Aeromonas[1]

Description

Species of *Aeromonas* are Gram-negative, non-spore-forming, rod-shaped, facultatively anaerobic bacteria that occur ubiquitously and autochthonously in aquatic environments. Although historically the *Aeromonas* genus has been placed in the family Vibrionaceae (Popoff, 1984), there have been proposals to place it in its own family, the Aeromonadaceae (Colwell, MacDonnell & De Ley, 1986). The aeromonads share many biochemical characteristics with members of the Enterobacteriaceae, from which they are primarily differentiated by being oxidase-positive. The genus includes at least 13 genospecies, among which are the mesophilic *A. hydrophila*, *A. caviae*, *A. sobria*, *A. veronii*, and *A. schubertii*, and the non-motile, psychrophilic *A. salmonicida*.

A. salmonicida is a fish pathogen and has not been associated with human infection. By contrast, the mesophilic species have been associated with a wide range of infections in humans (Janda & Abbott, 1996). Although members of the genus have classically been divided into three biochemically differentiated groups (typified by *A. hydrophila*, *A. caviae*, and *A. sobria*), these contain a number of genospecies, to which new species have been added (Carnahan & Altwegg, 1996). Currently the genus is made up of 17 DNA hybridization groups representing a range of genospecies and phenospecies (see Table 1).

The mesophilic aeromonads have been commonly isolated from patients with gastroenteritis although their role in disease causation remains unclear. They are also associated with sepsis and wounds, and with eye, respiratory tract, and other systemic infections (Janda & Duffey, 1988; Janda & Abbott, 1996; Nichols et al., 1996); see Table 2. Many of the systemic infections arise following contamination of lacerations and fractures with *Aeromonas*-rich waters.

The species principally associated with gastroenteritis are *A. caviae*, *A. hydrophila*, and *A. veronii* biovar sobria (Joseph, 1996); *A. caviae* is particularly associated with young children (under 3 years of age). Many studies have resulted

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***Table 1. Genospecies and phenospecies of the genus* Aeromonas**[a]

DNA hybridization group	Reference strain (^{T} = type strain)	Genospecies	Phenospecies
1	ATCC 7966^T	*A. hydrophila*	*A. hydrophila*
2	ATCC 51108^T	*A. bestiarum*	*A. hydrophila*
3	ATCC 33658^T	*A. salmonicida*	*A. salmonicida*
3	CDC 0434-84	*A. salmonicida*	*A. hydrophila*
4	ATCC 15468^T	*A. caviae*	*A. caviae*
5A	CDC 0862-83	*A. media*	*A. caviae*
5B	CDC 0435-84	*A. media*	*A. media*
6	ATCC 23309^T	*A. eucrenophila*	*A. eucrenophila*
7	CIP 7433^T	*A. sobria*	*A. sobria*
8	ATCC 9071	*A. veronii*	*A. veronii* biovar sobria
9	ATCC 49568^T	*A. jandaei*	*A. jandaei*
10	ATCC 35624^T	*A. veronii*	*A. veronii*
11	ATCC 35941	Unnamed	*Aeromonas* sp. (ornithine-positive
12	ATCC 43700^T	*A. schubertii*	*A. schubertii*
13	ATCC 43946	Unnamed	*Aeromonas* Group 501
14	ATCC 49657^T	*A. trota*	*A. trota*
15	CECT 4199^T	*A. allosaccharophila*[b]	*A. allosaccharophila*[b]
16	CECT 4342^T	*A. encheleia*[b]	*A. encheleia*[b]

[a] Modified from Carnahan & Altwegg, 1996.
[b] The taxonomic status of *A. allosaccharophila* and *A. encheleia* remains to be confirmed. A further new species, *A. popoffi* (unassigned DNA hybridization group), has also been proposed.

in the isolation of several species of *Aeromonas* from patients with gastroenteritis, and these have been extensively reviewed (Altwegg & Geiss, 1989; Janda, 1991; Joseph, 1996). There has been considerable debate as to whether the mesophilic aeromonads are primary enteropathogens, prompted largely by failure to establish significant infection in volunteer studies. In a study in which 57 people were challenged using five strains of *A. hydrophila* with doses ranging from 10^4 to 10^{10} organisms, only two individuals developed diarrhoea—one had mild diarrhoea after a dose of 10^9 organisms and the other developed moderate diarrhoea after a dose of 10^7 (Morgan et al., 1985). The value of these data is limited, as the strains used were poorly characterized and some were not demonstrably enterotoxigenic (Gosling, 1996). However, there have been reports of laboratory-acquired infections in microbiologists who (unintentionally) ingested significant doses of *Aeromonas* and developed self-limiting diarrhoea (Joseph, 1996).

