GLOBAL WATER PATHOGEN PROJECT

PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND EPIDEMIOLOGY ASPECTS

AEROMONAS

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Summary

The genus *Aeromonas*, consists of Gram-negative, oxidase positive bacilli that are considered autochthonous of aquatic environments and are commonly isolated from clinical and environmental samples. Typical habitats for these bacteria are freshwater (ground water, lakes, rivers and reservoirs), chlorinated and un-treated drinking water, bottled water, swimming pools, wastewater, reclaimed waters, brackish waters, and seawater. *Aeromonas* spp. can produce several diseases in wild and farmed freshwater and marine fish species impacting the economy of the aquaculture sector. Most common human clinical presentations of *Aeromonas* infections are diarrhea, wound and soft-tissue infections and bacteremia. Many infections are related to water exposure (traumatic accidents, near-drowning, natural disasters etc.), leech therapy (due to their symbiotic relationship with these bacteria) or consumption of contaminated water or food. Drinking water strains have recently been epidemiologically related to isolates from cases of human diarrhea. When investigated, *Aeromonas* is found at 25% of public ground drinking water systems in the USA, with concentrations ranging between 0.2 to 880 (mean 34.4) CFU/100 ml. However, due to growth in sewage, aeromonads occur in 100% of raw sewage samples (reaching 10^6 – 10^8 CFU/ml), and traditional biological treatment only reduce these loads by 1 to 2 logs. However, disinfection (with chlorination or ultraviolet radiation) is effective in removing *Aeromonas* to below routine detection limits, but regrowth occurs post treatment, such as when water is used for agricultural irrigation. Furthermore, irrigation water can affect food quality. Risk factors favoring *Aeromonas* abundance in water include: retention time or stagnant piped water, high turbidity and presence of organic matter, the presence of biofilms and low levels of disinfectant residual (chlorine, etc.). Recently genotypic identification has revealed that one of the most common species isolated, *A. hydrophila* is neither the prevailing species nor the principal pathogen. Rather, prevailing species in clinical cases and in contaminated water are *A. caviae, A. veronii* and *A. dhakensis*. Chironomid egg masses, and cyanobacterial blooms in surface water are recently identified habitats and reservoirs for *Aeromonas* spp. of potential public health concern.

1.0 Epidemiology of the Disease and Pathogen(s)

Gastroenteritis, septicemia and wound infections are the predominant presentation of *Aeromonas* in humans, though infections may also affect the hepatobiliary system, the respiratory tract, bone and/or joints etc. (Janda and Abbott, 1996; Janda and Abbott 1998; Figueras, 2005; von Graevenitz, 2007; Janda and Abbott, 2010; Parker and Shaw, 2011; Figueras and Beaz-Hidalgo, 2015). *Aeromonas* species, have been linked to major fish die-off events around the world, resulting in important economic losses to the aquiculture sector (Austin et al., 1998; Beaz-Hidalgo et al., 2012; Beaz-Hidalgo and Figueras, 2013; Hossain et al., 2013; Rasmussen-Ivey, 2016b). The implication of *Aeromonas* in human and animal infection comes from the fact that they possess or produce many virulence factors that can mediate the adhesion and invasion of host tissues, including structural components (flagella, lipopolysaccharide etc.), extracellular enzymes (hemolysins, lipases, etc.), secretion systems and associated toxins (Type I to Type VI secretion systems), iron acquisition systems and quorum sensing (QS) communication that is further explored in many studies cited in this chapter (Soler et al., 2002; Khajanchi et al., 2010; Pablos et al., 2010; Berg et al., 2011; Parker and Shaw, 2011; Senderovich et al., 2012; Beaz-Hidalgo and Figueras, 2013; Morinaga et al., 2013; Casabianca et al., 2015; Rasmussen-Ivey et al., 2016a).

1.1 Global Burden of Disease (World Health Organization)

In patients with diarrhea *Campylobacter* and *Salmonella* are the dominating bacteria with *Aeromonas* ranking third (Figueras and Beaz-Hidalgo, 2015). However, when *Campylobacter* was not investigated, like in a study in Nigeria, *Aeromonas* prevailed over *Salmonella* (Nzeako and Okafor, 2002). a study in Dhaka, Bangladesh performed between 2005–2008, the prevailing enteric bacteria were Vibrio spp. (42.9%, *Shigella* spp. (20.3%), *Aeromonas* spp. (12.8%) and *Salmonella* spp. (6.4%) (Ahmed et al., 2012). However, in Iran, *Aeromonas* was only second to *Shigella* (Soltan-Dallal and Moezardalan, 2004), while in Egypt, in a study of diarrhea cases in children (< 2 years), *Aeromonas* showed a similar isolation rate as *Shigella* and a higher rate than *Salmonella* (Mansour et al., 2012). Yet, isolation does not necessarily mean it was the primary cause of disease. Nonetheless, a higher isolation rate for *Aeromonas* than for *Salmonella* was also reported in Cuba (Bravo et al., 2012). Furthermore, in many studies *Aeromonas* has been found to be more prevalent than the enteropathogenic *E. coli* (Essers et al., 2000; Nzeako and Okafor, 2002; Soltan-Dallal and Moezardalan, 2004; Bravo et al., 2012). *Aeromonas* was the leading pathogen in a prospective matched case control study performed in Pakistan and Bangladesh that investigated diarrhea disease in children. Diarrhea mortality attributable to child infections with *Aeromonas* in 2013 was estimated to be 5.5 (per thousand), significantly lower than in 1990 when it was estimated at 12.3 (Kotloff et al., 2013). The frequency of *Aeromonas* diarrhea ranges from 10.6 to 1.62 infections per million people, but the disease impact on a global basis is unknown. Cases are sporadic rather than associated with large outbreaks and data are therefore derived from a limited number of studies, often retrospective studies as summarized in different reviews (Janda and Abbott, 2010; Figueras and Beaz-Hidalgo, 2015). *Aeromonas* cases are sporadic and reporting is not mandatory, making it hard to track disease, as indicated by Janda and Abbott (2010). The latter authors indicated that it was a reported microbe in California in 1988, and during a 12-month period they recorded 219 case of *Aeromonas* infections, yielding an incidence of 10.6 cases per million. A study conducted almost 10 years later in France involved 70 hospitals recoding data on *Aeromonas* over a 6-month period (Lamy et al., 2009). They reported 99 infections and a prevalence of 1.62 infections per million population. This value is much lower than that reported in the California study. However, in a study

performed in Valencia (Spain) the average annual incidence was reported to be 20 cases per million inhabitants (Esteve et al., 2015). Overall estimates of regional prevalence are summarized in Table 1.

In Asia *Aeromonas* seems to be more frequent, for instance in a 3-year study (2008 to 2010) performed by Wu et al. (2014) in South Taiwan they found 76 cases of *Aeromonas* bacteremia per million population, while in studies performed in the UK and the United States indicated an incidence of 1.5 cases per million population (Janda and Abbot, 2010; Batra et al., 2016). We have to consider that normally hospitals do not use a specific culture media for the recovery of Aeromonas, being generally discovered by chance from media dedicated to recover other bacterial pathogens.

Table 1. Incidence of *Aeromonas* Associated with Diarrhea Disease

Geographical Area of Study	Patient Age	Year of Data Collection Species Studied if Noted	Incidence in Relation to Feces Studied or Feces with Pathogens	Range[a] and/or Prevalence in Asymptomatic	Seasonality/ Other Comments	Reference
Africa						
Egypt (rural community)	Children <2y	2004 to 2007	1.4% (56/4001)	0.5% non-diarrhea cases (52/9539)	0.07 episodes/child/year. Seasonal peak. Significantly more common in cases of diarrhea	Cited by Ghenghesh et al., 2015
Egypt	Children	NR[b]	8.3% (29/350)	0% (0/50)	NR	Cited by Ghenghesh et al., 2015
Libya	Children	14.6%	4.6% (19/408)	17.8% ND (28/157)	NR	Adapted from Ghenghesh et al., 2015
Nigeria	NR	1990	2.6% (53/2350)	0.4% non-diarrheic (2/500)	NR	Cited by Joseph, 1996
South Africa	All ages	2003 to 2005	20.8% (56/269)	6.2% P<0.001) non-diarrheic (16/259)	NR	Samie et al., 2009
Tanzania	Children <5y	2012 to 2013	9.7%	ND	NR	Deogratias et al., 2014
Americas						
Brazil	All age groups	2010	7.2 % (29/400)	2.6 to 19.5%[a] (in other cited studies)	14.6% among patients of low socioeconomic status	Assis et al., 2014 and references therein
Brazil	All age groups	2010 to 2011	2.7% (5/182)	ND	NR	Prediger et al., 2012
Cuba	Children	1985 to 2005	7.1% (166/2322)	1.8% non-diarrheic (35/2072)	NR	Bravo et al., 2012
Peru	All ages	NR	52.4% (205/391) 12% (90/721)	8.7% non-diarrheic P<0.001) (12/138) 11 to 52.4%[a] (in other cited studies)	NR	Adapted from Joseph 1996
USA	All age groups	1965,1985, 1987 to 1988	1.6% (34/2118) 6% (15/246) 4% (355/8795)	0.2% P<0.001) (2/933) 7% (11/155) 1 to 7%[a] ND	Mean values from several studies	Adapted from Joseph 1996
USA	< 2 years (outpatients)	1985	3.7% (7/188)	1.9% in asymptomatic (2/106)	NR	Cited by Altwegg and Geiss 1989

