Health Canada Santé Canada

Your health and safety… our priority. *Votre santé et votre sécurité… notre priorité.*

Guidance on Waterborne Bacterial Pathogens

Canada

Health Canada is the federal department responsible for helping the people of Canada maintain and improve their health. *We assess the safety of drugs and many consumer products, help improve the safety of food, and provide information to Canadians to help them make healthy decisions. We provide health services to First Nations people and to Inuit communities. We work with the provinces to ensure our health care system serves the needs of Canadians.*

Published by authority of the Minister of Health.

Guidance on waterborne bacterial pathogens

is available on Internet at the following address:
www.healthcanada.gc.ca

Également disponible en français sous le titre :
Conseils sur les bactéries pathogènes d'origine hydrique

This publication can be made available on request in a variety of alternative formats.

Pub. Number: 130469
Cat.: H129-25/1-2014E-PDF
ISBN: 978-1-100-22966-9

Guidance on Waterborne Bacterial Pathogens

Prepared by the
Federal-Provincial-Territorial Committee on Drinking Water
of the
Federal-Provincial-Territorial Committee on Health and the Environment

Health Canada
Ottawa, Ontario

February 2013

This document may be cited as follows:

Health Canada (2013). Guidance on waterborne bacterial pathogens. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario (Catalogue No. H129-25/1-2014E-PDF).

The document was prepared by the Federal-Provincial-Territorial Committee on Drinking Water of the Federal-Provincial-Territorial Committee on Health and the Environment.

Any questions or comments on this document may be directed to:

Water and Air Quality Bureau
Healthy Environments and Consumer Safety Branch
Health Canada
269 Laurier Avenue West, Address Locator 4903D
Ottawa, Ontario
Canada K1A 0K9

Tel.: 613-948-2566
Fax: 613-952-2574
E-mail: water_eau@hc-sc.gc.ca

Other documents concerning Canadian drinking water quality can be found on the following website: www.healthcanada.gc.ca/waterquality

Table of Contents

February 2013

Guidance on waterborne bacterial pathogens

Background on guidance documents

The main role of the Federal-Provincial-Territorial Committee on Drinking Water is the development of the Guidelines for Canadian Drinking Water Quality. This role has evolved over the years, and new methodologies and approaches have led the Committee to develop a new type of document, guidance documents, to provide advice and guidance on issues related to drinking water quality for parameters that do not require a formal guideline under the Guidelines for Canadian Drinking Water Quality.

There are two instances in which the Federal-Provincial-Territorial Committee on Drinking Water may choose to develop a guidance document. The first would be to provide operational or management guidance related to specific drinking water–related issues (e.g., boil water advisories), in which case the document would provide only limited scientific information or health risk assessment. The second instance would be to make health risk assessment information available when a guideline is not deemed necessary.

The Federal-Provincial-Territorial Committee on Drinking Water establishes guidelines under the Guidelines for Canadian Drinking Water Quality specifically for contaminants that meet all of the following criteria:

1. exposure to the contaminant could lead to adverse health effects;
2. the contaminant is frequently detected, or could be expected to be found, in a large number of drinking water supplies throughout Canada; and
3. the contaminant is detected, or could be expected to be detected, at a level that is of possible health significance.

If a contaminant of interest does not meet all these criteria, the Federal-Provincial-Territorial Committee on Drinking Water may choose not to establish a numerical guideline or develop a guideline technical document. In such a case, a guidance document may be developed.

Guidance documents undergo a similar process as guideline technical documents, including public consultations through the Health Canada website. They are offered as information for drinking water authorities and, in some cases, to provide guidance in spill or other emergency situations.

Part A. Guidance on waterborne bacterial pathogens

This document provides information as background for those interested in drinking water quality and safety. There is a particular focus on waterborne bacterial pathogens that may not have the classical faecal–oral transmission route, as these may be less well known to the water industry and public health professionals. Although there is information available from many countries, this document emphasizes studies most relevant to the North American situation.

Throughout history, consumption of drinking water supplies of poor sanitary quality has been linked to illnesses in human populations. These illnesses most commonly present as gastrointestinal-related symptoms, such as diarrhoea and nausea. The organisms identified within this document as waterborne faecal bacterial pathogens are those that have been well established as having a history of being responsible for waterborne outbreaks of gastrointestinal illness.

There are standardized methods available for detecting and measuring certain pathogenic bacteria in drinking water. However, routine monitoring for these organisms still remains difficult and impractical. This is because there are a number of types of bacterial pathogens that can be present in human and/or animal wastes, which can vary significantly in their distribution, depending on the sources of contamination affecting the water supply; and because conducting detection and identification procedures for each possible type can be difficult, requiring significant resources. As a result, monitoring for a broad indicator of faecal contamination such as *Escherichia coli* is useful in verifying the microbiological quality and safety of the drinking water supply. The presence of *E. coli* in drinking water system indicates that the source or the system has likely been affected by recent faecal contamination; as a result, the water should be deemed as unsafe to drink.

In recent decades, there has been an increasing amount of interest in waterborne bacterial pathogens that occur naturally in the water environment and thus have the potential to be transmitted through water. The waterborne non-faecal bacterial pathogens discussed within this document can cause human infection, resulting in gastrointestinal and non-gastrointestinal illnesses (particularly respiratory illnesses). Routine monitoring for these pathogens remains difficult and expensive as well. As these organisms occupy different environmental niches and have primary sources other than human or animal faeces, there are at present no satisfactory microbiological indicators for their presence. To date, none of these organisms has been associated with outbreaks of illness as a result of ingestion of drinking water in Canada. However, as they do have the potential to be spread through drinking water, it is important to ensure that the treatment and disinfection strategies in place are capable of providing adequate control of these organisms. Health Canada maintains that the best means of safeguarding against the presence of waterborne pathogens (including non-faecal bacterial pathogens) in drinking water is the application of the multibarrier approach, which includes adequate treatment, a well-maintained distribution system and, in the case of enteric bacteria, source protection. Treatment and disinfection requirements in the provision of microbiologically safe drinking water are based on health-based treatment goals for the removal and inactivation of the enteric protozoa *Giardia* and *Cryptosporidium* and enteric viruses. These organisms present a significant challenge to water treatment and disinfection technologies because of their difficulty of removal, high infectivity and high disinfectant resistance. As a result, current drinking water treatment and disinfection practices applied to meet the treatment goals for viruses and protozoa are expected to be similarly capable of controlling waterborne bacterial pathogens in drinking water. This

approach can reduce both faecal and non-faecal pathogens to non-detectable levels or to levels that have not been associated with human illness.

Consequently, it remains unnecessary and impractical to establish maximum acceptable concentrations for the waterborne bacterial pathogens described in this document. The monitoring of *E. coli* continues to be used in the verification of the microbiological quality of drinking water. Information on the adequacy of drinking water treatment and on the microbial condition of the distribution system is provided by the monitoring of *E. coli* and other indicators, such as disinfectant residual and turbidity.

Under the multibarrier approach to safe drinking water, numerous process controls are required to function alongside bacteriological analysis in order to reliably produce drinking water of an acceptable quality. Important individual elements under this approach include:

- source water protection (where possible);
- optimized treatment performance (e.g., for turbidity reduction and particle removal);
- proper application of disinfection technologies;
- a well-designed and well-maintained distribution system; and
- maintenance of a disinfectant residual.

The potential for the introduction of waterborne bacterial pathogens into the distribution system and their ability to survive and regrow in biofilms are of concern in drinking water treatment. Recurring patterns of elevated heterotrophic plate counts downstream of water treatment may indicate the presence of biofilms, which could be a source of waterborne pathogens. As microorganisms in biofilms may survive, multiply and be released into the distribution system, water that meets the bacteriological guidelines may be recontaminated over time.

Additional considerations specific for aiding in the control of biofilms in the distribution system can include:

- use of proper construction materials;
- control measures to reduce levels of natural organic matter, scaling and corrosion;
- measures to prevent low flow rates or water stagnation and to control temperatures (where possible);
- maintenance activities, such as flushing and cleaning; and
- ensuring adequate disinfection after installation of new pipes and after maintenance or repair of existing pipes.

Contamination problems involving waterborne bacterial pathogens can occur in water systems beyond the water treatment plants' distribution network, such as plumbing systems or heating, ventilation and air conditioning systems. Specific information pertaining to guidance and requirements for these systems can be found by consulting the proper regulatory authority.

Part B. Supporting information

B.1 Waterborne faecal pathogens

Waterborne faecal pathogens are microorganisms that can occur in water as a result of contamination from human or animal faeces and cause gastrointestinal illness. They are often associated with bacteria from the Enterobacteriaceae family. Faecal bacteria that are well established as having a history of being responsible for waterborne outbreaks of gastrointestinal illness include pathogenic *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter* and *Yersinia*.