Understanding the clinical significance of enteric isolates of *Aeromonas* has been further complicated by the fact that some studies have demonstrated similar isolation frequencies from symptomatic and asymptomatic adults (Altwegg & Geiss, 1989), while others have shown significant correlations between diarrhoea

***Table 2. Relative frequency of occurrence of human infections associated with mesophilic* Aeromonas**[a]

Type of infection	Characteristics	Relative frequency[b]
Diarrhoea		
Secretory	Acute watery diarrhoea, vomiting	Very common
Dysenteric	Acute diarrhoea with blood and mucus	Common
Chronic	Diarrhoea lasting more than 10 days	Common
Choleraic	"Rice water" stools	Rare
Systemic		
Cellulitis	Inflammation of connective tissue	Common
Myonecrosis	Haemorrhage, necrosis with/without gas gangrene	Rare
Erythema gangrenosum	Skin lesions with necrotic centre, sepsis	Uncommon
Septicaemia	Fever, chills, hypotension, high mortality	Fairly common
Peritonitis	Inflammation of peritoneum	Uncommon
Pneumonia	Pneumonia with septicaemia, sometimes necrosis	Rare
Osteomyelitis	Bone infection following soft-tissue infection	Rare
Cholecystitis	Acute infection of gallbladder	Rare
Eye infections	Conjunctivitis, corneal ulcer, endophthalmitis	Rare

[a] Modified from Janda & Duffey, 1988, and Nichols et al., 1996.
[b] Frequency of occurrence relative to all cases of *Aeromonas* infection.

and enterotoxin-producing *Aeromonas* spp. (Gracey, Burke & Robinson, 1982; Bloom & Bottone, 1990; Joseph, 1996). Seasonal variations in isolation of *Aeromonas* from stools has also been reported, with highest recovery during the warmer months (Burke et al., 1984a; Moyer, 1987). The available evidence indicates that people are generally unaffected by enteric *Aeromonas* and that aeromonads may be a natural part of the gut flora, either transiently or in the longer term. A numbers of factors, including age, immunocompetence, infection dose, underlying illness, and expression of sufficient virulence factors by the infecting organism, affect the ability of *Aeromonas* spp. to cause disease (Nichols et al., 1996).

Although the pathogenesis of *Aeromonas* infections remains poorly understood, mesophilic *Aeromonas* spp. can express a range of virulence factors (Gosling, 1996), including attachment mechanisms and production of a number of toxins. Several studies have demonstrated that strains of *A. hydrophila* produce lectins and adhesins which enable adherence to epithelial surfaces and gut mucosa (Gosling, 1996). Additionally, two types of pili have been characterized from Hep-2-adherent *A. hydrophila* (Carrello et al., 1988; Gosling, 1996), and invasion of Hep-2 cells by faecally derived *A. hydrophila* has also been reported (Lawson, Burke & Chang, 1985).

Species of *Aeromonas* are capable of expressing a number of extracellular toxins and enzymes (Gosling, 1996; Howard, MacIntyre & Buckley, 1996). Early characterization of the toxins, however, resulted in confusion regarding their

number and activities. The primary toxins produced are haemolysins, of which the most significant is aerolysin, expressed by many strains of *A. hydrophila* and *A. sobria* (Janda, 1991; Howard, MacIntyre & Buckley, 1996). This is a heat-labile β-haemolysin, which exhibits phospholipase A and C activity. It is a pore-forming cytolysin able to insert into the cell membrane bilayer causing leakage of cytoplasmic contents. Haemolytic enterotoxins have been reported by some authors (Chopra, Houston & Kurosky, 1991; Gosling, 1996). A weak haemolysin, glycerophospholipid:cholesterol acyltransferase (GCAT), has been characterized from *A. hydrophila* and *A. salmonicida* (Howard, MacIntyre & Buckley, 1996); other haemolysins may also exist, but need to be isolated and purified before haemolytic activity can be confirmed. In addition, at least one cytotonic enterotoxin with similar activity to cholera toxin has been demonstrated (Ljungh, Eneroth & Wadström, 1982; Gosling et al., 1992; Gosling, 1996), and there may be several. Evidence for plasmid-encoded expression by *A. hydrophila* and *A. caviae* of a cytotoxin similar to Shiga-like toxin 1 has been reported (Haque et al., 1996). Species of *Aeromonas* also produce a range of cell-surface and secreted proteases which probably enhance virulence (Gosling, 1996). Expression of virulence factors, including haemolysins and proteases, by aeromonads has been shown to be influenced by environmental temperature (Eley, Geary & Wilcox, 1993; Mateos et al., 1993).