Geographical Area of Study	Patient Age	Year of Data Collection Species Studied if Noted	in Relation to Feces Studied or Feces with Pathogens	Range[a] and/or Prevalence in Asymptomatic	Seasonality/ Other Comments	Reference
USA (California)	All age groups	NR	NR	NR	10.6 cases / million population 1.4 / million persons aged 30 to 39 years) All infections wound infections 0.7 case / million)	Cited by Janda and Abbott 2010
USA	All age groups and infections	1998 to 1999	0.66% (17/2565)	ND	NR	Borchardt et al., 2003
USA	All age groups and infections	2004	NR	NR	1.5 cases / million population	Cited by Janda and Abbott 2010
Asia						
Bangladesh	All age groups	2005 to 2008	12.8% (1847/14428)	ND	NR	Ahmed et al., 2012
Bangladesh	Children	1997 to 1998	7.2% (125/1735)	3.3% non-diarrheic P<0.001 (27/830)	NR	Albert et al., 2000
China	NR	NR	4.6% (73/1577)	ND	NR	Zhang et al., 2015
India and Bangladesh	All age groups	1986 to 1988 1990 to 1991	11% (1987/18010)	5-33%[a]	NR	Adapted from Joseph, 1996
Indonesia	NR	1987	11% (186/1695)	4% non-diarrheic (14/338)	NR	Cited by Joseph, 1996
Iraq	Children	NR	8.3% (29/350)	ND	NR	Cited by Ghenghesh et al., 2015
Israel	All age groups	*A. caviae* (65%), *A. veronii* (29%), *A. taiwanensis* (6%)	1.6% (17/1033)	ND	NR	Senderovich et al., 2012
Israel	NR	1990 to 1992	1.7% (17/1005)	0% non-diarrheic P<0.001 (0/500)	NR	Cited by Joseph, 1996
			1.1% (146/13820)	ND		
Japan	Traveler's diarrhea	1986 to 1995	5.6% (1191/21257)	3.8% non-diarrheic (74/1958)	NR	Yamada et al., 1997
Japan	NR	1988	11.1% (29/262)	2.2% non-diarrheic P<0.001 (202/9104)	NR	Cited by Joseph, 1996
Saudi Arabia	Children	1991	14% (7/50)	16% non-diarrheic (24/150)	NR	Cited by Ghenghesh et al., 2015

Geographical Area of Study	Patient Age	Year of Data Collection Species Studied if Noted	Incidence in Relation to Feces Studied or Feces with Pathogens	Range[a] and/or Prevalence in Asymptomatic	Seasonality/ Other Comments	Reference
Saudi Arabia	All age groups	1991	0.4% (58/15548)	0% non-diarrheic P<0.001 (0/1368)	NR	Cited by Joseph, 1996
Taiwan	Adults	2010 to 2011 (15m) *A. veronii* (52.6%), *A. caviae* (36.8%), *A. dhakensis* (5.3%), *A. saranellii* (5.3%)	2.5% (13/514)	3.6% (6/167)	Diarrhea increased with age (P=0.07). Seasonal peak	Chen et al., 2015
Taiwan	Children	1994 to 1998	2.5% (54/2150)	NR	NR	Cited by Chen et al. 2015
Thailand	All age groups	1982	18% (37/207)	12% non-diarrheic P<0.001 (44/367)	NR	Cited by Joseph, 1996
Europe						
Finland	All age groups	1995	1.9% (249/13027)	0% non-diarrheic P<0.001 (0/343)	NR	Cited by Joseph, 1996
France	All age groups and infections	2006	19% (15/78)	NR	Nationwide study in 70 hospitals. Based upon an estimated 2006 population of 61 million, this represents a prevalence of 1.62 infections per million population	Lamy et al., 2009 and cited by Janda and Abbott 2010
France	All age groups	1965	0.67% (30/4426)	ND	NR	Cited by Joseph, 1996
Italy		1986	4% (21/561)	2% non-diarrheic (12/576)	NR	Cited by Joseph, 1996
Netherlands	All age groups	1989	0.61% (208/34311)	ND	NR	Cited by Joseph, 1996
Spain	Adults traveler diarrhea	1999 to 2001 *A. veronii* (50%), *A. caviae* (38.9%), *A. jandaei* (5.5%), *A. hydrophila* (5.5%)	2% (18/863)	NR	NR	Vila et al., 2003
Spain	All age groups	*A. caviae, A. veronii, A. hydrophila*	4% (32/800)	NR	NR	Pablos et al., 2010
Spain	All age groups	2004 to 2005	4 cases within the same families	NR	20 cases /million inhabitants 31 cases / 1,554,021 inhabitants of Valencia	Esteve et al., 2015
Sweden	Adults	1996 to 1997	2% (15/838)	0% in controls	3% travelled abroad previous 2 weeks (12/423)	Svenungsson et al., 2000

Geographical Area of Study	Patient Age	Year of Data Collection Species Studied if Noted	Incidence in Relation to Feces Studied or Feces with Pathogens	Range[a] and/or Prevalence in Asymptomatic	Seasonality/ Other Comments	Reference
Switzerland	Children	1990 to 1994	4.8% (15/312)	NR	NR	Essers et al., 2000
United Kingdom	All age groups	1983	9% (51/568)	8.5 to 11%[a] (33/1248) 2.61 to 3.3% non-diarrheic	NR	Cited by Joseph, 1996
United Kingdom	All age groups and infections	1990 to 2004	NR	47 to116 cases found annually	1.5 cases / million considering an estimated population of 53 million	Cited by Janda and Abbott 2010
United Kingdom	All age groups and infections	1993 to 1996	5.7% (164/2893)	4.2% (96/2264)	Mostly only after enrichment	Tompkins et al., 1999
Oceania						
Australia	All ages	1980 to 1981	12.3% (142/1156)	1.9% (22/1156)	NR	Cited by Altwegg and Geiss 1989

[a]Range; [b]NR: Not Reported; [c]ND:Not Done; Altwegg and Geiss (1989) and Joseph (1996) provide summary tables from several other studies.

1.1.2 Global distribution

Aeromonas cases have been described from all over the world, although the incidence of gastroenteritis appears higher in undeveloped or developing regions. In relation to bacteremia or septicemia the incidence is higher in Asian countries, probably due to a higher incidence of cirrhosis, which is an important underlying condition link to *Aeromonas bacteremia* (Figueras and Beaz-Hidalgo, 2015; Batra et al., 2016).

1.1.3 Symptomatology (morbidity and case-fatality ratios)

The predominant presentation of *Aeromonas* is diarrhea, followed by wound infection and bacteremia (Janda and Abbott, 2010; Figueras and Beaz-Hidalgo, 2015). The gastrointestinal disease is normally self-limited and does not require antibiotic treatment except in patients with underlying disease (hepatobiliary disease, malignancy etc.), re-hydration is essential in the case of children. Bacteremia affects predominantly (ca. 80%) patients with undelaying disease and mortality can range from 27 to 70% of the cases, while wound infections occur in healthy individuals normally after trauma (or burns) in contact with water and soil or leech therapy (Figueras and Beaz-Hidalgo, 2015). Delayed proper antibiotic treatment of wound infections (*Aeromonas* are generally resistant to ampicillin) may evolve into necrotizing fasciitis which can result in the need for limb amputation or else is a life-threatening presentation (11% mortality).

The role of *Aeromonas* in gastroenteritis has been questioned, but many arguments support its causative association with diarrheal disease (Figueras et al., 2007; Figueras and Beaz-Hidalgo, 2015; Teunis and Figueras, 2016). Few cases of Hemolytic Uremic Syndrome (HUS) have been attributed to *Aeromonas* spp., yet strains carried genes homologous to those of the Shigatoxins of *E. coli* O157:H7 responsible of HUS (Figueras et al., 2007; Alperi and Figueras, 2010; Palma-Martínez et al., 2016).

A re-evaluation and update of the Global Burden Disease Study of 2013, published in 2015, reported *Shigella* and *Aeromonas* distribution among patients with diarrhea and indicated that this had a significant ecological association with sanitation (GBD, 2015). Deaths from these pathogens including also non-typhoid *Salmonella* fell by 5.4% (28,062 deaths) from 1990 to 2013. Cases of death attributed to *Aeromonas* alone in 1990 and 2013 were 12,300 and 5,500 respectively in children younger than 5 years and 28,000 and 13,000 considering all ages, representing a rate of change for death (1990-2013) of -53.6%. The data considering all ages was very similar to that reported for *Campylobacter* enteritis 28,400 in 1990 and 14,100 in 2013 (GBD, 2015).