B.1.1 Pathogenic *Escherichia coli*

Escherichia coli are bacteria found naturally in the digestive tracts of warm-blooded animals, including humans. As such, *E. coli* are used in the drinking water industry as the definitive indicator of recent faecal contamination of water. *Escherichia coli* are Gram-negative, facultative anaerobic, rod-shaped bacteria, approximately 0.5–2 µm in size (AWWA, 2006). Whereas most strains of *E. coli* are non-pathogenic, some possess virulence traits that enable them to cause serious diarrhoeal infections in humans. These pathogenic *E. coli* are divided into groups based on the mechanisms with which they interact with the human intestinal tract and cause symptoms (e.g., some produce specific types of toxin, whereas others invade, bind to or cause structural alterations of intestinal cells) (Percival et al., 2004). The six groups are enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAEC) and diffuse adherent (DAEC) *E. coli* (Percival et al., 2004; AWWA, 2006). The EHEC group has emerged as a group that is of particular significance to the water industry (AWWA, 2006). This broad group contains many different serotypes that have been implicated as causes of human illness (Muniesa et al., 2006).

One member of the EHEC group, *E. coli* O157:H7, has been most commonly associated with pathogenic *E. coli* outbreaks worldwide (Muniesa et al., 2006) and has been implicated in a few waterborne outbreaks (Bruce-Grey-Owen Sound Health Unit, 2000; Schuster et al., 2005; Craun et al., 2006; Clark et al., 2010). In Canada, the Walkerton outbreak of 2000 was the first documented outbreak of *E. coli* O157:H7 infection associated with a Canadian municipal water supply and the largest multibacterial waterborne outbreak in the country to date (Bruce-Grey-Owen Sound Health Unit, 2000). Surveillance reports published for other countries have indicated that over the period from 1990 to the early 2000s, *E. coli* O157:H7 was identified as the causative agent of approximately 6% of the reported drinking water outbreaks in England and Wales (Smith et al., 2006) and roughly 7% of those reported in the United States (Craun et al., 2006).

Cattle and human sewage are the primary and secondary sources, respectively, of EHEC (Jackson et al., 1998; Percival et al., 2004; Gyles, 2007), but human sewage is the major source of the other pathogenic *E. coli* groups (Percival et al., 2004; AWWA, 2006). Transmission of pathogenic *E. coli* occurs through the faecal–oral route, and the primary routes of exposure are from contaminated food or water or by person-to-person transmission (Percival et al., 2004; AWWA, 2006). Pathogenic *E. coli* are not usually a concern in treated drinking water when treatment and distribution systems are properly operated and maintained. However, outbreaks of *E. coli* O157:H7 involving consumption of drinking water contaminated with human sewage or cattle faeces have been documented in North America (Olsen et al., 2002), including some fatal outbreaks (Swerdlow et al., 1992; Novello, 1999; Bruce-Grey-Owen Sound Health Unit, 2000).

The probability of becoming ill depends on the number of organisms ingested, the health status of the person and the resistance of the person to the organism or toxin (LeChevallier et al., 1999).

With the exception of EHEC, most pathogenic *E. coli* require a high number of bacteria to be ingested in order to produce illness. Infectious dose estimates for non-EHEC strains range from 10^5 to 10^{10} organisms (Percival et al., 2004). EHEC strains, in contrast, have a very low infectious dose. It has been suggested that ingestion of fewer than 100 cells may be sufficient to cause infection (Percival et al., 2004; Pond, 2005). The onset and duration of pathogenic *E. coli*–related illness will be strain dependent, but symptoms can begin in as little as 8–12 hours and last from a few days up to a few weeks (Percival et al., 2004).

Pathogenic *E. coli* can cause diarrhoea that ranges in severity from mild and self-limiting to severe and life-threatening (Percival et al., 2004; AWWA, 2006). Most non-EHEC illness is marked by a watery diarrhoea that can be accompanied by vomiting, abdominal pain, fever and muscle pain, depending on the group or strain involved.

EHEC illness can begin with watery and bloody diarrhoea in combination with vomiting, but in some cases can progress to the more serious and potentially life-threatening symptoms of haemorrhagic colitis (grossly bloody diarrhoea) and haemolytic uraemic syndrome (kidney failure). These symptoms are caused by shiga-like toxins, potent toxins that are related to *Shigella dysenteriae* toxins (Percival et al., 2004; AWWA, 2006). It has been suggested that up to 10% of *E. coli* O157:H7 infections can progress to haemolytic uraemic syndrome (Moe, 1997; Sherman et al., 2010). Children and the elderly are most susceptible to the complications that arise from EHEC infections (Percival et al., 2004). One area of recent interest has been the possible long-term health effects in adults as a result of contracting haemolytic uraemic syndrome from *E. coli* O157:H7, as these to date have been largely unknown (Clark et al., 2010). Clark et al. (2010) reported on the results of a health study among persons who developed gastrointestinal illness or remained asymptomatic following exposure to *E. coli* O157:H7 and *Campylobacter* during the Walkerton outbreak in May 2000. The authors concluded that increases in the incidences of hypertension, cardiovascular disease and indicators of kidney impairment were evident in persons who experienced acute gastroenteritis during the outbreak. Further study in this area is required, but because such waterborne outbreaks are rare, there are very limited opportunities for such studies.

B.1.1.1 Treatment technology

In the majority of treatment and disinfection studies involving pathogenic *E. coli*, the EHEC strain O157:H7 has been selected as the model organism because of its health significance and prominence in foodborne and waterborne outbreaks. Regardless, review of the evidence generated to date suggests that the proper application of water treatment and disinfection technologies will be capable of controlling strains of pathogenic and non-pathogenic *E. coli* in drinking water (Percival et al., 2004; AWWA, 2006).

In terms of chlorine and monochloramine effectiveness, laboratory studies have demonstrated *E. coli* O157:H7 log inactivation capabilities of up to 4 log at concentrations and contact times that would be encountered in municipal drinking water treatment (Rice, 1999; Wojcicka et al., 2007; Chauret et al., 2008).

For ultraviolet (UV) disinfection, Zimmer-Thomas et al. (2007) observed log inactivations of 4.5 log or greater for *E. coli* O157:H7 at all tested doses of low-pressure and medium-pressure UV. These included UV doses commonly used in water disinfection (20 and 40 mJ/cm^2, low pressure), as well as low doses intended to be representative of compromised UV dose delivery (5 and 8 mJ/cm^2, low and medium pressure). In UV inactivation experiments,

Sommer et al. (2000) observed considerable divergence in sensitivity of different pathogenic (including enterohaemorrhagic) strains of *E. coli*. The authors further demonstrated that a UV dose of 125 J/m^2 (equivalent to 12.5 mJ/cm^2) was sufficient to produce a 6 log inactivation of all of the strains under study.

Further information on treatment technologies for *E. coli* can be found in the *E. coli* guideline technical document (Health Canada, 2012).

B.1.1.2 Assessment

Studies have shown that the survival and susceptibility to disinfection of pathogenic *E. coli* strains approximate those of typical *E. coli* (LeChevallier et al., 1999; Rice, 1999). Also, although routine examination methods for *E. coli* are not designed to distinguish pathogenic *E. coli* strains from the general *E. coli* population, the latter will always occur in greater concentration in faeces, even during outbreaks. Pathogenic *E. coli* will not occur in the absence of generic *E. coli*. As a result, the presence of *E. coli* is the best available indicator of faecal contamination and the potential presence of faecal pathogens, but is not a specific signal for the presence of pathogenic *E. coli.*

B.1.2 *Salmonella* and *Shigella*

Salmonella and *Shigella* are agents of gastrointestinal illness that belong to the same microbiological family as *E. coli*, Enterobacteriaceae.

Salmonella are non-spore-forming, facultative anaerobic, Gram-negative bacilli that are 2–5 µm long and 0.8–1.5 µm wide (AWWA, 2006). *Salmonella* is a complex taxonomic genus consisting of over 2000 different varieties or serological types that can cause infections in animals and humans (AWWA, 2006). According to experts, the genus is officially made up of only two species, *Salmonella enterica* and *Salmonella bongori* (Percival et al., 2004; AWWA, 2006). *Salmonella enterica* is the species of most relevance for human infections, and it can be further broken down into six subspecies, of which one, *Salmonella enterica* subsp. *enterica*, contains the majority of serotypes that are associated with cases of human gastroenteritis (Percival et al., 2004). By convention, when referring to *Salmonella* serotypes, the serotype is adopted as the species name (e.g., *Salmonella enterica* subsp. *enterica* serovar *enteritidis* becomes *Salmonella enteritidis*).

The vast majority of *Salmonella* serotypes encountered in developed countries are zoonotic pathogens. Reservoirs for these organisms include poultry, pigs, birds, cattle, cats, dogs, rodents and turtles (AWWA, 2006). Infected humans and, as a result, sewage are also sources of *Salmonella*. Transmission of *Salmonella* occurs through the faecal–oral route, predominantly through food. By comparison, drinking water is not often implicated as a source of *Salmonella* infection (Percival et al., 2004). As *Salmonella* is a zoonotic pathogen, runoff from agricultural lands can provide a mechanism for the transfer of animal faecal wastes to source waters.