There is abundant evidence to suggest associations between mesophilic aeromonads and diarrhoea, and production of enterotoxins has been demonstrated. Further work is needed to clarify the pathogenic mechanisms of *Aeromonas* spp. and substantiate the causative role of these organisms in gastroenteritis. There is also a need for reliable data on human infective doses for well-defined strains of putative enteropathogenic aeromonads, pending the establishment of an appropriate animal model for the study of *Aeromonas*-associated diarrhoea.

Health significance of *Aeromonas* in drinking-water

The health significance of detecting mesophilic aeromonads in public water supplies is not well understood: no clearly defined point-source outbreak has been documented and establishing epidemiological links is difficult.

Reports from Australia (Burke et al., 1984a; 1984b) have suggested that there may be a connection between cases of *Aeromonas*-associated diarrhoea and the numbers of *Aeromonas* in the drinking-water. In later studies following increases in numbers of aeromonads in treated water in the Netherlands, some of the strains isolated demonstrated strong cytotoxic properties (van der Kooij, 1988). Following a review of data available at the time, the health authorities in the Netherlands in 1985 introduced "indicative maximum values" for *Aeromonas* densities in drinking-water. The values were based on a national survey of aeromonads in drinking-water in the Netherlands and have been defined as follows: 20 cfu/100 ml as a median value over a 1-year period in water leaving

the treatment facility; 200 cfu/100 ml as the 90th-percentile value of the *Aeromonas* counts of drinking-water collected from the distribution system in a 1-year period (Trouwborst, 1992). It should be noted that these values were based on assessment of achievability in the Netherlands, motivated by a precautionary approach, and not on the public health significance of the occurrence of *Aeromonas* in drinking-water.

Virulence factors

Several studies have demonstrated that many mesophilic aeromonads isolated from drinking-water can exhibit toxigenic factors. Millership, Barer & Tabaqchali (1986) found that cytotoxicity was demonstrated by 28% of *Aeromonas* isolates (mainly *A. hydrophila*) from chlorinated and unchlorinated drinking-water but by none of the strains of *A. caviae* (which represented 50% of the isolates). More recently Holmes, Niccolls & Sartory (1996) found that 20% of *Aeromonas* isolates exhibited phenotypic characteristics associated with enterotoxicity; of these, 75% were *A. hydrophila*, 14% *A. sobria*, 9% *A. caviae*, and the remainder *A. schubertii*. In contrast, Burke et al. (1984b) reported that 61% of aeromonads isolated from an unchlorinated municipal water supply in Australia were enterotoxigenic, and 64% produced haemolysins. Notermans et al. (1986) found that all of 26 drinking-water isolates of *A. hydrophila* and 9 of 22 isolates of *A. sobria* exhibited the haemolytic enterotoxin Asao toxin and cytotoxicity to Vero cells, while none of 14 isolates of *A. caviae* was positive. Similarly, Krovacek et al. (1992) found that 100% of *A. hydrophila* and 70% of *A. sobria* in Swedish chlorinated and unchlorinated drinking-water were haemolytic, but that less than 30% of the isolates were enterotoxigenic. Kirov et al. (1994) found that 53.6% of isolates of *A. hydrophila* hybridization group 1 (HG1) and 55.9% of HG3 from water expressed two or more virulence factors.

Epidemiology

Despite the association of virulence factors with drinking-water aeromonads, there is increasing evidence that strains isolated from the environment generally belong to different groups from strains associated with gastroenteritis. Havelaar et al. (1992) typed 187 *Aeromonas* isolates from human diarrhoeal stools and 263 from drinking-water. There was little similarity between the strains from stools and those from drinking-water. This was particularly true of *A. caviae*, which was the dominant aeromonad in both sets of samples. Other studies have indicated that *A. hydrophila* prevalence may be related to hybridization groups. Both Kirov et al. (1994) and Hänninen (1994) found that HG1 was associated with clinical specimens, while HG3—and to a lesser extent HG2—predominated in water and environmental samples. It appears that this may be reflected in the maximum growth temperatures (t_{max}) of the homology groups. Hänninen, Salmi & Siitonen (1995) have reported that hybridization groups of *Aeromonas* associated with

clinical samples (HGs 1, 4, 9/10 and 13) generally had a t_{max} of 40–44 °C, while isolates from freshwater (HGs 3 and 11) had t_{max} values between 36.5 and 37.5 °C.

