evidence that all Aeromonas sobria strains were highly cytotoxic to Vero cell lines, but the cytotoxicity of Aeromonas hydrophila/caviae depended on the temperature of incubation: it was 100% at 5°C, 80% at 0°C and 89.2% at 31°C.

High temperatures destroy aeromonads. According to reports, D-values of Aeromonas spp. are as followed: for Aeromonas hydrophila at 48°C in saline solution – from 3.49 to 6.64 min, and in raw milk – from 3.20 to 6.23 min [66]; for Aeromonas hydrophila at 48°C in egg suspension – from 3.62 to 9.43 min, and at 60°C - from 0.026 to 0.040 min [76]; for Aeromonas hydrophila at 60°C – 7 min, for Aeromonas bestiarum – 4 min

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and for *Aeromonas salmonicida* – 3 min [26]. HANDFIELD et al. [27] showed that the 10-min treatment at 56°C reduced cytotoxicity of *Aeromonas hydrophila* up to 40%, but did not eliminate it completely.

Higher salt concentrations [16] inhibit the growth of *Aeromonas* spp. All 16 studied *Aeromonas* spp strains were capable to grow at 0.34 M NaCl, 9 – at 0.68 M NaCl, 2 strains (*Aeromonas enteropelogenes* and *Aeromonas trota*) – at 0.85 M NaCl, only *Aeromonas trota* – at 1.02 M NaCl, and no growth was detected at 1.71 M NaCl.

The combinations of different factors had an adverse effect on the growth of *Aeromonas* spp. The investigations of PALUMBO et al. [64], PALUMBO [67], PALUMBO et al. [65], PALUMBO and WILLIAMS [63] on the combined effects of temperature (from 5°C to 42°C), pH (5.3–7.3), NaCl (0.5–4.5%), NaNO2 (0–200 µg/ml), different acids, side microflora and modified atmosphere packaging have shown that the aerobic growth of *Aeromonas hydrophila* K144 was inhibited by a combination of low temperature, low pH, salt and nitrite concentration. At 28°C, most clinical *Aeromonas hydrophila* isolates tolerated 4% NaCl and acid pH, but at 4°C only a limited number of strains grew at 3% NaCl and low pH. The growth of the experimental *Aeromonas hydrophila* K144 strain in minced pork stored at 5°C was inhibited by 3% NaCl, pH <6 and the natural meat microflora, but it was shown to grow in vacuum-packed pork minced meat. The aerobic growth of *Aeromonas hydrophila* in brain heart infusion broth was limited by NaCl concentrations over 2% in combination with organic acids (acetic, lactic, citric and tartaric) and temperature decreasing from 19°C to 5°C. Acetic and lactic acids were effective to control the growth of the tested microorganism. STECCHINI et al. [80] confirmed that the combined effect of NaCl (0.1%, 1.5% and 3%) and ascorbic acid (0, 1 and 2 mmol/l) resulted in increased death rate of *Aeromonas hydrophila* bacterial cells.

VELAZQUEZ et al. [88] tested the antibacterial effect of four different phosphates against two *Aeromonas hydrophila* strains (ATCC 7965 and one isolated from food). The growth was completely inhibited at concentrations between 0.5 and 3.0%. Most efficient against both strains was 0.5% disodium pyrophosphate.

The research of ELLEMBERG and HOOVER [18] proved that the 15-min exposure of contaminated pork meat to high hydrostatic pressure (253 MPa) resulted in reduction of *Aeromonas hydrophila* counts by 7 log10 cfu/g, and no growth was observed after a 15-min stay at 128–203 MPa and storage at 4°C for 14 days. However, if fridge-stored meat is put at 30°C, after a 10-hour lag stage, *Aeromonas hydrophila* begins to grow. It was therefore concluded that *Aeromonas hydrophila* was capable to recover and grow after being exposed to high hydrostatic pressure.