1.2 Taxonomic Classification of the Agent(s)

The genus *Aeromonas* described by Stainer in 1943 is classified within the family Aeromonadaceae (Martin-Carnahan and Joseph, 2005). By 2008 there were 20 described species in the genus, which by the time of publication had expanded to 32 species (Beaz-Hidalgo et al., 2015a; Marti and Balcazar, 2015; Martínez-Murcia et al., 2016; Hoel et al., 2017). Moreover, of four additional new species that remain to be described, two were recovered from human feces, one from shellfish, and one from water associated with a cyanobacterial bloom (Beaz-Hidalgo et al., 2015a). The rapid expansion of the genus responds to the introduction of molecular identification methods like the 16S rRNA-RFLP designed to obtain species specific patterns for all the species described up to 2000, so a different pattern could represent a potential new species (Borrell et al., 1997; Figueras et al., 2000; Beaz-Hidalgo et al., 2009; Alperi et al., 2010; Beaz-Hidalgo et al., 2015a). However, the addition of 11 new species from 2010 up to 2015 was accredited to routine sequencing of housekeeping genes like rpoD or gyrB etc. (Beaz-Hidalgo et al., 2015a; Beaz-Hidalgo et al., 2015b). This approach avoids the misidentification produced by conventional or automatic phenotypic identification methods (Soler et al., 2003; Figueras et al., 2009; Beaz-Hidalgo et al., 2010; Aravena-Román et al., 2011; Senderovich et al., 2012; Morinaga et al., 2013). Errors can also be generated using almost complete and especially short partial sequences (ca. 300 bp) of the 16S rRNA gene (Alperi et al., 2008; Beaz-Hidalgo et al., 2010). The sequences of the 16S rRNA gene can be used to assign the strains to the genus but it may lead to speciation errors due to the high similarity of the sequences (>99%) among certain species of the genus (Alperi et al., 2008; Figueras et al., 2011).

Aeromonas are considered autochthonous of the aquatic environments, and in agreement with that, water was the origin in the description of 11 of the 32 (34.4%) species, with seven from fish and two from shellfish (Table 1). The second most frequent origin, with nine species (28.1%) is human.

1.2.1 Physical description of the agent

Aeromonas are Gram-negative, glucose-fermenting rod-shaped bacteria that are oxidase and catalase positive, that do not produce acid from Inositol, are able to grow at 0% NaCl but not at 6% NaCl, and the majority of the species (only few exceptions described below) are resistant to the vibriostatic agent O/129 (2,4-diamino-6,7-di-isopropypteridine; 150 mg/disc).

1.2.2 New variants

The species *Aeromonas cavernicola* and *Aeromonas*

australiensis, recovered from the water of a brook in a cavern in the Czech Republic and from a treated effluent used for irrigation in Western Australia, respectively, show sensitivity to the vibriostatic agent O/129 (Aravena-Román et al., 2013; Martínez-Murcia et al., 2013; Figueras and Beaz-Hidalgo, 2015). Sensitivity to the vibriostatic agent O/129 has, however, been reported for two strains of *Aeromonas eucrenophila* and in one strain of *Aeromonas veronii* (Abbott et al., 2003; Figueras and Beaz-Hidalgo, 2015), and very recently for the new proposed species "*Aeromonas aquatilis*" isolated from lake water in Finland (Figueras et al., 2017). Additional atypical biochemical behavior has also been described for other species (Figueras and Beaz-Hidalgo, 2015).

1.2.3 Recent specific pathotypes

Recently it has become apparent that the classical importance attributed to *Aeromonas hydrophila*, is actually the result of a bias produced by culture and biochemical identification methods (Figueras et al., 2005; Soler et al., 2003; Figueras et al., 2009; Beaz-Hidalgo et al., 2010; Aravena-Román et al., 2011; Aravena-Román et al.,2013; Wu et al., 2014; Morinaga et al., 2013; Figueras and Beaz-Hidalgo, 2015; Beaz-Hidalgo et al., 2015b). It has now been demonstrated using molecular methods that *A. hydrophila* is not the most prevalent in water nor in clinical cases, though it can result in important mortalities in fish (Hossain et al., 2013; Hossain et al., 2014; Rasmussen-Ivey et al., 2016b). Furthermore, the discovery of the new clinical species *Aeromonas dhakensis* (previously known as *A. aquariorum* and *A. hydrophila* subsp. dhakensis), which was previously misidentified using biochemical methods as *A. caviae*, *A. hydrophila* or *A. veronii* (Martinez-Murcia et al., 2008; Figueras et al., 2009; Aravena-Román et al., 2011; Esteve et al., 2012; Putchucheary et al., 2013; Morinaga et al., 2013; Chen et al., 2014; Chen et al., 2016), has changed the panorama of the important prevalent clinical species.

Up to 2014, the species *A. caviae*, *A. veronii* and *A. hydrophila* were considered to be the most relevant clinical species representing ca. 92% of the strains isolated from clinical specimens (Janda and Abbott, 2010). However, a review performed in 2015 including 645 strains from nine studies, which identified isolates using reliable molecular methods demonstrated that 95.4% of the isolates were in order: *A. caviae*, 29.9%; *Aeromonas dhakensis*, 25.5% (previously *Aeromonas aquariorum*); *A. veronii*, 22%; and *A. hydrophila* 18% (Figueras and Beaz-Hidalgo, 2015). The species *A. dhakensis* was most frequent in Malaysia than the typical dominating clinical species *A. caviae* and *A. veronii* or showed an almost equal frequency than the latter two in Australia and Taiwan (Figueras and Beaz-Hidalgo, 2015; Chen et al., 2016). Furthermore *A. dhakensis* is considered to be more virulent than *A. hydrophila* and shows resistance to cefotaxime, while the rest of the species were sensitive, so confusion between the species may have important clinical consequences (Esteve et al., 2012; Wu et al., 2013; Chen et al., 2014, Chen et al., 2016).

Another important aspect is that *Aeromonas* show the capacity to integrate many genetic mobile elements (Shigatoxin genes, antibiotic resistance genes, Type 3 Secretion System etc.) so strains with these characteristics may be much more virulent (Alperi and Figueras, 2010; Hossain et al., 2013; Adler et al. 2014).

Importantly, no surrogates or indicators have been identified that predict the presence of *Aeromonas*, despite some authors reporting high correlations with indicators of fecal pollution and heterotrophic bacteria (Holmes et al., 1996 and references therein). The correlation with fecal indicators only occurs when there was a strong influence of nutrient enrichment (say via sewage pollution) that results in aeromonads growing (Table 2). Hence, when the source of pollution is diffuse the correlation with the fecal indicators tends to fail (Araujo et al., 1990; Holmes et al., 1996). Classically, *Aeromonas* may be recovered from municipal drinking water systems that meet quality standards based on fecal indicators organisms (Pablos et al., 2009). Also, despite *Aeromonas* spp. being a fraction of the heterotrophic or total bacterial plate count (HPC) no significant correlation either at 22 or 37°C have been reported (Gavriel et al., 1998; Holmes et al., 1996; Pablos et al., 2009). The association sometimes described with the HPC is probably because both groups grow at low levels of chlorine residuals and in the presence of organic matter in drinking water.

Table 2. Summary of Studies Reporting Detection of *Aeromonas spp.* in Surface Waters and Reclaimed Waters

Area (Year of Study)	Sample Type	Percent Positives (# of Samples)	Concentration CFU or MPN/100ml	Method (Sample Volume)	Reference
Nigeria (2011)	Lakes	36% (54/150)	Presence/ Absence	Culture enrichment, phenotypic identification (2.5 ml)	Bello et al., 2016

Area (Year of Study)	Sample Type	Percent Positives (# of Samples)	Concentration CFU or MPN/100ml	Method (Sample Volume)	Reference
Poland	River Water	100%	1.8 E+03 to 5.5 E+05	Membrane filtration (100 ml)	Niewolak, and Opieka, 2000
	River Sediments		7.9 E+03 to 1.6 E+05/g	(1g)	
	Seawater	83.1% (69/83)	1E+04		
Spain (1995)	River water	88% (22/25)	E+04 to E+06	Membrane filtration, culture and phenotypic identification (100 ml)	Borrell et al., 1998
	Lakes	95.5% (105/110)	E+03 to E+05		
Spain[a] (2001)	Seawater impacted by sewage	26.5% (30/113 samples)	Presence/absence	Membrane filtration, culture and molecular identification species-specific 16S rDNA-RFLP (100 ml)	Soler et al., 2002
Spain[b] (2001)	River water and reservoirs some impacted by sewage	63.7% 72/113 samples	Presence/absence	Membrane filtration, culture and molecular identification species-specific 16S rDNA-RFLP (100 ml)	Soler et al., 2002
Spain[c] (2013, 6 month)	Reclaimed water (inlet and outlet lagooning water)	100% (12/12 samples)	Mean 1.94E+05	MPN five tubes (2.5 ml)	Fernandez-Cassi et al., 2016
Spain[d] (2013)	Reclaimed irrigation water	63.6% (7/11)	7.0E+02 to 2.45E+04	MPN five tubes, verification by culture (direct plating and after enrichment) and molecular identification of the colonies (phylogeny with rpoD sequences) (10 ml)	Latif-Eugenín et al., 2016a
USA (1993)	River water	100%	Summer range 1.8E+03 to 4.0E+05	Membrane filtration, culture and phenotypic identification (NS)	Pettibone, 1998
USA[e] (2010, 6 month)	Lake water	Prevalence range from 1.1% to 29.0% (mean = 16.0%, SD = 10.2%).	Range 475 to 3.7 E+04 (mean = 3.4 E+03 SD = 2.7 E+04)	Enumeration by membrane filtration, culture (EPA method) 1065), genetic identification (8 µl to 25 ml)	Skwor et al., 2014