It has been claimed that drinking-water supplies are responsible for the increased incidence of *Aeromonas*-associated gastroenteritis. Ghanem, Mussa & Eraki (1993) considered that, since 90% of the domestic water supplies in Cairo were positive for aeromonads, and that 56% of isolates produced enterotoxins, the supplies were a major source of *Aeromonas* infections. Investigating a case of long-term diarrhoea in a child aged 18 months, Krovacek et al. (1989) concluded that the cause was *A. hydrophila* from a private, unchlorinated well in which counts ranged from 70 cfu/100 ml to 6.4×10^4 cfu/100 ml. The majority of isolates were enterotoxin-producers.

Although these reports (Burke et al., 1984a, 1984b; Krovacek et al., 1989; Ghanem, Mussa & Eraki, 1993) indicate a possible relationship between *Aeromonas* in drinking-water and increased incidence of aeromonad-related illness, the evidence is tenuous. In one case comparing the typing of faecal and water isolates, the two groups proved to be unrelated (Moyer et al., 1992). Following a number of cases of diarrhoea in children using a small community water supply, *Aeromonas* was isolated from water-treatment and distribution samples; ribotyping and DNA hybridization showed that isolates from faeces were of different ribotypes and DNA hybridization groups (HGs 1 and 4) from drinking-water isolates (predominantly HGs 2, 3 and 5A) (Moyer et al., 1992).

Monitoring and assessment

Routine monitoring for *Aeromonas* in piped and non-piped water supplies cannot be justified on the basis of present knowledge of their role in human infection. Monitoring or periodic surveys may be required in some circumstances, for instance where especially vulnerable populations are exposed; and further research is justified.

Membrane filtration is the procedure most commonly used for the enumeration of *Aeromonas* from treated water; it employs a variety of culture media, most of which contain ampicillin. For drinking-water, the most widely used medium is ampicillin–dextrin agar (ADA) (Havelaar, During & Versteegh, 1987; Havelaar & Vonk, 1988). An alternative, which gives the same selectivity and sensitivity, is Ryan's *Aeromonas* medium (Holmes & Sartory, 1993). These media, however, contain selective agents and are nutrient-rich, and their use may result in low recovery of some aeromonads from low-nutrient or chlorinated waters, weighting any data in favour of the more robust, rapidly growing strains (Gavriel & Lamb, 1995; Holmes, Niccolls & Sartory, 1996). The incubation regime is typically 28–30 °C for 24–48 hours. *Aeromonas* species are sensitive to the presence of copper at concentrations as low as 10 µg/l, and a complexing agent (50 mg/l sodium ethylenediamine tetraacetate, Na_2EDTA, or sodium nitrilotriacetate, Na_3NTA) should therefore be added to samples from domestic and other properties containing copper piping to reduce die-off (Versteegh et al., 1989;

Schets & Medema, 1993). Pre-enrichment with alkaline peptone water before subculturing to selective media has proved successful for recovery of *Aeromonas* from water (e.g. well water) in which the number of organisms is low (Moyer et al., 1992).

Several different media have been used for the recovery of *Aeromonas* from environmental waters; m-aeromonas agar (Rippey & Cabelli, 1979), ADA, starch–ampicillin agar (Palumbo et al., 1985), pril–xylose–ampicillin agar (Rogol et al., 1979) and SGAP-10C agar (Huguet & Ribas, 1991) are the most widely used (Moyer, 1996). All these media contain ampicillin and some have been shown to result in under-recovery of certain species such as *A. sobria*, *A. veronii* and *A. schubertii* (Gavriel & Lamb, 1995).

Primary identification of isolates as members of the genus *Aeromonas* is relatively simple. Many laboratories should be able to assign the mesophiles to one of the classical complexes (*A. hydrophila*, *A. caviae*, and *A. sobria*), but identification to phenospecies or genospecies level through biochemical testing can be problematic because of taxonomic complexities within the genus (Millership, 1996).