Shigella are facultative anaerobic, non-sporulating, non-motile, Gram-negatives rods 0.3–1.5 µm in diameter and 1–6.5 µm in length (AWWA, 2006). The taxonomy of *Shigella* is much simpler than that of *Salmonella*. The genus is categorized into four major serological groups: *dysenteriae*, *flexneri*, *boydii* and *sonnei*. *Shigella sonnei* and *Shigella flexneri* are the two species of importance as causes of gastrointestinal illness in developed countries (Percival et al., 2004). Infected humans are the only significant reservoir (AWWA, 2006). Transmission is faecal–oral, through drinking water or through food that has been contaminated with human faecal wastes. Person-to-person transmission is also a significant route of exposure for *Shigella*, particularly among children. *Shigella* is a human-specific pathogen and is not expected to be found in the

environment (AWWA, 2006). Thus, contamination of water supplies is suggestive of a source of human faecal contamination, such as from sewage or on-site wastewater disposal systems.

Numerous outbreaks linked to contaminated drinking water have been reported worldwide (Boring et al., 1971; White and Pedersen, 1976; Auger et al., 1981; Arnell et al., 1996; Angulo et al., 1997; Alamanos et al., 2000; R. Taylor et al., 2000; Chen et al., 2001). Schuster et al. (2005) reported that *Shigella* and *Salmonella* were identified as the causative agents in 9 and 16 confirmed, proposed or suspected drinking water outbreaks in Canada, respectively, over the years 1974–2001. In the United States, *Salmonella* and *Shigella* accounted for approximately 2% and 5% of drinking water outbreaks reported from 1991 to 2002, according to U.S. surveillance data (Craun et al., 2006). Common causes of waterborne outbreaks by these organisms are poor source water, inadequate treatment or post-treatment contamination (e.g., by cross-connections) (AWWA, 2006). Both organisms give rise to acute, self-limiting gastrointestinal illness with symptoms of diarrhoea, vomiting and abdominal pain. *Shigella*-associated illness is more dysenteric in nature, marked by a more watery diarrhoea containing blood and mucus (AWWA, 2006). Once infected, recovering individuals may continue to shed either of these organisms in their faeces for days up to several weeks or months. Published reports regarding the median infective doses for these two organisms have suggested that they may be as low as 10^3–10^5 organisms for *Salmonella* serotypes and 10^2–10^3 organisms for *Shigella flexneri* and *Shigella sonnei* (Hunter, 1997; Kothary and Babu, 2001). The factors that contribute to the virulence of these organisms are still under investigation. Both possess mechanisms that enable the bacteria to invade, survive, replicate and disrupt the function of the human intestinal lining (Percival et al., 2004). In addition, *Shigella sonnei* and *Shigella flexneri* are known to produce an exotoxin that affects intestinal water absorption and retention (Percival et al., 2004).

B.1.2.1 Treatment technology

Salmonella and *Shigella* survival characteristics in water and their susceptibility to disinfection have been demonstrated to be similar to those of coliform bacteria, including *E. coli* (McFeters et al., 1974; Mitchell and Starzyk, 1975; Chang et al., 1985; Koivunen and Heinonen-Tanski, 2005). It is generally recognized that a properly operated facility will be sufficient in controlling *Salmonella* and *Shigella* in treated drinking water (AWWA, 2006).

B.1.2.2 Assessment

The absence of *E. coli* during routine verification should be an adequate indication of the sufficient removal and inactivation of *Salmonella* and *Shigella*.

B.1.3 *Campylobacter* and *Yersinia*

Campylobacter are pathogenic bacteria found primarily in the intestinal tracts of domestic and wild animals, especially birds. Poultry, cattle, sheep and pigs are considered significant reservoirs for these organisms (Percival et al., 2004; AWWA, 2006). *Campylobacter* are motile, Gram-negative, slender, curved rods 0.2–0.5 µm wide and 0.5–5 µm long. *Yersinia* can be found in the faeces of wild animals as well as domestic livestock such as cattle, pigs and sheep (Percival et al., 2004). *Yersinia* are facultative anaerobic, Gram-negative, non-sporulating rods 0.5–0.8 µm in diameter and 1–3 µm in length (AWWA, 2006). It is the *Campylobacter* species *C. jejuni*, *C. coli* and *C. upsaliensis* and the *Yersinia* species *Y. enterocolitica* that are most important to the water industry (AWWA, 2006). Human sewage also contains large numbers of both of these organisms.

Both *Campylobacter* and *Yersinia enterocolitica* are transmitted through the faecal–oral route, mostly through contaminated food and sometimes through water (Percival et al., 2004). Person-to-person transmission of *Campylobacter* or *Yersinia enterocolitica* is uncommon (Percival et al., 2004; AWWA, 2006).

Waterborne outbreaks of gastroenteritis involving *Campylobacter jejuni* and *Yersinia enterocolitica* have been recorded on numerous occasions, with improper treatment, post-treatment contamination or consumption of untreated water supplies being the most frequent causes (Eden et al., 1977; McNeil et al., 1981; Mentzing, 1981; Vogt et al., 1982; Taylor et al., 1983; Lafrance et al., 1986; Sacks et al., 1986; Thompson and Gravel, 1986). In a review of Canadian data on waterborne outbreaks for the period spanning from 1974 to 2001, Schuster et al. (2005) reported that *Campylobacter* was implicated in 24 outbreaks and was second only to *Giardia* (51 outbreaks) in outbreaks where a causative agent was identified. The most notable Canadian waterborne outbreak was the May 2000 Walkerton outbreak involving *Campylobacter* and *E. coli* O157:H7, where faecally contaminated well water was not properly treated before consumption (Clark et al., 2003). No outbreaks of *Yersinia*-related gastroenteritis have been reported for municipal drinking water supplies in North America over the past two decades (Schuster et al., 2005; Craun et al., 2006).

Gastroenteritis caused by *Campylobacter* typically presents as flu-like symptoms and/or abdominal pain, followed by a profuse watery diarrhoea caused by the presence of an enterotoxin similar to cholera toxin (AWWA, 2006). An important characteristic of *Campylobacter* is the high infectivity potential; as few as 1000 organisms can cause infection (Black et al., 1988; Hara-Kudo and Takatori, 2011). *Yersinia enterocolitica* can cause a variety of symptoms, depending on the age of the person infected, but the most commonly observed are gastrointestinal illness, fever and occasionally vomiting in children (AWWA, 2006). The gastrointestinal illnesses caused by both organisms are considered to be self-limiting (Percival et al., 2004).

B.1.3.1 Treatment technology

Studies have demonstrated the susceptibility of *Campylobacter* species and *Yersinia enterocolitica* to disinfectants commonly used in water treatment (Blaser et al., 1980; Wang et al., 1982; Sobsey, 1989; Lund, 1996; Rose et al., 2007). It is generally recognized that treatment technologies effective in the removal and inactivation of *E. coli* will be effective against these pathogenic bacteria (AWWA, 2006).

B.1.3.2 Assessment

Studies have suggested a lack of a correlation between indicator organisms (e.g., *E. coli*, total coliforms) and the presence of *Campylobacter* and *Yersinia* in raw surface water supplies (Carter et al., 1987; Lund, 1996; Hörman et al., 2004). Thus, *E. coli* may not be an adequate indicator of the presence of both *C. jejuni* and *Y. enterocolitica* in source waters at all times. However, as it is expected that properly operated treatment and disinfection technologies are effective in controlling these organisms in treated drinking water, it is expected that the *E. coli* guideline is sufficiently protective against their potential presence.

B.2 Waterborne non-faecal pathogens

Although *E. coli* is the best available indicator of recent faecal contamination, there are waterborne illnesses that result from pathogens not transmitted by the faecal–oral route. These

pathogens are usually bacteria naturally found in source waters. Those that clearly have a public health impact include *Legionella*, *Mycobacterium avium* complex, *Aeromonas* and *Helicobacter pylori.* The detection of faecal indicators does not provide any information on the potential presence of non-faecal pathogens. No indicators are currently known for such pathogens.

B.2.1 *Legionella*

Legionellae are recognized human pathogens that can cause two different types of illness: Legionnaires' disease, which is a serious respiratory illness involving pneumonia, and Pontiac fever, which is a milder flu-like illness without pneumonia. Legionellae are free-living aquatic bacteria that occur widely in water environments. The presence of *Legionella* is more of a concern for water systems beyond municipal water treatment and distribution systems, such as cooling towers and hospital and residential plumbing systems. *Legionella* species exhibit a number of survival properties that make them relatively resistant to the effects of chlorination and elevated water temperatures. The organisms are also capable of colonizing drinking water distribution system biofilms (Lau and Ashbolt, 2009).