KOTHARI et al. [42] investigated the sensitivity of *Aeromonas hydrophila* to seven different phytocompounds and found out that curcumin was the most effective with MIC of 175 µg/ml, followed by tannic acid. Gallic acid had no influence on the growth of *Aeromonas hydrophila*.

Smoke condensates had an antimicrobial effect with regard to *Aeromonas hydrophila*. *Aeromonas hydrophila* contaminated salted rainbow trout samples, were cold-smoked with four different smoke condensates, vacuum-packed and stored at 4°C for 21 days [83]. It was concluded that all tested smoke condensates exhibited an antimicrobial effect against *Aeromonas hydrophila* in vacuum-packed cold-smoked trout.

Lactic acid microflora inhibits the growth of *Aeromonas* spp. LEWUS et al. [45] reported that lactic acid bacteria, isolated from meat and producing a bacteriocin, suppressed the growth of *Aeromonas hydrophila*. The growth of aeromonads was inhibited by lactic acid bacteria isolated from salmon and identified as *Lactococcus lactis* subsp. lactis, *Lactococcus lactis* subsp. cremoris, *Lactobacillus curvatus*, *Leuconostoc mesenteroides*, *Lactobacillus sakei* and *Carnobacterium maltaromaticum* [8]. That is a probable reason for the rapid inactivation of *Aeromonas* spp. in the early production stages of the Spanish fermented sausages Chorizo and Longaniza, as observed by ENCINAS et al. [20].

Having investigated chilled pork and turkey meat contaminated with *Aeromonas hydrophila* and after modified atmosphere packaging (MAP), MANO et al. [47] demonstrated that the growth was strongly inhibited in both MAP meats stored at 1°C. No growth of *Aeromonas hydrophila* was present in samples stored in an atmosphere with 40/60 CO2/O2 at 1 and 7°C. Growth was however detected in samples packed under 100% N2 and stored at 1 and 7°C, as well as in turkey meat packed under 20/80 CO2/O2 and stored at 7°C.

*Aeromonas* spp. is fairly sensitive to disinfectants such as 2-chlorophenol and glutaraldehyde [33]. The microorganism is inactivated at 25°C after treatment with 5 ppm NaOCl for 1 min, and with 2.5 ppm NaOCl for 5 min; after exposure to quaternary ammonium compounds at a concentration of 1:12500 for 1 min; with iodophores (10 ppm) for 10 min. SISTI et al. [79] established that at 20°C, 0.31 mg/ml chlorine killed 50% of *Aeromonas hydrophila* population for 10 min, and that *Aeromonas veronii* biovar sobria and *Aeromonas caviae* bacterial cultures were more sensitive than *Aeromonas hydrophila*.

According to KERSTERS and VERSTRAETE [39] the counts of *Aeromonas* spp. decreased rapidly by 2 to 3 log in oxidized groundwater with high ferrous ion (460–1070 µM) concentrations. *Aeromonas* spp. is sensitive to ionised radiation [33]. Their D-values in substrates as phosphate buffer, brain heart infusion broth and minced fish meat within the temperature range –15°C to 22°C, were between 0.131 and 0.274 kGy. D-values for *Aeromonas hydrophila*, *Aeromonas veronii*, *Aeromonas caviae*, *Aeromonas jandaei* and *Aeromonas salmonicida* in saline solution at 0-4°C ranged between 0.031 and 0.046 kGy [56].

**Conclusion**

*Aeromonas* spp. is widely prevalent in foods and could be isolated from fish, meat, milk, vegetables and water. The microbial density of mesophilic species in foods ranges from <105 cfu/g to 108 cfu/g. According to available reports, most *Aeromonas* spp. positive samples were established in fish. Aeromonads are part of the natural aquatic microflora and therefore, fish is contaminated either in basins, or during the
processing. Meat could be contaminated when carcasses are washed with water containing Aeromonas spp., or during cutting or mincing. Motile representative of the gender are isolated washed with water containing processing. Meat could be contaminated when carcasses are stored produce.

References