As yet, there are few published immunological or molecular methods for detecting mesophilic *Aeromonas* spp. in water compared with the number currently available for *Escherichia coli* and other members of the Enterobacteriaceae. A polymerase chain reaction (PCR) procedure based on 16S rRNA (Khan & Cerniglia, 1997) has been successfully used for the detection of *A. caviae* and *A. trota* in seafood and water samples, and PCR amplification of 16S rDNA sequences has been used to identify environmental isolates of *Aeromonas* (Dorsch et al., 1994).

Control

Environmental occurrence

Aeromonads are ubiquitous in aquatic environments and readily isolated from both nutrient-rich and nutrient-poor environments (Holmes, Niccolls & Sartory, 1996). Typical numbers of *Aeromonas* in a range of aquatic environments are given in Table 3. As *Aeromonas* are autochthonous to fresh and marine waters their recovery is to be expected. However, increasing levels of pollution may result in substantially greater populations, and may also affect distribution of the organisms (Holmes, Niccolls & Sartory, 1996). Several studies have shown that *A. caviae* tends to predominate in waters with a high degree of organic loading (Araujo, Arribas & Pares, 1991; Stecchini & Domenis, 1994); *A. caviae* and *A. hydrophila* are almost equally distributed in less polluted waters, and *A. sobria* becomes more frequent in unpolluted and brackish waters (Holmes, Niccolls & Sartory, 1996). Aeromonad densities have also been related to trophic status, and populations in some waters have a seasonal variation, with highest numbers occurring in the warmer months (Rippey & Cabelli, 1989). Relationships between aeromonad densities and parameters relating to trophic status or sewage

Table 3. Typical numbers of* Aeromonas *species in aquatic environments[a]

Environment	Typical counts (cfu/ml)
Domestic sewage sludge	$>10^8$
Crude sewage	10^6–10^8
Treated sewage	10^3–10^5
Wastewater	10^2–10^7
Rivers receiving sewage discharges	10–10^4
Clean rivers, lakes, storage reservoirs	1–10^2
Seawater	10^{-2}–10^2
Drinking-water, post-treatment	10^{-2}–10
Drinking-water, in distribution system	10^{-2}–10^3
Groundwaters	<1

[a] Reproduced, with permission, from Holmes, Niccolls & Sartory (1996).

contamination will vary according to the site, season and region (Rhodes & Kator, 1994).

Effects of drinking-water treatment

As aeromonads can occur in large numbers in some water sources (particularly lowland rivers and reservoirs), there is potential for them to enter distribution systems if water treatment is ineffective (Holmes, Niccolls & Sartory, 1996). A survey of a treatment works in Belgium demonstrated the following cumulative reduction of aeromonads at different stages of the treatment process under summer and winter conditions (Meheus & Peeters, 1989):

following flocculation/sedimentation	30–60%
following rapid sand filtration	70–90%
following granular activated carbon	80–90%
following hyperchlorination/direct filtration	99–100%

In the same study reductions following slow sand filtration were 98–100%. Neither the mode of cleaning nor the age of the filters appeared to influence the elimination of *Aeromonas*.

Studies of five water-treatment plants in Belgium (Huys et al., 1995; Kersters et al., 1995) reported a mean reduction of 99.7% in aeromonad numbers following flocculation–decantation and chlorination. Slow sand filtration reduced aeromonad numbers by 98.9%. Increased levels of *Aeromonas* were obtained from the effluents of activated carbon filters. Following slow sand filtration at a plant that treated surface water, there was a marked shift from a predominance of *A. hydrophila* and *A. sobria* to a predominance of *A. caviae*.

In a study on the impact of the type of material used for rapid gravity filters at a treatment works (Holmes, Niccolls & Sartory, 1996), coagulation and

clarification resulted in a mean reduction of 90% and aeromonads were undetectable after post-clarifier chlorination. There was a marked difference between sand-based rapid gravity filters and those employing granulated activated carbon (GAC). *Aeromonas* were recovered on only one occasion from the sand filters as chlorine levels were maintained through the beds. However, chlorine was rapidly removed by the activated carbon filters resulting in concentrations of less than 0.1 mg/l in the effluent. *Aeromonas* were recovered throughout the year, with greater numbers between July and September when water temperatures were highest.