[a]A. caviae (47.7%), A. bestiarum (13.6%), A. salmonicida (11.4%), A. media (9.1%), A. popoffii (6.8%), A. hydrophila (4.5%), A. veronii (2.3%), A. jandaei (2.3%), A. schubertii (2.3%); [b]A. veronii (43%), A. hydrophila (14%), A. caviae (13%), A. bestiarum (8%), A. popoffii (8%), A. media (7%), A. salmonicida (3%), A. jandaei (3%), A. sobria (1%); [c]Inlet water corresponded to a secondary treated wastewater; [d]A. caviae (71.4%) and A. media (20%) dominated in the secondary treated wastewater, A. salmonicida (22.5%),18.5% each (A. media, A. allosaccharophila), and A. popoffii (14.8%) represented 74.0% of the strains in the irrigation water; [e]A. veronii (80%), A. hydrophila (2%)

1.3 Transmission

1.3.1 Routes of transmission

Water and contaminated food are considered the main sources of *Aeromonas* transmission (Janda and Abbott, 2010; Figueras and Beaz-Hidalgo, 2015). In the case of water, both dermal exposure and ingestion are relevant, because water contact can also result in wound or burn infections.

So far, few food or waterborne outbreaks have been described involving *Aeromonas* (Altwegg et al., 1991; Krovacek et al., 1995; Granum et al., 1998; Vally et al. 2004; Ramalivhana et al. 2010; Zhang et al. 2012; Ventura et al., 2015). However, the most important is that the an epidemiology link between the source of infection and clinical isolates have been demonstrated in several studies (Teunis and Figueras, 2016). For instance, the same *Aeromonas* strain (genotype) that caused diarrhea was isolated from drinking water (Khajanchi et al., 2010; Pablos et al., 2010), from a shrimp cocktail (Altwegg et al., 1991) and from the household environment (Demarta et al., 2000 and references therein). The genetic relatedness of *Aeromonas* isolates obtained from HIV/AIDS patients suffering from gastroenteritis and those recovered from their household drinking-water was demonstrated also in a study performed in South Africa by Ramalivhana et al. (2010).

An *Aeromonas* wound infection outbreak associated with a muddy football game occurred in Australia and affected 26 people that received game-related scratches and abrasions that became infected when exposed to the mud irrigated with river water (Vally et al., 2004). *Aeromonas* plays an important role in infections produced in survivals from natural disasters, for instance it was the most isolated microbe (22.6%, 145 isolates) of all isolated bacteria (n=641 recovered from the infected wounds of the southern Thailand tsunami survivors (Hiransuthikul et al., 2005) and something similar occurred in 2005, after hurricane Katrina in New Orleans (Presley et al., 2006). In all these cases, exposure to water was again the source of infection, in fact the floodwater during Katrina showed concentrations of *Aeromonas* around 10^7 CFU·ml^{-1} (Presley et al., 2006).

In Tainan (Taiwan) Chen et al. (2014) investigated 80 patients with wound infections produced by *Aeromonas* and found *A. dhakensis* to be the prevailing species (43.6%), and also that cases with this species were more associated with water environments that cases produced by *A. hydrophila* (32.4% vs. 0%, $P > 0.05$).

In 96% of *Aeromonas* infections identified in burn patients, contact with ambient untreated water to extinguish flames was recognized as the main risk factor associated with the infection (Azzopardi et al. 2011).

1.3.2 Reservoirs: human, animal, and environmental reservoirs

The high concentration of *Aeromonas* in wastewater, and their frequent isolation from chironomid egg masses, which may infest drinking water systems indicate reservoirs for this genus (Figueras et al., 2011; Beaz-Hidalgo et al. 2012; Laviad and Halpern, 2016), in addition to wet soils and fish. The latter may be relevant because 80% of aquiculture-grown trout are carriers of *Aeromonas*, excreting the bacteria at a rate of between 10^5 and 10^6 CFU/hour/fish (Beaz-Hidalgo and Figueras, 2013). These carriers increase the likelihood of transmission of the microbe to other susceptible fish or to humans. Despite the common view that certain *Aeromonas* spp. cause diseases in fish (i.e., *A. salmonicida*) but do not infect humans, cases of *Aeromonas* diarrhoea and bacteraemia have been linked to having eaten raw fish or shellfish on the preceding days to diarrhea. Furthermore *A. salmonicida* have been isolated from human samples and recently linked to an episode of peritonitis in a 68-year-old diabetic woman, who had to be treated by continuous ambulatory peritoneal dialysis after she ate fish (Yang et al. 2008; Aravena-Román et al., 2011).

1.3.3 Incubation period

The incubation period for *Aeromonas*-associated infections is 1-2 days as also described in cases of traveler's diarrhea (Carnahan and Joseph, 1991; Vila et al, 2003; Gascón, 2006). For example, Carnahan et al. (1991) reported a case of the accidental ingestion of approximately 10^9 cells of the type strain of *Aeromonas trota* (ATCC 49657T) by a 28-year-old laboratory worker, with no preexisting health problems, the sudden onset of a "rice-water" diarrhea occurred within 24h, and lasted for over a period of 2 days.

1.3.4 Period of communicability

Limited data on *Aeromonas* shedding levels were found. The amount of *Aeromonas* recovered from persons with diarrhea ranged from 10^3 to 10^{10} CFU/gram (g) while in those patients that did not show diarrhea concentrations ranged from 10^5 to 10^6 CFU/g (George et al., 1985).

1.3.5 Population susceptibility

Aeromonas-associated infections (diarrhea, wound infections, bacteremia) occur worldwide. Wound infections occur in previously healthy persons after accidents and trauma, and burn victims are highly susceptible to *Aeromonas* infections. Diarrhea and bacteremia may also occur in healthy people despite many cases in patients

suffering from underlying diseases, mainly cirrhosis or other liver diseases or those with immune disorders such as HIV infection (Figueras, 2005; Janda and Abbott, 2010; Figueras and Beaz-Hidalgo, 2015). However, the most susceptible people for diarrhea, as occurs with other enteropathogens, are less than 2 to 5 years old children, the elderly people or patients with underlying conditions.

Aeromonads may preferentially colonize the bowels of persons with hematologic malignancies such as leukemia on the basis of the 8% incidence found in neutropenic/bone-marrow transplantation patients versus a 0.24% rate in other hospitalized persons (Sherlock et al. 1987). In this sense Janda and Abbott (2010) suggested that it is plausible that patients with hematologic cancers, tumors of the gastrointestinal tract, or other underlying pathological anomalies of the alimentary canal are more susceptible to *Aeromonas* colonization and infections. Diseases such as HUS and colitis can be further complicated with *Aeromonas* gastroenteritis (Janda and Abbott, 2010).

An increasing susceptibility to *Aeromonas* infections after antibiotic treatment has also been reported (Sanchez-Cespedes et al., 2009; Dias et al., 2014).

1.4 Population and Individual Control Measures

1.4.1 Vaccines

Vaccines are only available to treat fish disease (Beaz-Hidalgo and Figueras, 2013). However, the sporadic number of cases does not seem to justify the need from a human vaccine.

1.4.2 Hygiene measures

No information available, but general hygienic measures discussed in other sections of this book may be useful and relevant to control aeromonads that are known to be able to grow in various water/soil environments.

2.0 Environmental Occurrence, Persistence and Survival

2.1 Detection Methods

As indicated elsewhere, no specific culture methods are being use for the recovery of *Aeromonas* in the clinical setting and they are accidentally recovered from media use to detect other enteropathogens; i.e. MacConkey, xylose lysine dextrose (XLD) agar, Hektoen Enteric (HE) agar, *Salmonella–Shigella* (SS) agar, cefsulodin–Irgasa–novobiocin (CIN) agar, etc. (Moyer et al., 1992; Janda and Abbott, 2010; Beaz-Hidalgo and Figueras, 2013; Figueras and Beaz-Hidalgo, 2015). In contrast to clinical microbiology, many culture media have been developed for the isolation of *Aeromonas* from water and food (Palumbo et al., 1985; Havelaar et al., 1987; Neyts et al., 2000; Latif-Eugenín et al., 2016a and references therein). The incubation conditions are typically at 28–30°C for 24–48 hours (WHO, 2002). The Ampicillin Dextrin Agar (ADA= M-*Aeromonas* Agar, Biolife, Italy) is one of the most commonly employed for the recovery of *Aeromonas* from water (Havelaar et al., 1987; Borrell et al., 1998; Latif-Eugenín et al., 2016a) and a method have been developed also by the USA Environmental Protection Agency modifying the ADA medium with the addition of vancomycin, ADA-V (EPA Method 1605) to eliminate interfering bacteria from the analysis of finished water by membrane filtration. Pre-enrichment with alkaline peptone water before sub-culturing to selective media has proved successful for recovery of *Aeromonas* from water (e.g. well water) or food in which the number of organisms is low (Moyer et al., 1992; Latif-Eugenín et al., 2016a). In fact, in a recent study we have demonstrated that enrichment is essential in order to increase the number of shellfish positive samples, and that the culture media may favor the recovery of certain species from food and water (Latif-Eugenín et al., 2016a).