The bacteria themselves are weakly Gram-negative, small, motile rods that have precise nutritional requirements and as a result do not grow well on culture media. At least 50 different *Legionella* species have been identified, and approximately half of these species have been associated with disease. *Legionella pneumophila* (serogroup 1) is the agent responsible for most cases of illness in humans. Other than *L. pneumophila*, species causing far fewer infections but still considered to be clinically relevant include *L. micdadei*, *L. bozemanii*, *L. longbeachae* and *L. dumoffi* (Reingold et al., 1984; Doyle and Heuzenroeder, 2002; Roig et al., 2003).

B.2.1.1 Sources and exposure

Legionella species are naturally present in a wide range of freshwater environments, including surface water (Fliermans et al., 1981; Palmer et al., 1993) and groundwater (Brooks et al., 2004; Costa et al., 2005). The bacteria are not considered to be enteric pathogens and are not transmitted via the faecal–oral route. However, *Legionella* can occasionally be detected in human faecal samples, as diarrhoea is a symptom of illness in a small percentage of cases (Rowbotham, 1998). Similarly, animals are not reservoirs for *Legionella* (U.S. EPA, 1999a).

Legionella can be isolated from human-made systems (e.g., cooling towers, hot water tanks, showerheads, aerators) and are most frequently associated with biofilms (Lau and Ashbolt, 2009). In general, the amount of legionellae in source waters is low compared with the concentrations that can be reached in human-made systems (Mathys et al., 2008). In an investigation of biofilm formation and *Legionella* colonization on various plumbing materials (Rogers et al., 1994), the detection of *Legionella* was greater in biofilm than in free water but varied in time and with different plumbing materials. Biofilms are important for the survival of the fastidious legionellae: they provide protection to *Legionella*, which are also able to utilize nutrients supplied by other organisms in this nutrient-rich environment (Borella et al., 2005; Temmerman et al., 2006; Lau and Ashbolt, 2009).

Some naturally occurring waterborne protozoa, such as *Acanthamoeba*, *Hartmanella*, *Naegleria*, *Valkampfia* and *Echinamoeba*, can also harbour *Legionella* organisms (Rowbotham, 1986; Kilvington and Price, 1990; Kramer and Ford, 1994; Fields, 1996). *Legionella* can infect and remain within the protozoan cyst form, where they are protected from disinfectants (Kilvington and Price, 1990; Thomas et al., 2004; Declerck et al., 2007). They are also able to multiply within these protozoa, which has been proposed as the only way that *Legionella* can replicate within aquatic systems (Abu Kwaik et al., 1998; Thomas et al., 2004). Thus, as well as

offering protection, this association suggests a mechanism for the increase and transport of *L. pneumophila* in human-made systems (Declerck et al., 2009).

Temperature is an additional factor that influences *Legionella* colonization of water systems. Temperatures between 20°C and 50°C are hospitable for colonization, although legionellae typically grow to high concentrations only at temperatures below 42°C (Percival et al., 2004).

Plumbing systems outside of public water supply systems (e.g., in residential buildings, hotels, institutional settings) are most commonly implicated in *L. pneumophila* infections (Yoder et al., 2008). As *Legionella* is a respiratory pathogen, systems that generate aerosols, such as cooling towers, whirlpool baths and showerheads, are the more commonly implicated sources of infection. The hot water supply system is commonly pinpointed as the origin of the contamination (Hershey et al., 1997; McEvoy et al., 2000; Borella et al., 2004; Oliver et al., 2005; Burnsed et al., 2007; Yoder et al., 2008). However, the cold water supply, when held at about 25°C, which is within the range of *Legionella* multiplication, has also been implicated (Hoebe et al., 1998; Cowgill et al., 2005). *Legionella* infection can occur when people breathe in aerosolized water containing the bacteria or aspirate water containing the bacteria. The bacteria have not been found to be transmitted from person to person (U.S. EPA, 1999a).

Legionella contamination is particularly troublesome in hospitals, where susceptible individuals can be exposed to aerosols containing hazardous concentrations of *L. pneumophila*. Large buildings such as hotels, community centres, industrial buildings and apartment buildings are most often implicated as sources of outbreaks (Reimer et al., 2010). Studies have shown that contamination of domestic hot water systems with *Legionella* can occur in single-family homes (Joly, 1985; Alary and Joly, 1991; Stout et al., 1992a; Marrie et al., 1994; Dufresne et al., 2012). In a study of hot water plumbing systems in homes in the Québec area, Alary and Joly (1991) reported that *Legionella* was detected in 39% (69/178) of hot water tanks with electric heaters and in 0% (0/33) of tanks with oil- or gas-fired heaters. The authors further observed that in a proportion of those homes whose hot water tanks tested positive for *Legionella*, the organism could also be detected at distal locations, such as faucets (12%) and showerheads (15%). The position of the heat source in the design of electrically heated hot water tanks observed at the time of the study was cited as the reason for the difference in contamination between the two water heater types. In the electric tanks, the heating elements were located above the bottom of the heater, which could allow bottom sediments and water below the heating element to remain at the lower temperatures (< 50–60°C) permissible for *Legionella* growth (Alary and Joly, 1991). Although the presence of the bacteria in the home can increase the risk of infection in susceptible individuals, it does not necessarily mean that occupants will develop the illness. In addition, although outbreaks generally do not occur in residential settings, individual cases have been identified as originating from residential plumbing (Falkinham et al., 2008).

Similarly, evidence has been provided that sporadic cases of Legionnaires' disease can plausibly be acquired from aerosols in residential plumbing systems (Stout et al., 1992b; Straus et al., 1996; Lück et al., 2008). In a study conducted in the province of Quebec, Dufresne et al. (2012) observed that among 36 legionellosis-confirmed patients residing in homes with domestic hot water tanks, residential and clinical isolates of *Legionella* were microbiologically related by pulse-field gel electrophoresis in 14% (5/36) of the cases. Similar studies conducted in Pittsburgh, Pennsylvania (Stout et al., 1992b), and the state of Ohio (Straus et al., 1996) showed comparable results.

Persons thought to be at the highest risk of contracting Legionnaires' disease are those with lung conditions or compromised immune systems (e.g., persons receiving transplants or

chemotherapy, persons with diabetes or kidney disease). The risk of infection is higher among persons 40–70 years of age, and the disease is seen more frequently in males than in females (Percival et al., 2004). Other risk factors include smoking and excessive use of alcohol. Legionnaires' disease is considered a very rare cause of pneumonia in children. In contrast, age, gender and smoking do not seem to be risk factors for Pontiac fever (Diederen, 2008).

The concentration of *Legionella* required to cause infection is not well understood (Armstrong and Haas, 2008). It has been suggested that amoebae harbouring *Legionella* may increase the potential for infectivity by providing a mechanism to expose humans to hundreds of *Legionella* cells if inhaled or aspirated in an aerosol (Rowbotham, 1986; Greub and Raoult, 2004).

B.2.1.2 Health effects

As mentioned previously, there are two distinct illnesses caused by *Legionella*: Legionnaires' disease and Pontiac fever. Collectively, these illnesses are referred to as legionellosis. Legionnaires' disease is a serious respiratory illness involving pneumonia. Other features include fever, cough and headache, chest and muscle pain, and a general feeling of unwellness (malaise) (Fields et al., 2002). The time from the point of infection to the onset of symptoms is about 2–10 days, and the disease period can last up to several months. One problem in diagnosing Legionnaires' disease is a lack of any specific symptom that distinguishes it from other bacterial pneumonias. Reported rates of Legionnaires' disease in Canada over the period 2000–2004 (the latest year for which data have been published) ranged from 0.13 to 0.20 cases per 100 000 population (PHAC, 2006). The mortality rate of Legionnaires' disease in the United States as of 1998 was reported at roughly 10% and 14% for community-acquired and hospital-acquired cases, respectively (Benin et al., 2002). Early diagnosis and antibiotic therapy are keys to successfully treating Legionnaires' disease.

Pontiac fever is a less serious respiratory illness that does not involve pneumonia and is more flu-like in nature. The time to the onset of symptoms is 24–48 hours (AWWA, 2006). The disease is self-limiting and typically resolves without complications in 2–5 days. No known fatalities have been reported with this illness. Pontiac fever is difficult to distinguish from other respiratory diseases because of a lack of specific clinical features. Experts have speculated that the disease may be caused by exposure to a mixture of live and dead *Legionella* cells and non-*Legionella* endotoxin (Diederen, 2008). Antibiotic treatment is typically not prescribed because of the short, self-limiting nature of the disease.

B.2.1.3 Treatment technology

Successful control of *Legionella* in drinking water supplies requires focused attention not only on the organisms themselves, but also on the control of free-living amoebae and biofilms that support their persistence.