Low numbers of *Aeromonas* have been reported in the final waters of 20 plants treating surface waters and groundwaters in the Netherlands (Havelaar, Versteegh & During, 1990). The maximum count of 470 cfu/100 ml was recorded from a plant treating deep aerobic groundwater. The high counts obtained at some works were often associated with filter beds with long operational periods (over 25 years) without replacement of filter material, or with filter units that were operated intermittently to meet variable water demand.

Clearly, water treatment can significantly reduce levels of *Aeromonas*, but these bacteria are capable of establishing significant populations in GAC-based treatment processes. Low numbers may be recovered from the final waters of water-treatment plants that meet water quality standards for the hygienic indicator organisms.

Changes in piped distribution systems

Aeromonads are readily isolated from municipal drinking-water systems, sometimes at quite high levels (Havelaar, Versteegh & During, 1990; Krovacek et al., 1992; Stelzer et al., 1992; Holmes, Niccolls & Sartory, 1996). Knøchel & Jeppesen (1990) examined drinking-water in Denmark and found that only 28% of samples were positive, with counts ranging from 1 to 40 cfu/100 ml; *A. hydrophila* made up 97% of isolates. In contrast, Ghanem, Mussa & Eraki (1993) reported that 90% of domestic water supplies in areas of Cairo contained *Aeromonas*, while from a survey of three distribution systems in Sweden, Krovacek et al. (1992) reported that 85% of samples were positive for presumptive *Aeromonas*, with a maximum count of 860 cfu/100 ml. *A. hydrophila* accounted for 67% of the strains isolated; the remainder were *A. sobria*. Stelzer et al. (1992) recorded a maximum count of 240 *Aeromonas*/100 ml in a drinking-water supply in Germany, with an isolation frequency for *A. hydrophila* of 37% and for *A. sobria* of 57%. The highest counts were obtained from points furthest (>10 km) from the treatment works. Havelaar, Versteegh & During (1990) reported regrowth of aeromonads in 16 of 20 distribution systems examined in the Netherlands. Geometric mean counts varied between 1 and 440 cfu/100 ml; a maximum count of 3300 cfu/100 ml was obtained from a system supplying water abstracted from a river source. Growth of *Aeromonas* generally occurred in the peripheral parts of distribution systems and was associated particularly with drinking-water

derived from anaerobic groundwaters containing methane. *Aeromonas* densities usually showed a seasonal pattern, with peak values occurring in late summer when water temperatures were highest. All three classical species were recovered during the survey, with either *A. hydrophila* or *A. caviae* tending to predominate. Although *A. sobria* predominated in one system, this species tended to be recovered in low numbers only. LeChevallier et al. (1982) reported that the aeromonads that occurred in 27% of samples taken over an 18-month period from a chlorinated supply in Oregon, USA, consisted solely of *A. sobria*.

Although species of mesophilic *Aeromonas* are commonly resident in drinking-water distribution systems, there are few data on the factors affecting their occurrence. However, it is generally reported that higher rates of isolation and larger populations occur during the warmer months and at the peripheries of distribution systems (LeChevallier et al., 1982; Havelaar, Versteegh & During, 1990; Stelzer et al., 1992, Holmes, Niccolls & Sartory, 1996). For chlorinated water, Burke et al. (1984a) reported that *Aeromonas* occurrence was positively correlated with water temperature and negatively correlated with residual chlorine levels. A seasonal variation in mean *Aeromonas* counts closely paralleled mean water temperature in samples that were either unchlorinated or had free chlorine values consistently below 0.3 mg/l. Isolation of *Aeromonas* spp. from drinking-water lacking chlorine was generally associated with water temperatures greater than 14.5 °C.

A study of a large supply system in central England resulted in a model of best fit relating the probability of occurrence of *Aeromonas* to temperature, free chlorine, and age of water (Holmes, Niccolls & Sartory, 1996). The probability of occurrence of *Aeromonas* increased significantly when the mean seasonal temperature exceeded 14 °C and this was exacerbated where the mean free chlorine concentration fell below 0.1 mg/l. The impact of water age was significant only when the mean free chlorine level was less than 0.1 mg/l. There was no relationship between *Aeromonas* incidence and coliforms or heterotrophic plate counts. Strains of *A. hydrophila*, *A. caviae*, and *A. sobria* isolated from drinking-water can grow at 4 °C (Holmes, Niccolls & Sartory, 1996) and are thus capable of growth throughout the year in many geographical areas.