It have been suggested that the inability to isolate *Aeromonas* during the winter months or from cold waters may result not from cell death, but from the entry of cells into a viable but nonculturable (VBNC) state (Wai et al. 2000; Rahman et al. 2001; Maalej et al. 2004a: Maalej et al. 2004b; Casabianca et al., 2015). *Aeromonas* have been described to achieve a VBNC state when cultured in freshwater at 4-5°C (Wai et al., 2000; Mary et al., 2002, Mary et al.,2003). How this VBNC state impact currently analytical results it is not completely known. The use of qPCR analysis indicated the possible presence of a considerable nonculturable population (3.4×10^3 – 2.4×10^6 cells/100 ml) of *Aeromonas* in water samples (Robertson et al., 2014). However, early studies from Holmes et al. (1996 and references therein) demonstrated that although growth of *Aeromonas* was delayed and reduced at 4°C, all the tested species showed the ability to increase their concentration at this temperature. Furthermore, these authors recovered the bacteria in winter contrary to what was described in other studies. Other authors using microcosms of sterile seawater showed that when at 5°C there were less than 10 CFU/100 ml of *Aeromonas* spp. and when they shifted the temperature to 23°C culturable cells appeared after 24 h and increased to a maximum of 10^6 CFU/ 100 ml within three days of the temperature shift (Table 3). Authors indicated that temperature and physiological state affected bacterial behavior and that the apparent recovery of culturability without nutrient addition was largely due to the development of a few culturable cells at the expense of the damaged ones. Pianetti et al. (2005) demonstrated that counts obtained with flow cytometry did not correlate with the results obtained by traditional culture methods or with optical density measures when analyzing the survival of *Aeromonas* in different water types (Table 3). In fact, flow cytometry indicated that there were viable cells when optical density measures were low or the bacteria were no longer detectable by culture. The authors concluded that flow cytometry was a most appropriate technique to study VBNC aeromonads.

Table 3. Summary of Studies Reporting Detection of *Aeromonas spp.* in Ground, Rain and Drinking Water

Area (Year of Study)	Sample Type	Percent Positives (# of Samples)	Concentration CFU/100ml	Method (Sample Volume (mL))	Reference
Australia[a]	Rain water	16 (20/125)	Presence/ absence	Enumeration by membrane filtration, culture and phenotypic identification. (100)	Simmons et al., 2001
Brazil[b]	Drinking water	6 (12/200 chlorinated drinking water samples)	Presence/ absence	Filtration, culture enrichment, phenotypic identification (500)	Razzolini et al., 2008
		1.6 (1/62 taps)			
		8 (11/138 reservoirs).			
Nigeria (2011)	Boreholes	25 (31/124)	Presence/ absence	Culture enrichment, phenotypic identification (2.5)	Bello et al., 2016
Palestine (2006 to 2007)	Rainwater	52 (22/42)	Presence/ absence	Filtration, culture enrichment, PCR (100)	Daoud et al., 2011
Portugal[c] (2004 to 2007)	Untreated drinking water (fountains, wells and mines)	34.4 (33/96)	NR	Enumeration by membrane filtration, culture and genetic identification (phylogeny of gyrB gene). (100)	Carvalho et al., 2012
Scotland (UK)	Drinking water Reservoirs	67.7 (21/31)	217	Enumeration by membrane filtration and selective culture (300)	Gavriel et al., 1998
Several Arab countries	Tap water	21 (65/305)	NR	NR	Adapted from Ghenghesh et al., 2015
	Wells	48 (515/1069)			
	Water reservoirs	66 (78/118)			
	Mineral water	24 (160/659)			
Spain (1995)	Drinking water (DW)	95.5 (105/110)	1.0 E+03 to 1.0 E+05	Membrane filtration, culture and phenotypic identification (100)	Borrell et al., 1998
		8.9 (9/101)			
	Untreated DW	64.4 (29/45)	1-6 E+02		

Area (Year of Study)	Sample Type	Percent Positives (# of Samples)	Concentration CFU/100ml	Method (Sample Volume (mL))	Reference
		42.4 (14/33 samples from treatment plant).	Mean range 0 to 6.8; range 0 to 24		
Spain[d] (2005 to 2006)	Drinking water	36.4 (12/33 samples from storage facility).	Mean range 0 to 5; concentration range 0 to 7	Membrane filtration culture enrichment, phenotypic identification (100)	Pablos et al., 2009
		13.6 (5/66) samples from artesian fountains. Globally 26.5%	Mean range 0 to -50.3; range 0 to 190		
USA (49 States and Puerto Rico)[e] (2005)	Ground water (from Public Water Systems, PWS)	3.2 ((95/2982) samples from 17.8% of (31/174) PWS)	Mean 0.4 SD (7.9)	Enumeration by membrane filtration, culture (EPA method 1065), phenotypic identification (500)	Egorov et al., 2011
USA (49 States and Puerto Rico) (2005)	Surface water (from Public Water Systems, PWS)	1.7 ((35/2060 samples from 11 (9.2 %) of 119 PWS)	Mean 1.6 SD (32.1)	Enumeration by membrane filtration, culture (EPA method 1065), phenotypic identification (500)	Egorov et al., 2011
USA[f]	Raw and treated drinking water	58.8% (10/17) by qPCR	3.4E+03 to 3.0E+05 GC/100ml	Real-time PCR Centrifugation, DNA extraction and qPCR (50)	Robertson et al., 2014
		4.7% (8/17) by selected media	no growth was obtained		
	Groundwater	25 (5/20)	10.4 to 644	(250)	Massa et al., 2001

[a]Households showing GI cases 1 month before sampling were more likely to have Aeromonas in their water than households without symptoms (odds ratio 3.22, 95% CI 1.15 to 9.01, p=0.021); [b]4.6% of drinking water samples in a previous study performed in Brazil; [c]*A. hydrophila* (26%), *A. media* (23%), *A. bestiarum* (12%), *A. eucrenophila* (11%), *A. veronii*, (6%) the rest <6%; [d]Winter peak at water temperature <14; [e]Summer peak but not dependent of temperature; [f]Nonculturable *Aeromonas* population ranging from 3.4 x 10^1 to 2.4 x 10^4 cells.mL-1 was detected in the drinking water; NR: Not Reported

A recent study by Casabianca et al. (2015) demonstrated that in response to a stress condition two different strains of *Aeromonas*, one from fish and the other from seawater reduced their culturability and virulence expression but with a different behavior. The strain from fish was not detected at day 35, while the one recovered from seawater remained culturable. The strain isolated from fish showed a first reduction in expression of the virulence but maintained it for longer period that the seawater strain, possibly as a strategy to colonizing a new host.

Affiliation of recovered isolates to the genus *Aeromonas* is sometimes difficult, because strains maybe confounded with members of other genera, especially with Vibrio species and in fact aeromonads were previously included in the family Vibrionaceae (Janda and Abbott, 2010; Figueras et al., 2011). Probes for the characterization of colonies or for the detection of *Aeromonas* directly from water or food samples have been developed (Chacón et al., 2002; Latif-Eugenín et al., 2016b). There are several methods designed for the detection and identification of *Aeromonas* species described in several reviews (Borrell et al., 1997; Figueras et al., 2000; Martínez-Murcia et al., 2011). However, none of these methods, except the sequences of housekeeping genes like for instance the rpoD or gyrB genes, are useful for the proper characterization of all currently know species and for identifying new species (Martínez-Murcia et al., 2011; Beaz-Hidalgo et al., 2015a). The MALDI-TOF mass-spectrometry have also been use for the characterization of *Aeromonas* recovered from wastewater with promising results but its precision depends upon

having a good updated database (Banerjee et al., 2017).

2.2 Data on Occurrence

2.2.1 Excreta in environment

No data available fecal waste, and dry latrines, except studies that demonstrated the capacity of *Aeromonas* to biodegrade night soils, but its common occurrence in excreta and ability to grow in nitrified soils/water (Vally et al. 2004) makes it a likely member associated with open defecation.

2.2.2 Municipal sewage and human feces

Aeromonas show a high prevalence (ca. 100% samples positive) in sewage, and this highly prevalence in sewage and wastewater at similar counts to fecal coliforms, indicates that they can multiply in this environment (Holmes et al., 1996). This high prevalence in wastewater has been verified by metagenomic studies (McLellan et al., 2010; Ye et al., 2011; VandeWalle et al., 2012; Al-Jassim et al., 2015).