Physical removal mechanisms used during drinking water treatment, such as conventional filtration (i.e., coagulation, flocculation and sedimentation), will reduce the number of *Legionella* present in finished water. Disinfection strategies shown to be effective in reducing the number of *Legionella* present include the use of chlorine, monochloramine, chlorine dioxide ozone and UV. However, it must be noted that chlorine dioxide (Health Canada, 2008) and ozone (U.S. EPA, 1999b) are generally not effective in maintaining a disinfectant residual in the distribution system and UV does not provide a disinfectant residual. In comparison with *E. coli*, *Legionella* cells have been shown to be more resistant to chlorination (Delaedt et al., 2008; Wang et al., 2010).

Survival strategies exhibited by the organisms (colonization of biofilms and residence within free-living amoebae) also further protect *Legionella* from the action of disinfectants.

In the distribution system, currently recommended disinfectant residuals are sufficient to keep the concentration of non-biofilm-associated *Legionella* at levels that have not been associated with disease (Storey et al., 2004; Delaedt et al., 2008).

Various alternative disinfection methods have been examined for their potential to control *Legionella* colonization in municipal drinking water distribution systems. Monochloramine has been shown to be more effective than chlorine as a residual disinfectant against legionellae. Weintraub et al. (2008) observed that converting from chlorine to monochloramine for residual disinfection in a municipal distribution system resulted in a significant reduction in the number of distribution samples, point-of-use sites and water heaters positive for *Legionella* colonization. Pryor et al. (2004) studied the impact of changing the secondary disinfectant from chlorine to chloramine on the microbiological quality of the drinking water in a utility. Samples were taken from the source water, from the distribution system and at the point of use (i.e., showerheads). The authors found that there was a decrease in the colonization rate and the variety of *Legionella* species present in samples from the distribution system and showerheads when the disinfectant was changed from chlorine to choramine. However, despite the decrease in the variety of *Legionella* species, there was no indication of a decrease in *L. pneumophila* at the point of use (Pryor et al., 2004).

Kool et al. (1999) reported that hospitals supplied with water containing monochloramine for secondary disinfection were less likely to have reported outbreaks of Legionnaires' disease than those supplied with water containing free chlorine. Monochloramine is considered better able to penetrate into biofilms (LeChevallier et al., 1980), more stable and therefore able to maintain its concentration over greater distances in the distribution system (Kool et al., 1999).

It has been suggested that ozone may be a more effective disinfectant against *Legionella* than chlorine, but its main drawback is that it does not provide a disinfectant residual (U.S. EPA, 1999b; Kim et al., 2002; Blanc et al., 2005). Loret et al. (2005) observed that ozone at a concentration of 0.5 mg/L was effective in reducing *Legionella*, protozoa and biofilms in a model distribution system, but it was not as effective as chorine (2 mg/L) or chlorine dioxide (0.5 mg/L). Conversely, ozone at a concentration of 0.1–0.3 µg/mL (0.1–0.3 mg/L) was shown to be as effective as free chlorine at 0.4 mg/L in inactivating *Legionella* suspensions, producing a 2 log reduction of the organism within 5 minutes in laboratory experiments (Dominique et al., 1988). There are also a variety of supplemental strategies that can be used to control *Legionella* in the plumbing systems of large commercial, industrial and residential buildings.

Hyperchlorination has been employed as a control strategy in large buildings, including hospitals. However, studies have demonstrated that *Legionella* residing in biofilms or in cysts of *Acanthamoeba polyphaga* can survive following exposure to free chlorine at 50 mg/L (Kilvington and Price, 1990; Cooper and Hanlon, 2010). There is also the concern of an increased potential for corrosion of plumbing systems with continued high concentrations of chlorine (Kool et al., 1999).

Chlorine dioxide has demonstrated similar advantages over chlorine as a residual disinfectant for *Legionella* control when applied to a small distribution system such as a hospital complex. Sidari et al. (2004) observed significantly decreased *Legionella* concentrations at hot and cold point-of-use sites of a hospital's plumbing system upon switching to chlorine dioxide as the residual disinfectant.

In plumbing systems of hospitals and large buildings, thermal disinfection (elevating hot water to temperatures above 70°C, and flushing use points such as taps and showerheads) has

been routinely employed either on its own or in conjunction with chemical disinfection. Typically this is recognized as a temporary control strategy, as *Legionella* recolonization can occur within weeks to months of treatment (Storey et al., 2004).

Use of copper–silver ionization systems has also received much study and has shown effectiveness in controlling *Legionella* in drinking water supplies (Stout et al., 1998; Kusnetsov et al., 2001; Stout and Yu, 2003; Cachafeiro et al., 2007). Stout et al. (1998) observed that copper–silver ionization (mean copper and silver concentrations of 0.29 mg/L and 0.054 mg/L, respectively, in the hot water tank) was more effective than a superheat-and-flush method in reducing the recovery of *Legionella* from a hospital plumbing/distribution system. In a survey of the experiences of hospital systems using copper–silver ionization, Stout and Yu (2003) reported that following installation of the disinfection systems, the percentages of hospitals reporting (1) cases of Legionnaires' disease and (2) positive *Legionella* samples at more than 30% of the sites measured had both been reduced to 0% from 100% and 47%, respectively, before the systems had been installed. It is important to note that use of these systems should include monitoring of copper and silver concentrations in the water, as these concentrations will increase. In addition, pretreatment may be required to address pH and hardness challenges (Bartram et al., 2007).

Additional measures used for large plumbing systems include temperature control; control of water system design and construction to prevent the accumulation of biofilms, sediments or deposits; and nutrient control strategies (Bartram et al., 2007; Bentham et al., 2007).

General recommendations regarding the control of *Legionella* in domestic plumbing systems involve maintaining proper water temperatures. The National Plumbing Code of Canada includes requirements of a minimum water temperature of 60°C in hot water storage tanks, to address the growth of *Legionella* (NRCC, 2010). Where increased hot water temperatures create an increased risk of scalding for vulnerable groups (e.g., children and the elderly), appropriate safety measures should be applied to limit the temperature to 49°C. Thermostatic or pressure-balanced mixing valves can be installed to control the water temperature at the tap to reduce the risk of scalding (Bartram et al., 2007; Bentham et al., 2007).

Control of *Legionella* in water systems outside of plumbing systems also requires controlling its growth in biofilms. The heating, ventilation and air conditioning industry has guidelines for reducing *Legionella* growth in cooling systems (ASHRAE, 2000). Hotel and lodging industry requirements for the operation and maintenance of plumbing facilities, including procedures for the proper disinfection of plumbing equipment in their facilities, are generally specified under various public health regulations and/or legislation. To obtain information on these requirements, the appropriate provincial or territorial ministry of health should be consulted.

B.2.1.4 Assessment

The increasing importance of *Legionella* as a cause of human infection can in part be linked to continued human development and the resulting dependence on human-made plumbing systems (Fields et al., 2002). Despite being ubiquitous in source waters, *Legionella pneumophila* and other *Legionella* species have been recovered only in low concentrations from Canadian drinking water supplies (Dutka et al., 1984; Tobin et al., 1986), and people do not get infected with *Legionella* by consuming drinking water. As *Legionella* is a respiratory pathogen, infection can occur if people breathe in contaminated aerosols. Thus, the presence of *Legionella* becomes a problem only when they are able to grow to high numbers in water systems such as showers, cooling towers or whirlpool baths that generate aerosols or sprays. These plumbing systems have been implicated in outbreaks, but are mainly outside of the control of municipal water treatment

and distribution. For these reasons, the presence of the organism in low numbers in the distribution network is not sufficient evidence to warrant remedial action in the absence of disease cases (Dufour and Jakubowski, 1982; Tobin et al., 1986).

Owing to the existence of *Legionella* outside of faecal sources in nature, *E. coli* is not expected to be a reliable indicator of the presence of these bacteria. No suitable indicators have been identified to signal increasing concentrations of *Legionella* in a building's plumbing system. There is some evidence that increasing *Legionella* concentrations are accompanied, or preceded, by elevated heterotrophic plate count (HPC) measurements (WHO, 2002). However, the correlation between HPC and *Legionella* is not consistent.

Legionella have also been included on the U.S. Environmental Protection Agency's (EPA) Candidate Contaminant List as one of the priority contaminants for regulatory decision-making and information collection (U.S. EPA, 2009). Guidelines or regulations that have been developed for *Legionella* in Canada, the United States and other countries worldwide relate to control of the organism in water environments outside of municipal water distribution networks (e.g., piped water systems, cooling towers, health care facilities) (Cunliffe, 2007).