It is apparent that water temperature and free chlorine are factors that significantly influence the growth of *Aeromonas* in drinking-water supplies. Mesophilic aeromonads are nutritionally versatile. Studies using a drinking-water isolate of *A. hydrophila* demonstrated its ability to utilize a variety of organic compounds, including carbohydrates, amino acids, carboxylic acids, and long-chain fatty acids, at low concentrations (10 µg/l) (van der Kooij & Hijnen, 1988; van der Kooij, 1991); mixtures of compounds at individual concentrations of 0.1 or 1 µg/l (expressed as carbon) enhanced growth. These results demonstrate that aeromonads are capable of growth in the presence of the low concentrations of nutrients that would be available from biofilms and sediments within distribution systems. Thus the organic carbon content (assimilable organic carbon, AOC,

or biodegradable organic carbon, BDOC) of the water would also be expected to be a determining factor in the occurrence of these organisms, but this has yet to be studied within water distribution systems. In a study on biofilm formation characteristics, various types of drinking-water demonstrated large differences in biofilm formation rates (van der Kooij & Veenendaal, 1993). A highly significant correlation between biofilm formation rate of groundwater-derived drinking-water and *Aeromonas* density in drinking-water during distribution has also been demonstrated (van der Kooij et al., 1995).

Typically, *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. Knøchel (1991) found that strains of *A. hydrophila*, *A. sobria*, *A. caviae*, and *A. veronii* were generally more susceptible to chlorine and monochloramine than coliforms and pseudomonads, and Medema et al. (1991) 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 & Finch, 1987). This is probably due to association of the organisms with biofilms. Mackerness, Colbourne & Keevil (1991) found that *A. hydrophila* became readily established within a mixed heterotrophic bacterial biofilm and was unaffected by addition of 0.3 mg/l monochloramine. 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. A key mechanism for the control of aeromonads in drinking-water is therefore the removal of biodegradable compounds (i.e. improving the biostability of the water). Where reasonably practical, limiting the concentrations of biodegradable compounds (e.g. treatment with granular activated carbon treatment or, for anaerobic groundwaters, by aeration) may be the preferred option. Such measures would also help to control the regrowth of heterotrophic bacteria and the proliferation of invertebrates within the distribution system.

Conclusions and recommendations

Health risk assessment

Although aeromonads are frequently isolated from drinking-water systems, and some strains may exhibit enterotoxigenic properties, further epidemiological studies are required to ascertain any significance in relationships between cases of *Aeromonas*-associated diarrhoea and presence of these organisms in drinking-water. Current evidence indicates that the predominant aeromonads typically

found in drinking-water do not belong to the same DNA homology groups as those isolated from cases of gastroenteritis. It also appears that, if species of *Aeromonas* are primary enteropathogens, high numbers are required to initiate disease. As numbers in drinking-water are generally low compared with those found in foods (10^3–10^5 cfu/g), treated drinking-water probably represents a very low risk. The virulence of enterotoxigenic *Aeromonas* for risk groups (newborn infants and immunocompromised individuals), however, remains to be ascertained. To date there is no firm evidence that direct transmission occurs via drinking-water, but in the absence of more definitive proof of their public health significance it would be advisable to control excessive numbers of aeromonads in drinking-water supplies.

Risk management strategies

The mesophilic aeromonads are a ubiquitous component of the natural bacterial flora of aquatic environments. When temperature and nutrient conditions allow, they can rapidly proliferate in unchlorinated drinking-water supply systems and where chlorine residuals tend to be low (e.g. in the extreme parts of extensive distribution systems). The key factors in controlling *Aeromonas* proliferation are temperatures below 14 °C (although the organisms are capable of growth at 4 °C), free chlorine residuals above 0.1–0.2 mg/l, and the limitation of organic carbon compounds that would serve as nutrients. Control of the development of biofilms within water supply systems will reduce, but not prevent, the proliferation of *Aeromonas*. As *Aeromonas* are associated with biofilm development, significant increases in numbers in a drinking-water supply are indicative of a general deterioration of bacteriological quality. The increasing use of granulated activated carbon in water treatment may allow proliferation and dissemination of *Aeromonas*. Limiting the numbers of aeromonads released into distribution systems thus requires effective management of filter beds and maintenance of adequate final chlorination. Control of aeromonad numbers in piped distribution systems is achieved primarily by limiting regrowth possibilities; this will also limit the numbers of heterotrophic bacteria and improve the efficacy of chemical disinfection in the distribution system.

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