2.2.3 In animal manure

Abu-Elala et al. (2015) investigated the incidence of *Aeromonas* species in fish farms that use untreated poultry manure as a direct feed for the fish and as pond fertilizers. They found that the moribund Nile tilapia bared *A. veronii* and *A. hydrophila* and that disease chicken from the same area carried *A. caviae* after the genetic identification using the gyrB. The species reported were the ones that predominate in association with human infections, hence the authors alerted the public health authorities about the risks from the use of poultry manure. The main drawback of this study was the limited number of strains examined, the fact that the strains from poultry did not come directly from the manure use but from disease chickens in the area, and finally the lack of further epidemiologic genotyping of the isolates. The number of bacteria present in organic manure use in aquaculture systems have also been studied in the Kainji Lake Basin area, Nigeria (Ogbondeminu and Okaeme, 1986). In the latter study they found high levels of fecal coliforms (i.e. 10^5 CFU/100ml) in addition to the presence of aeromonads and other potential pathogenic bacteria both in the water and in the fish. In conclusion of the study it was recognized that pathogens represent a possible occupational risk for the fish handlers.

Motile aeromonads were isolated by Ceylan et al. (2009) from rectal swabs from feces of clinically healthy sheep (12/120, 10%), cattle (7/85, 8.2%) and horses (1/20, 5%), while in another study only 8.8% of 520 samples from pigs and 4.6% of 481 samples from cows were found to show the presence of these bacteria (Gray and Stickler, 1989). Its presence in livestock may favor the transmission between animals and to humans, but the contaminated feces may also impact surface water quality during rain and storm water runoff. Despite the low prevalence found in healthy horses, 22/40 fecal samples (55%) from horses with diarrhea were positive for *Aeromonas* (Hathcock et al., 1999). In contrast, a study that tried to determine the impact of manure bacteria excreted by horses and mules used as porters along the John Muir Trail that crosses several USA National Parks, no *Aeromonas* was detected (Derlet and Carlson, 2002).

Aeromonas have been reported in a pilot-scale lagoon processing swine manure installation with an incidence that ranged between 4.7% in the decanted and filtered swine manure to 25% in the receiving fish ponds. Whereas fecal coliforms decreased across the system, aeromonads increased in some of the lagoon ponds, with densities that ranged between 2 to 190 x 10^4 CFU/100 ml (Chikh et al., 1997).

2.2.4 In surface waters

Aeromonas are highly abundant in river water and in lakes and reservoirs (88% and 95% respectively) at concentrations of up to 3.4 x 10^4 and 6.9 x 10^3 CFU/100 ml, respectively (Borrell et al., 1998). *Aeromonas* have been found in association with Finnish waters where cyanobacterial blooms were suspected to have caused adverse human health effects (fever, gastrointestinal symptoms) from where 116 strains were recovered and identified with partial sequences of the 16S rRNA gene (Berg et al. 2009; Berg et al., 2011). A re-identification of these strains sequencing the rpoD gene lead to the description of three new *Aeromonas* species i.e. *A. aquatica*, *A. finlandiensis* and *A. lacus* (Beaz-Hidalgo et al. 2015a).

2.2.5 In ground water

The incidence of *Aeromonas* in ground water is generally low and when detected concentrations are typically < 100 CFU/100 ml (Holmes et al., 1996; WHO, 2002; Borchardt et al., 2003). However, Havelaar et al. (1990) recorded 470 CFU/100 ml from a plant treating deep aerobic groundwater and similarly high counts at some works have been associated with filter beds with long operational periods (over 25 years) without filter material replacement or with filter units that were operated intermittently to meet variable water demand (WHO, 2002). High levels have also been related with the intrusion of fecal contamination (WHO, 2002).

2.2.6 In drinking water

Aeromonas have be isolated from treated drinking water distribution systems in many studies from different countries (van der Kooij, 1988; Havelaar et al., 1990; Holmes et al., 1996; Gavriel et al., 1998; Borrell et al., 1998; WHO, 2002; Figueras et al., 2005; Egorov et al., 2011; Ghenghesh et al., 2015). The persistence of these bacteria in the network have been associated to its capacity to regrowth in the system (Havelaar et al., 1990; Holmes et al., 1996 and references therein; van der Kooij et al., 2015). Prevalence may change considerably depending on water temperature, chlorine residual concentration, and location within the drinking water distribution system/residence time of the water. Culture conditions are also important;

i.e. inclusion or not of an enrichment step and composition of the culture medium (Egorov et al., 2011; Latif-Eugenín et al., 2016a). The incidence found in different selected studies is shown in Table 4 but may vary considerably, even within the same country. For instance, in studies performed in Spain relative lower incidences of 6.9 and 8.9% were reported by Borrell et al. (1998) and Figueras et al. (2005) respectively for different drinking water systems, while 26.5% was reported by Pablos et al. (2009). Positive samples with concentrations that ranged from 10 to 600 CFU/100 mm were associated with undetected levels of chlorine or that ranged between 0.05 and 1.5 ppm (Borrell et al., 1998; Figueras et al., 2005). Concentrations one or two orders of magnitude higher than those reported by Figueras et al. (2005) have been described for finished drinking water leaving the treatment plant (10^2-10^4 CFU/100 ml), and in the distribution system (10^4 -10^5 CFU/100ml) (Holmes et al., 1996; WHO, 2002). A study performed in the USA provided adjusted odds ratios of *Aeromonas* detection of 1.6 (95% confidence limits 1.0, 2.5) for the summer season, 3.3 (1.8, 6.2) for turbidity above 0.5 nephelometric units and 9.1 (3.5, 24) at 0 mg/L of total chlorine compared with 0.25 mg/L (Egorov et al., 2011). Adequate residual chlorine and low turbidity were concluded to be essential for preventing regrowth of aeromonads in drinking water. However other authors report *Aeromonas* independently of the presence of adequate levels of chlorine in the system, suggesting that the bacteria maybe protected in some way (Holmes et al., 1996; Gavriel et al., 1998; Figueras et al., 2005; Pablos et al., 2009). For instance, chlorine residual ranging from 0.21 and 0.72 mg/L were reported in 94.2% (33/35) of the positive sampler (Pablos et al., 2009). In the latter study the higher number of positive samples were found at the period of lower temperatures (14°C) and in association with rainfall events, which may increase the organic load to the water (Pablos et al., 2009). However, temperature exceeding 14°C and mean free chlorine levels 0.1 mg/L were generally the factors considered to increase the presence of *Aeromonas* in drinking water distribution networks (Holmes et al., 1996). Recent studies have suggested that other factors different from water temperature might influence the summer peak of *Aeromonas* fund in some drinking water systems (Egorov et al., 2011).

Distribution system biofilms may be colonized by several species, but the dominance of a single persistent strain is common (Figueras et al., 2005; Martínez-Murcia et al., 2000; Pablos et al., 2009). Persistent clones within the distribution systems and in biofilms have been described in other studies (Martínez-Murcia et al., 2000; Figueras et al., 2005; Rahman et al., 2007). For instance, the same three persistent clones of the species *A. veronii* (misidentified at that time as the species *A. culicicola* now synonym of *A. veronii*) corresponded to 46% of the identified isolates that were dominating over time and space because they were recovered in several sampling occasion and from distant sites (up to 4 km) in the water supply system (Figueras et al. 2005).

Species prevalent in drinking water have commonly been considered different from those from clinical cases (Holmes et al., 1996), but recent studies have shown that the most prevailing species identified using molecular methods are: *A. veronii,* followed by *A. salmonicida*, *A. hydrophila*, *A. media*, *A. jandaei* and *A. caviae* (Figueras et al., 2005; Pablos et al., 2009; Carvalho et al., 2012). Hence, the classical idea that the species present in water differed from those that may cause disease (Holmes et al., 1996) was wrong as is also the idea that all environmental *Aeromonas* separate into sub-groups that differ from clinical isolates. Genotyping 55 isolates of *A. veronii* from environmental and clinical origins demonstrated that strains grouped in intra-specific lines of descent comprising clinical strains linked to isolates from environmental sources (Martínez-Murcia et al. 2000). Supporting the revised view that at least some environmental isolates are genetically similar to the clinical isolates.

As commented above the factors that influence the occurrence and population sizes of *Aeromonas* spp. in drinking water distribution systems include organic content, temperature, the residence time of water in the distribution network, and the presence of residual chlorine (Figueras and Borrego, 2010). Control and removal of biofilms and eradication of *Aeromonas* is a slow process and may need concentrations of chlorine in excess of 0.2 mg/L. Henc, keeping adequate levels of chlorine throughout the complete distribution system, reducing dead ends in the network, as well as the retention time and the biofilms are the strategies that can help to reduce the presence of *Aeromonas* in the drinking water systems. Apart from chlorination, the removal of biodegradable compounds (i.e. improving the biostability of the water) is another strategy to reduce *Aeromonas* in drinking-water, this can be achieved by treatment with granular activated carbon or, for anaerobic ground waters, by aeration (WHO, 2002). Another strategy has been to introduce *Aeromonas* as an operational indicator within the drinking water monitoring program. In the Netherlands, the public health authorities defined maximum values for *Aeromonas* densities, i.e., 20 CFU/100 ml as a median value over a 1-year period in water leaving the treatment facility, and 200 CFU/100 ml as the 90th-percentile value of the counts of drinking-water collected from the distribution system in a 1-year period (WHO, 2002; Figueras and Borrego, 2010).