B.2.2 *Mycobacterium avium* complex

The *Mycobacterium avium* complex (Mac)[1] is a group of environmental mycobacteria that can cause illness in humans. The group consists of *Mycobacterium avium* (includes subspecies *avium*, *sylvaticum* and *paratuberculosis*); and *Mycobacterium intracellulare* (Cangelosi et al., 2004). Mac organisms are considered ubiquitous in natural waters. Transmission is primarily through contact with contaminated waters via either ingestion or inhalation (AWWA, 2006). Mac-related disease comes mainly in the form of lung infections and occurs largely in persons who have suppressed immune systems (Percival et al., 2004).

Mycobacteria themselves are motile, rod- to coccoid-shaped bacteria that have characteristically high levels of waxy lipids in their cell walls. They are Gram negative, but are more commonly considered to be "acid-fast" because of the way their cell walls respond to diagnostic staining procedures (AWWA, 2006). Mac organisms are also referred to as "non-tuberculous" or "atypical" mycobacteria. This is to distinguish them from the more well-known mycobacteria species that are responsible for tuberculosis and leprosy, which are not a concern for drinking water (Nichols et al., 2004). Other environmental mycobacteria are known that have been linked to skin infections through waterborne contact, but these are also of lesser importance to drinking water supplies (Nichols et al., 2004).

B.2.2.1 Sources and exposure

Mac organisms are natural inhabitants of water and soil environments (Falkinham, 2004). Water is considered the main reservoir (Percival et al., 2004; Vaerewijck et al., 2005), and the organisms can be encountered in natural aquatic systems worldwide, including marine waters and freshwater lakes, streams, ponds and springs (Falkinham, 2004; Percival et al., 2004). Mac organisms can be encountered in drinking water supplies, but generally in low numbers and at low frequency (Peters et al., 1995; Covert et al., 1999; Falkinham et al., 2001; Hilborn et al., 2006). However, Mac bacteria can survive in distribution system biofilms and grow there to

[1] For the purpose of this document, the acronym Mac will be used for *Mycobacterium avium* complex instead of the usual MAC to avoid confusion with maximum acceptable concentration (MAC).

reach significant populations (Falkinham et al., 2001). Counts of *M. intracellulare* in biofilms were observed to reach 600 colony-forming units (CFU) per square centimetre, on average (Falkinham et al., 2001). Feazel et al. (2009) observed that mycobacteria were enriched in plumbing system (showerhead) biofilms, reaching counts 100 times, based on higher numbers of genes being sequenced, above those in water samples. In another study, Tsintzou et al. (2000) observed a statistically significant decrease in the presence of environmental mycobacteria in drinking water samples after the replacement of the city's water distribution network. The authors attributed the reduction to the absence of distribution system biofilms (Tsintzou et al., 2000). Surveys of water samples from water treatment plants and residential dwellings have reported Mac isolation rates of 2–60% (von Reyn et al., 1993; Glover et al., 1994; Peters et al., 1995; Covert et al., 1999; Hilborn et al., 2006). Hilborn et al. (2006) recovered *M. avium* from roughly 50–60% of point-of-use samples (cold water taps) served by two water treatment plants. Concentrations ranged from 200 to > 300 CFU/500 mL (Hilborn et al., 2006). Von Reyn et al. (1993) isolated Mac organisms from 17–25% of water supply samples collected from hot water taps at patient care facilities (hospitals and clinics).

Other studies have reported a failure to isolate Mac organisms from water systems, instead detecting only other non-Mac mycobacteria (von Reyn et al., 1993; Le Dantec et al., 2002a; September et al., 2004; Sebakova et al., 2008). It has been suggested that the likelihood of exposure to Mac bacteria in water is diverse in various areas of the world, but, in general, may be less in developing countries (von Reyn et al., 1993; September et al., 2004). Mac organisms in biofilms have also been found in other human-made systems, such as cooling towers (Pagnier et al., 2009), ice machines (LaBombardi et al., 2002), nebulizer reservoirs, toilets and sinks (AWWA, 2006) and water meters (Falkinham et al., 2001). Studies have reported the isolation of non-tuberculous mycobacteria from groundwater, although *M. avium* has not been frequently detected (Falkinham et al., 2001; Vaerewijck et al., 2005).

Similar to *Legionella*, the growth and survival of Mac organisms can be enhanced by their ability to invade and survive in free-living amoebae, such as *Acanthamoeba polyphaga* or *A. castellanii* (Cirillo et al., 1997; Steinert et al., 1998). A key difference between Mac and *Legionella*, however, is that Mac organisms are able to replicate outside of amoebae, in biofilms (Steinert et al., 1998; Vaerewijck et al., 2005).

The ubiquitous nature of Mac organisms results from their ability to survive and grow under varied conditions. Mycobacteria can survive in water with few nutrients. Archuleta et al. (2002) observed that *M. intracellulare* was capable of surviving for over a year in reverse osmosis–deionized water. Mac organisms have also been shown to grow in natural waters over wide ranges of pH (5–7.5), salinity (0–2%) and temperature (10–51°C) (Sniadack et al., 1992; Falkinham et al., 2001). Water conditions that have been identified as being more favourable for the growth of Mac organisms include high levels of humic and fulvic acids, high zinc concentrations, low pH and low dissolved oxygen levels (Kirschner et al., 1992, 1999; Vaerewijck et al., 2005).

Infection through contact with *M. avium* and *M. intracellulare* has been well documented (Wendt et al., 1980; Grange, 1991; Glover et al., 1994; Montecalvo et al., 1994; von Reyn et al., 1994; Kahana et al., 1997; Aronson et al., 1999; Mangione et al., 2001). Inhalation of contaminated aerosols, during contact with contaminated hot tubs, spa pools or similar facilities, is most frequently cited as the route and source of infection (Kahana et al., 1997; Mangione et al., 2001; Rickman et al., 2002; Cappelluti et al., 2003; Lumb et al., 2004; Sood et al., 2007). Person-to-person transmission of the organisms is thought to be uncommon (Falkinham, 1996; Nichols et al., 2004). Evidence of the link between water supplies, particularly hot water supplies and Mac

infection, has also been provided (von Reyn et al., 1994; Tobin-D'Angelo et al., 2004; Marras et al., 2005). Von Reyn et al. (1994) reported the detection of the same strain of *M. avium* in patients and hospital potable water supplies to which they had been exposed, but not in water supplies collected from patients' homes. Marras et al. (2005) documented a case of Mac-associated hypersensitivity pneumonitis where the patient strain was recovered from the shower and bathtub from the patient's home, but not the hot tub. Despite these links, it has been suggested that hospital and domestic drinking water–related cases represent a small proportion of Mac-related illness (von Reyn et al., 1994; Phillips and von Reyn, 2001). The infectious dose of Mac has not been well established. Rusin et al. (1997) proposed an oral infectious dose for mice of 10^4–10^7 organisms. True estimations of the inhaled infectious dose would be dependent upon (among other factors) the virulence of the organism and the immune status of the host.

B.2.2.2 Health effects

Mac organisms largely cause opportunistic infections in humans. Infections occur mostly in individuals who have weakened or suppressed immune systems (e.g., patients with acquired immunodeficiency syndrome [AIDS], the elderly or the very young) or persons with underlying respiratory conditions, such as cystic fibrosis. Mac-related disease rarely occurs in healthy people (Field et al., 2004). Mac organisms have low pathogenicity, so individuals can become colonized with the organisms without exhibiting any adverse health effects.

The main symptom of Mac lung infection is a chronic productive cough (cough with phlegm, saliva or mucus) (Field et al., 2004). Other symptoms can include fever, night sweats, fatigue and weight loss (Percival et al., 2004). However, it has been suggested that the secondary symptoms are less common unless the individual has extensive lung disease (Crow et al., 1957; Field et al., 2004). In those individuals with human immunodeficiency virus (HIV) or AIDS, Mac infection can spread to other parts of the body, including joints, skin, blood, liver and brain; the disease can be debilitating and life-threatening for these patients (Percival et al., 2004).

The true prevalence of Mac infections is not known, as it is not a reportable illness in Canada or the United States. Estimates of the rate of Mac-related pulmonary disease in the United States range from 1–2 cases to 5 cases per 100 000 persons per year, based on epidemiological studies conducted in various U.S. cities (Marras and Daley, 2002). Marras et al. (2007) estimated the prevalence of pulmonary non-tuberculous mycobacteria in Ontario to range from 9 to 14 positive isolations per 100 000 population over the years from 1997 to 2003. The authors further reported that, overall, Mac organisms were isolated in roughly 60% of the cases.

Mac diseases are treatable, but the clearing of these infections can be difficult, and treatment can have a high rate of failure (Field et al., 2004). Mycobacteria have demonstrated strong resistance to antimicrobial agents (Daley and Griffith, 2010). Antibiotics are delivered at high doses and often require a long administration period (e.g., several months to over a year) (Percival et al., 2004; Daley and Griffith, 2010).