Aeromonas have been isolated together with Vibrio cholerae during the cholera outbreak in Haiti (Figueras and Beaz-Hidalgo, 2015). The occurrence of *Aeromonas* in untreated drinking water is much higher than in treated drinking water, Table 4 (Borrell et al., 1998; Carvalho et al., 2012).

Table 4. Treatment Reductions for Aeromonas for Domestic Wastewater

Area of Study	Treatment	Treatment Conditions	Initial Concentration CFU or MPN/ml	Final Concentration CFU or MPN/ml	Treatment Reductions Log_{10}	Quantifi-cation Method	Reference
Brazil	Sewage stabilization pond	3 serial ponds with a total retention time of 20 days	Total inflow 6.61E+08	Total outflow 2.14E+08 anaerobic pond 1.66E+05 facultative pond	2.57	MPN	Martone-Rocha et al., 2010
Germany	Combined Sewage Overflow Disinfection with Performic acid	12 to 24 $mg \cdot L^{-1}$	1E+05 to 1E+06	NR	1.8	MPN	Tondera et al., 2016
Mauritania	Activated sludge, primary and secondary treatment followed by retention in oxidation channel enriched with oxygen	Influent water 18,000 m3·d-1	4.90E+03 raw wastewater	2.29E+03 oxidation pond 1.82E+03 treated effluent	0.45	Plate Count	Lafdal and Malang, 2012
Morocco	Stabilization pond	2500 m^2 Ponds depth ca 2m and mean retention time 11 days	1.04E+05 to 5.63E+06	NR	1.74 globally 1.92 warm months 1.52 cold months 1.02 overall	Spread platting	Hassani et al., 1992
Spain	Lagooning	16,864 m^2 Ponds depth range 1.95 to 3.15 m	1.08E+06	NR	0.03 warm months 2 cold months	MPN	Fernandez-Cassi et al., 2016
Spain	Tertiary treatment by UV and chlorination	Activated sludge +UV and chlorine	8.2E+06 to 2.4E+08	NR	>2	MPN	Latif-Eugenín, 2015

CFU: Colony Forming Units, MPN: Most Probable Number, NR: Not Reported.

Table 5. Treatment Reductions for Aeromonas for Drinking Water and Fomites

Area of Study	Treatment	Treatment Conditions	Initial Concentration Log_{10} and Conditions	Treatment Reductions Log_{10}	Quantification Method	Reference
Brazil (Biofilm formed on stainless steel surfaces)	Detergents containing essential oils	*Thymus vulgaris* (thyme) The thyme reduced the numbers in biofilm *Cymbopogon citratus* (lemongrass) Lemongrass solution to reduce the Biofilm	7.60 / $CFU \cdot cm^{-2}$ In biofilms	3.84 / $CFU \cdot cm^{-2}$ 4.51 /$CFU \cdot cm^{-2}$	Counting colony forming units $CFU \cdot cm^{-2}$	Millezi et al., 2013
USA (Household water treatment)	Combined coagulation-flocculation disinfection	Coagulation/ flocculation hypochlorite 5 $mg \cdot L^{-1}$, and 30 minutes contact time.	demand free buffer natural surface water fecally-contaminated surface water.	7.7 6.8 4.0	enumerated according to EPA Method 1605	Casanova and Sobsey, 2015
USA (secondary Disinfection)	Silver as a secondary disinfectant to replace or reduce the level of chlorine	8 hours of exposure to 100 $\mu g \cdot L^{-1}$ of silver	reduction at both pH 7 and pH 9 within 9h	>6 log_{10}	Plate count	Silvestry-Rodriguez et al., 2007

Also, roof-harvested drinking water showed an incidence of *Aeromonas* that ranged between 7-32% (Daoud et al., 2011; Dobrowsky et al., 2014; Ahmed et al., 2014). These bacteria have also been isolated in mineral bottle and sached-packed drinking water with unreported clinical impact (Korzeniewska et al., 2005b; Venieri et al., 2006; Ahmed et al., 2013).

2.2.7 Seawater

Aeromonas spp. grow in seawater and may range from 10^{-2}-10^{2} CFU/100 ml, being present in waters that do not show fecal pollution (Holmes et al. 1996) but may also exceed the density of fecal coliforms (Araujo et al., 1990). The number of positive samples can be high (83.1%) when waters are impacted by fecal pollution and counts may reach 10^{4} (Borrell et al., 1998), but concentration decrease at 500 m from the shoreline (Araujo et al. 1990). Experimental studies inoculating *Aeromonas* into sweet water (low salinity seawater) demonstrate their capacity to grow and survive (Araujo et al., 1990; Monfort and Baleux, 1991).

2.2.8 Sludge

Aeromonads are a predominant group present in activated sludge (Ochiai et al. 2013; Lade et al., 2014). Furthermore, their biofilm behavior may also be governed by N-acyl homoserine lactone (AHL) quorum sensing (Liebana et al., 2016).

2.2.9 Soil and sediments

Few studies dedicated to investigate the presence of *Aeromonas* in soils were identified (Pepi et al., 2007; Wang et al., 2009). *Aeromonas* have been described to represent 9-20% of cultivable bacteria in biofilms from freshwater sediment (Peduzzi et al., 1992; Szabo et al., 2011). Brandi et al. (1996) have suggested that soil may represent an important reservoir for *Aeromonas* because they have demonstrated that aeromonads can multiply and survive for long periods of time in soil (140 days), maintaining their virulence properties and getting into direct contact with skin/wounds.

2.2.10 Irrigation water and on crops

Aeromonas have been frequently recovered from irrigation water (Pianietti et al., 2004; Carvalho et al. 2012; Aravena-Román et al., 2013; Al-Jassim et al., 2015; Latif-Eugenín, 2015). The prevalence of *Aeromonas* in reclaimed water used for irrigation was relatively high (63.6%) and all the studied vegetables irrigated with the same water were positive (Latif-Eugenín et al., 2016a). In vegetables, *A.*

caviae (75%) was the most common species, among which a strain isolated from lettuce had the same genotype (ERIC pattern) as a strain recovered from the irrigation water (Latif-Eugenín et al., 2016a). Also, the same genotype of the species *Aeromonas saranellii* (a species described from human infections) was recovered from parsley and tomatoes demonstrating that the irrigation water was the likely source of contamination and confirming the potential risk for public health (Latif-Eugenín et al., 2016a). Concentrations of *Aeromonas* found in the irrigation water ranged from 7.0 x 10^2 CFU/100 ml to 2.45 x 10^4 MPN/100 ml. A quantitative microbial risk assessment (QMRA) study of *Aeromonas* infection risks in farmers exposed by dermal contact with irrigation water showed a (per event) probability of transmission that ranged from 1.9 x 10^{-3} for exposure to primary treated wastewater to 2.4 x 10^{-5} for the chlorinated effluent (Al-Jassim et al., 2015). The study estimated that the median exposure doses of *Aeromonas*, over a 95% confidence interval per irrigation event, decreased from 7.0 x 10^2 cells using untreated influent wastewater to 9.4 x 10^1 cells using effluent treated wastewater and to 9.0 cells using the chlorinated effluent. These authors consider that despite *Aeromonas* persistence in chlorinated effluent the annual microbial risk for the farmers remained within the accepted probability of 10^{-4} infection/year.

2.2.11 Fish and shellfish

As commented earlier fish are important reservoirs of *Aeromonas* spp. Several species of the genus are able to produce septicemia, and ulcerative and hemorrhagic diseases in fish, causing significant mortality in both wild and farmed freshwater and marine fish species (Beaz-Hidalgo and Figueras, 2013; Hossain et al., 2013; Jubirt et al., 2015).

Several studies have documented the presence of *Aeromonas* in shellfish and that recovery may vary from 31.3 to 67% (Borrell et al., 1998; Evangelista-Barreto et al., 2006; Ottaviani et al., 2006; Woodring et al., 2012). Concentrations in the shellfish may range 10^2 to 4.0x10^6 CFU/100 g (Borrell et al., 1998; Evangelista-Barreto et al., 2006). In our experience the incidence in shellfish depends on the culturing approach utilized (Latif-Eugenín, 2015), 65% of the water samples from the shellfish growing area and 54.5% of the shellfish studied were positive by direct culturing. However, as expected, when culture was performed after pre-enrichment step the number of positive samples increased both from water (75%) and from shellfish (90%). As more fecal contamination occurred within the harvesting area the higher the probability of finding shellfish positive samples (Latif-Eugenín, 2015). The mean concentration of *Aeromonas* in the waters was one order of magnitude higher that in the shellfish (10^4 vs 10^3 CFU/100 ml).

2.2.12 Air

The presence of *Aeromonas* in air have been poorly studied. Using a validated new and rapid quantitative molecular method for enumeration of four live potential Gram-negative bacterial pathogens in airborne particulate matter associated with biomass burning, *Aeromonas* was not detected despite other bacteria being present i.e. *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Kaushik and Balasubramanian, 2013). However, in a previous study performed by the same research group (Kaushik et al., 2012) and that investigated the influence of prevailing air quality on the microbial composition of rainwater they found *Aeromonas* in one of the 25 target bacteria-positive rainwater samples of the 50 samples studied. Suggesting aeromonads only represented some 2% of cultured bacteria and the dominating targeted bacteria in the positive samples were *E. coli* 21/25 (42%), *P. aeruginosa* 16/25 (32%) and *K. pneumoniae* 6/25 (12%).

3.0 Reductions by Sanitation Management - Considering Regrowth of This Genus

As commented above, environmental growth and regrowth from release of excreta is a reality and significant limitation for the control of exposures to *Aeromonas* spp. either from water systems or excreta deposits, as seen in water distribution systems (van der Kooij et al., 2015). Therefore, while log-reductions by treatments is expected to follow that seen for coliforms, regrowth potential is considerably higher for certain strains, some of which maybe human pathogens. Systems that include sand filters or wetlands are particularly likely to support high number of aeromonads.

3.1 Excreta and Wastewater Treatment Waterless Sanitation

3.1.1 On-Site

3.1.1.1 Pit latrines, vault toilets, dry toilets

No specific data relating to aeromonads was identified for these dry sanitation options. While the presence of *Aeromonas* in urine is restricted to the cases of infection, of the few reported cases, excreted concentrations of over 10^7 CFU/ 100 ml are expected. Importantly, multi-drug resistant strains of a range of Gram-negative bacteria including aeromonads were most commonly identified from healthcare center samples (Agyepong et al., 2018; Mohammed et al., 2016). Noting that novel species has been identified to cause urinary-tract infections (Hua et al., 2004). Hence the reuse of collected urine is an emerging pathway to also consider for aeromonads and antimicrobial resistance (AMR) control (see related chapter in this book, Piotrowska et al., 2017). In general, however, aeromonad growth (along with most human enteric bacterial pathogens) is probably controlled in stored urine that normal stays at pH > 9 (Höglund et al., 2002; Benchokroun et al., 2003).

No data was identified on the impact of adding ash, lime or soil to collected excreta, although aeromonads would likely be inhibited at pH > 9.

3.1.1.2 Composting

Recently the genome of a strain referred as *A. hydrophila* YL17 recovered from a compost pile have been obtained by Lim et al. (2016). However, that strain was mislabel because on the basis of the values obtained when comparing the genome of this strain with the available genomes of other *Aeromonas* spp. using the average nucleotide identity index and a phylogenetic analysis suggested the correct identity was *A. dhakensis* (Rasmussen-Ivey et al., 2016b). Its detection in compost is relevant because *A. dhakensis* is a species that has high potential to be virulent in humans (Figueras and Beaz-Hidalgo, 2015; Chen et al., 2016). The same authors have also isolated an *A. caviae* YL12 strain from a plant material compost pile that demonstrated quorum sensing activity (Lim et al., 2014), again this species and strain characteristics have often been implicated in clinical cases (Figueras and Beaz-Hidalgo, 2015). For other residuals (solids) management: with the intention of reuse as fertilizer for agriculture/food, etc. no data were found.

3.1.2 Waste stabilization ponds

Given the ability of aeromonads to grow in soil/aquatic habitats and in wastewater, they are likely members to grow within water-based sanitation systems. There is some data on reductions of aeromonads by lagooning or retention in water stabilization ponds (Monfort and Baleux, 1990; Boussaid et al., 1991; Benchokroun et al., 2003; Martone-Rocha et al., 2010; Fernandez-Cassi et al., 2016). According to the study of Monfort and Baleux (1990) the reduction of the *Aeromonas* spp. was seasonally related with a higher concentration in the treated effluent during summer than winter. In a study performed by Fernandez-Cassi et al. (2016) the concentration of *Aeromonas* spp. in the inlet water was 1.08 x 10^6 MPN·100 ml^{-1} and 1.94x10^5 MPN·100 ml^{-1} in the outlet water. This represented a 90% reduction in *Aeromonas* spp. during the storage period, but during the lower demand period that had longer retention time a 2 log_{10} reduction was recorded (mean concentration of *Aeromonas* in the inlet water was 1.65 x 10^6 MPN·100 ml^{-1} while in the outlet was 1.76 x 10^4 MPN·100 ml^{-1}). Despite such reductions, total elimination of these bacteria from the system was not achieved. Benchokroun et al. (2003) demonstrated using microcosms that there was a strong synergy between alkaline pH, exogenous dissolved sensitizers and dissolved oxygen on sunlight inactivation of *Aeromonas*, and concluded that the exogenous photooxidation could be considered as the main factor explaining differences in the seasonal removals of these bacteria in waste stabilization ponds.

3.1.3 Wastewater treatment facilities

As described above, metagenomic studies of the bacterial communities in wastewater revealed that *Aeromonas* spp. are a dominant group detected (VandeWalle et al., 2012; Shanks et al., 2013). Hence, their elimination requires disinfection, not secondary biological treatment. Hence, any release of raw sewage to the environment is likely to contain significant loadings of aeromonads, such as via combined sewer overflows.

3.1.3.1 Secondary treatment

Aeromonas spp. are present in high quantities ranging from 10^6 CFU/100 ml to 10^8 CFU/100 ml in secondary effluent (Boussaid et al., 1991; Monfort and Baleux, 1991; Hassani et al., 1992; Holmes et al., 1996; Martone-Rocha et al., 2010; Figueira et al., 2011; Igbinosa and Okoh, 2012; Fernandez-Cassi et al., 2016).

Physical treatments such as with micro- or ultra-filter membrane bioreactors would remove most bacteria effectively (see treatment section within this book). Given the facultative anaerobic nature of aeromonads, anaerobic or anoxic reactors would likely have limited impact, but their efficacy appears undocumented (Morrison et al., 2017).

3.1.4 Biosolids/Sewage sludge treatment

In a study evaluating the regrowth of bacteria after dewatering of thermophilic and mesophilically digested biosolids, in general, for thermophilic processes, even when a statistically significant ($p < 0.05$) sudden increase and regrowth in fecal coliforms, *E. coli* and enterococci, that was not seen for *Salmonella* or *Aeromonas* spp. (Chen et al., 2011). In contrast, for the mesophilic process evaluated, while regrowth occurred for coliforms, enterococci and *Salmonella*, it did not appear to occur for aeromonads. Suggesting *Aeromonas* risks are likely low from handling municipal biosolids.

3.2 Disinfection as a Tertiary (or Post Primary) Treatment

3.2.1 Chlorine, combined chlorine etc.

The most efficient treatment was an 8 mg/L chlorine at a temperature of 20°C for 30 min in a study performed by Martínez-Hernández et al. (2013) that used sodium hypochlorite as a disinfectant in a wastewater treatment plant, where the untreated effluent showed presence of *Aeromonas*. Lower concentrations may be effective, so long as the chlorine demand of the water is first met, and a residual is present. Not meeting the chlorine demand will result in combine chlorine, of which monochloramine is thekey disinfectant, and need to exceed 0.3 mg/L for some time to be effective against aeromonads (Mackerness et al., 1991)

3.2.2 Ultraviolet (UV) and ozonation

Chlorination (3 to 6 mg·L^{-1} of sodium hypochlorite with a contact time of 30 to 90 min) in combination with UV treatment two banks with four medium pressure lamps each, with a UV dose of 25–30 mJ·cm^{-2}, according to the UV supplier) removed *Aeromonas* below the detection limit, however, regrowth after storage of this water have been observed (Latif-Eugenín et al., 2016a) and is expected in the absence of a chlorine residual.

While ozonation is a generally effective wastewater disinfection process used in aquaculture (3 log_{10} reductions of aeromonads with a Ct of 0.12-0.50 mg min/L) (Colberg and Lingg, 1978), loss of efficacy has been a concern in certain water matrices. Hence, the use of combined UV/ozonation at aquaculture facilities, combining ozone dosages of only 0.1–0.2 mg min/L with a UV irradiation dosage of approximately 50 $mJ \cdot cm^{-2}$ appear to consistently reduce bacteria counts over 3-4 log_{10} to near zero (Sharrer et al., 2007).

3.2.3 Sunlight / Advance oxidation processes

Various applications of titanium dioxide (TiO_2) with sunlight or UV lamps has been examined to increase solar disinfection (see treatment Section in this book). While few of these studies have focused on aeromonads, a thin-film fixed-bed reactor (TFFBR) utilizing TiO2, was examined for the aquaculture pathogen *A. hydrophila* ATCC 35654 (Khan et al., 2012). High sunlight intensities (>/= 600 $W \cdot m^{-2}$) and low flow rates (4.8 L/hour) provided optimum conditions for inactivation of *A. hydrophila* ATCC 3564, with greater overall inactivation and fewer sub-lethally injured cells than at low sunlight intensities or high flow rates.

With regards to desiccation, aeromonads appear relative more susceptible than fecal indicators or other enteric bacterial pathogens (Janning et al., 1994).

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