B.2.2.3 Treatment technology

Water treatment technologies commonly used, including chemical disinfection and physical removal methods, have been tested for their ability to inactivate or remove mycobacteria from water supplies. Of these technologies, the most effective has been physical removal using conventional filtration (i.e., coagulation, flocculation and sedimentation). In one study, Falkinham et al. (2001) observed that water treatment plants treating surface water sources reduced mycobacteria numbers by 2–4 log through filtration and primary disinfection. A significant association between the frequency of detection of *M. avium* and high raw water

turbidity was also reported. The authors were careful to note that reducing turbidity could represent one approach to reducing mycobacteria in drinking water, but that this procedure alone may not be completely sufficient to eliminate *M. avium* from the distribution system (Falkinham et al., 2001). It is important to note that even with good removal of organisms from the source water, the number of Mac organisms may increase in the distribution system (Falkinham et al., 2001). Mac organisms are more resistant than other microorganisms to commonly used disinfectants. The high concentration of mycolic acid and the hydrophobic surface characteristics of mycobacteria are primarily responsible for their high resistance to chemical disinfection (LeChevallier, 2004).

In a study to evaluate the change in the microbiological population of a water distribution system by changing the secondary disinfectant from chlorine to chloramine, the authors reported the presence of mycobacteria from sites where the chlorine residual was above 3 mg/L. In this same study, samples from the distribution system and at the point of use (i.e., showerheads) were analyzed, and the authors reported that the colonization rate of mycobacteria in samples from the distribution system and showerheads increased when the disinfectant was changed from chlorine to chloramine (Pryor et al., 2004).

For chlorination, Le Dantec et al. (2002b) reported varying chlorine sensitivities among a collection of various mycobacteria isolated from the distribution system (note: Mac organisms were not isolated in this study). The authors calculated that a CT value of 60 mg·min/L (e.g., 0.5 mg/L for 2 hours) would result in a 1.5–4 log reduction for environmental mycobacteria. R.H. Taylor et al. (2000) provided data on the susceptibility of environmental and patient isolates of *M. avium* to various disinfectants: chlorine, monochloramine, ozone and chlorine dioxide. The mean $CT_{99.9}$ values (i.e., the CT values for a 3 log reduction) for the individual disinfectants were 51–204 mg·min/L for chlorine, 91–1710 mg·min/L for monochloramine, 0.10–0.17 mg·min/L for ozone and 2–11 mg·min/L for chlorine dioxide. The authors did note that there was significant variation in the susceptibility of different strains (R.H. Taylor et al., 2000).

In another study using chlorine dioxide, Vicuña-Reyes et al. (2008) reported $CT_{99.9}$ values ranging from 3 to 36 mg·min/L (5–30°C), prompting the authors to conclude that the disinfectant can be effective in controlling mycobacteria. Compared with the CT values necessary to inactivate *E. coli*, the CT values necessary for inactivation of Mac have been reported to range from near equivalency to a few times greater (monochloramine); to tens to hundreds of times greater (ozone, chlorine dioxide); to over 2000 times greater (chlorine) (R.H. Taylor et al., 2000). Data have been provided suggesting that mycobacteria are more sensitive than *Cryptosporidium* oocysts to chlorine, monochloramine, chlorine dioxide and ozone and are as sensitive as or more sensitive than *Giardia* to all of these, with the exception of free chlorine (Jacangelo et al., 2002; LeChevallier, 2004).

In UV disinfection studies, Hayes et al. (2008) demonstrated that patient and environmental strains of *M. avium* and *M. intracellulare* exhibited a greater than 4 log reduction at UV fluences less than 20 mJ/cm^2. The authors concluded that Mac organisms in free suspension could be readily inactivated by UV doses commonly employed in drinking water treatment (Hayes et al., 2008). LeChevallier (2004) reported that UV values required to inactivate mycobacteria are in the range of those required for other vegetative bacteria.

As stated above, there may be an increase of Mac organisms in the distribution system relative to levels leaving the treatment plant. By residing within biofilms or free-living amoebae, Mac organisms can further increase their resistance to inactivation. Steed and Falkinham (2006) observed that *M. avium* and *M. intracellulare* cells in biofilms were up to 1.8–4 times more resistant than cells in free suspension when exposed to chlorine. As with *Legionella*, successful

control of Mac organisms requires control of the free-living amoebae and biofilms that support their persistence.

Mac organisms have also demonstrated resistance to elevated temperatures. Several authors have reported recovery of *M. avium* from hot water systems at temperatures between 50°C and 57°C (du Moulin et al., 1988; von Reyn et al., 1994; Covert et al., 1999; Norton et al., 2004). Additional factors thought to play a role in encouraging growth in the distribution system include high assimilable organic carbon levels, as well as distribution system materials and construction (e.g., pipe materials, gaskets, coatings, corroded pipes, dead ends, spaces, long storage times) (Falkinham et al., 2001). Similar to the distribution system control strategies described for *Legionella* (Bartram et al., 2007; Bentham et al., 2007), temperature control; control of water system design and construction to prevent the accumulation of biofilms, sediments or deposits; and nutrient control strategies should also prove effective in the control of Mac organisms.

B.2.2.4 Assessment

No suitable indicators have been identified to signal increasing concentrations of Mac organisms in water systems. For example, studies have found no relationship between the numbers of non-tuberculous mycobacteria recovered from reservoir water and coliform counts, HPC and total and free chlorine levels (Glover et al., 1994; Aronson et al., 1999). There is some evidence that *M. avium* presence is associated with turbidity in raw waters (Falkinham et al., 2001), but further exploration of this issue is needed.

Currently, the presence of mycobacteria in water is not regulated by any country or international organization, including Canada. The U.S. EPA has identified *M. avium* and *M. intracellulare* as waterborne health-related microbes that need additional research on their health effects, their occurrence in water and their susceptibility to treatment methods. These organisms have also been included in a list of candidate contaminants for possible regulation by the U.S. EPA (2009). At the present time, there is not sufficient information to warrant actions based on the presence of the organisms in the absence of disease.

B.2.3 *Aeromonas*

The genus *Aeromonas* has gained public health recognition as including organisms that can cause opportunistic infections in humans. Species of *Aeromonas* have been associated with gastroenteritis; however, understanding of the role that the organisms play in causing diarrhoeal illness is currently incomplete. Skin, wound and soft tissue infections with *Aeromonas* species as a result of exposure to contaminated water in non–drinking water scenarios have been well documented. It is believed that drinking water has the potential to serve as a route of transmission, but direct evidence of *Aeromonas* as a cause of drinking water–acquired gastrointestinal illness is lacking.

Aeromonads are Gram-negative, short, rod-shaped bacteria that share some similarities with *Vibrio* and *E. coli.* They are universally found, occurring naturally in virtually all water types. The genus *Aeromonas* contains more than 17 distinct genetic species. Three species—*A. hydrophila, A. veronii* biovar *sobria* (syn. *A. sobria*) and *A. caviae*—account for roughly 85% of human infections and are therefore considered to be the species of most importance for drinking water systems (Janda and Abbott, 1998, 2010).

B.2.3.1 Sources and exposure

Aeromonas species can be found in virtually all surface water types (freshwater, marine and estuarine) in all but the most extreme conditions of pH, salinity and temperature (Percival et al., 2004; AWWA, 2006). They are less frequently detected in groundwater, with their presence in these systems typically indicating well contamination (Havelaar et al., 1990; Massa et al., 1999 Borchardt et al., 2003).

Aeromonads are recognized animal pathogens (Percival et al., 2004; AWWA, 2006). The organisms have been isolated from the gastrointestinal tracts and infected tissues of a number of cold-blooded and warm-blooded animals, most notably fish, birds, reptiles and domestic livestock (U.S. EPA, 2006; Janda and Abbott, 2010). They have also been recovered from retail food items, such as meat, poultry and dairy products (Janda and Abbott, 2010). It has been suggested that animals may be an environmental reservoir for *Aeromonas* (Janda and Abbott, 2010).

The organisms are not considered to be natural faecal pathogens (U.S. EPA, 2006). *Aeromonas* species are not normally found in human faeces in high numbers (Janda and Abbott, 2010); however, a small percentage of the population can carry the bacteria in their intestinal tracts without showing symptoms of disease (von Graevenitz, 2007). The prevalence of *Aeromonas* in human faecal samples worldwide has been roughly estimated to be 0–4% for asymptomatic persons and as high as 11% for persons with diarrhoeal illness (Burke et al., 1983; U.S. EPA, 2006; von Graevenitz, 2007; Khajanchi et al., 2010). Individual studies have observed rates as high as 27.5% and 52.4% for asymptomatic persons and diarrhoeal illness cases, respectively (Pazzaglia et al., 1990, 1991). Numbers of *Aeromonas* are much higher in sewage, with concentrations greater than 10^8 CFU/mL having been reported (Percival et al., 2004).

Levels of *Aeromonas* in clean rivers, lakes and storage reservoirs have generally been reported to be in the range of 1–10^2 CFU/mL (Holmes et al., 1996). *Aeromonas* concentrations in surface waters receiving sewage contamination and nutrient-rich waters in the warmer summer months may reach 10^3–10^5 CFU/mL (Holmes et al., 1996; U.S. EPA, 2006). Groundwaters generally contain less than 1 CFU/mL (Holmes et al., 1996). Drinking water immediately leaving the treatment plant typically contains concentrations in the range of < 1–10^2 CFU/mL (Holmes et al., 1996; U.S. EPA, 2006; Pablos et al., 2009; Janda and Abbott, 2010) , with potentially higher concentrations in drinking water distribution systems (Payment et al., 1988; Chauret et al., 2001; U.S. EPA, 2006). Concentrations in individual environments can be expected to vary; however, the organisms can survive over wide ranges of pH (5–10) and temperature (2–42°C) (Percival et al., 2004). Water temperature is particularly important to *Aeromonas* growth. In temperate climes during the warmer months of the year, the bacteria have been shown to be more readily detected in source waters and water distribution systems (Chauret et al., 2001; U.S. EPA, 2006; Janda and Abbott, 2010). Aeromonads are also very versatile nutritionally. They are capable of growing to elevated numbers in water with high organic content and can also survive in low-nutrient waters (Kersters et al., 1996).

Similar to other bacteria, *Aeromonas* species can enter into a viable, non-culturable state under stressful conditions in aquatic environments. There is some debate at to what effect this state has on a species' viability and pathogenicity. Maalej et al. (2004) reported that cells of a strain of *A. hydrophila* rendered non-culturable under marine stress conditions lost their haemolytic and cytotoxic properties, but that these could be regained following recovery at warmer temperatures. In contrast, Mary et al. (2002) observed that viable, non-culturable cells of *A. hydrophila* lost their viability and that this could not be regained following a temperature

upshift to 25°C. It has been suggested that survival properties may differ depending on the species and strain of *Aeromonas* (Brandi et al., 1999; Mary et al., 2002).

The organisms have been detected in the distribution systems of chlorinated drinking water supplies worldwide (Chauret et al., 2001; Emekdas et al., 2006; Långmark et al., 2007; September et al., 2007). As with other bacterial pathogens, the formation of biofilms and the presence of free-living amoebae have been identified as factors contributing to higher concentrations of *Aeromonas* encountered in drinking water distribution systems relative to finished water (September et al., 2007; Rahman et al., 2008). During an assessment conducted as part of their Unregulated Contaminant Monitoring Regulations, the U.S. EPA (2002) provided data indicating that *Aeromonas* could be detected in 11% of municipal systems serving more than 10 000 persons and 14% of systems serving fewer than 10 000 persons. The concentrations of *Aeromonas* reported were less than 10 CFU/100 mL in 78% of the samples (U.S. EPA, 2002). Limited studies have been conducted on *Aeromonas*–protozoa interactions within municipal supplies. Rahman et al. (2008) observed that the bacteria may use the free-living amoeba *Acanthamoeba* as a reservoir to improve transmission and for protection from disinfectants.

Exposure to *Aeromonas* species through direct contact of wounds or skin follicles with contaminated waters has been reported for recreational-type water environments, such as lakes, rivers, swimming pools and hot tubs (Gold and Salit, 1993; Manresa et al., 2009). Unusual water situations brought about by floods or disaster events can be expected to create similar opportunities for *Aeromonas* exposure. Wound infection with species of *Aeromonas* was a problem among victims of the tsunami in Thailand as a result of exposure to contaminated floodwaters (Hiransuthikul et al., 2005). Exposure to *Aeromonas* in contaminated floodwaters was also expected among victims and rescue workers following Hurricane Katrina (Presley et al., 2006). Person-to-person transmission of *Aeromonas* resulting in infection is not expected to occur (U.S. EPA, 2006).

The evidence for acquiring *Aeromonas* infection through the ingestion of drinking water is not well established, and this route of transmission is the subject of some debate (von Graevenitz, 2007). The presence of *Aeromonas* in finished drinking water supplies and distribution samples has been well documented, suggesting a possible route of transmission (LeChevallier et al., 1980; Payment et al., 1988; Kuhn et al., 1997; Borchardt et al., 2003; Emekdas et al., 2006; de Oliveira Scoaris et al., 2008). However, other findings have been cited that oppose this suggestion. Epidemiological investigations have demonstrated little evidence of direct connections between patient isolates of *A. hydrophila* and isolates recovered from their drinking water supplies. Borchardt et al. (2003) observed that *Aeromonas* isolates were infrequently found in stool samples of gastroenteritis patients and that those detected were not genetically related to isolates recovered from drinking water. Additionally, researchers have cited the virtual absence of reported outbreaks of diarrhoea against the near-universal presence of *Aeromonas* in water environments as evidence supporting the transmission of these organisms by a mechanism other than through drinking water (von Graevenitz, 2007; Janda and Abbott, 2010). Some researchers have speculated that for many faecal isolates of *Aeromonas*, colonization of the human gastrointestinal tract may only be fleeting (Janda and Abbott, 2010).

The ingested dose of *Aeromonas* necessary to cause gastrointestinal infections is uncertain. Limited study has suggested that a high dose is required (U.S. EPA, 2006; Janda and Abbott, 2010). In an early volunteer feeding study, Morgan et al. (1985) reported that only 2 of 57 individuals developed diarrhoea following ingestion of *A. hydrophila* strains at doses of up to 10^{10} CFU. It has been speculated that the concentrations required to cause illness are much higher

than the numbers that would typically be found in treated drinking water supplies (U.S. EPA, 2006).

Recently, in a large survey of clinical and waterborne strains of *Aeromonas* collected from across the United States and worldwide, Khajanchi et al. (2010) reported detecting three isolates belonging to the *A. caviae* group that were genetically indistinguishable and possessed the same virulence factors. The authors suggested that these findings provided the first evidence of human infection and colonization by a waterborne *Aeromonas* strain.

B.2.3.2 Health effects

Aeromonas-associated diarrhoea has been encountered worldwide, mostly in normally healthy persons across all age groups (Janda and Abbott, 2010). Having low stomach acidity, receiving antimicrobial therapy and having compromised immune function (e.g., from HIV infection or through underlying disease, especially liver disease) are thought to be associated risk factors (Merino et al., 1995; Percival et al., 2004; von Graevenitz, 2007; Janda and Abbott, 2010). The association between *Aeromonas* and gastrointestinal illness is controversial (von Graevenitz, 2007; Janda and Abbott, 2010). Case reports and a small number of foodborne outbreaks have linked the presence of *Aeromonas* to cases of diarrhoeal disease (U.S. EPA, 2006; Janda and Abbott, 2010). However, at present, no outbreaks of gastrointestinal illness have been reported for which a strain of *Aeromonas* has been definitely identified as the causative agent (Janda and Abbott, 2010). Furthermore, researchers have been unable to find an animal model in which *Aeromonas*-mediated gastrointestinal illness can be replicated (U.S. EPA, 2006; Janda and Abbott, 2010).

Where *Aeromonas* species have been associated with gastroenteritis, the most common symptom is watery diarrhoea, accompanied by fever and abdominal pain (Janda and Abbott, 2010). Far less commonly, *Aeromonas* has been identified in association with other forms of gastrointestinal illness, ranging from a dysenteric type of illness with bloody stools to a chronic or subacute watery diarrhoea (Janda and Abbott, 2010). *Aeromonas* infections can also be asymptomatic, with individuals shedding the bacteria in their stools, but not showing any symptoms of disease (Percival et al., 2004).

Aeromonas species have been positively isolated from skin, wound and soft tissue infections (Percival et al., 2004; Janda and Abbott, 2010). These can range in scope from mild irritations (e.g., pus-filled lesions) to cellulitis (inflammation below the skin) to, in extreme cases, necrotizing fasciitis (flesh-eating disease) (Janda and Abbott, 2010). These are often the result of trauma or penetrating injury from occupational or recreational water exposure and are generally seen more frequently in adults than in children. *Aeromonas* has also recently been implicated in respiratory infections. However, these have been rare and have largely been caused by near-drownings or aspirations of contaminated waters unrelated to drinking water supplies (Janda and Abbott, 2010)

Factors responsible for the pathogenicity and virulence of *Aeromonas* species or strains are poorly understood. A number of potential virulence components have been identified that would appear to enable the organisms to behave as human pathogens. These include components such as pili, fimbriae and flagella for attachment and colonization; external lipopolysaccharides, capsules or surface layers to assist in evading host defences; and toxins, haemolysins, proteases and other enzymes for causing damage to host cells (von Graevenitz, 2007; Janda and Abbott, 2010). Current studies have been unable to specifically pinpoint which combination of factors would make a strain of *Aeromonas* behave as an enteropathogen (Janda and Abbott, 2010). Research has identified that a known diarrhoea-causing strain of *A. hydrophila* possesses four

prospective virulence factors: two haemolysins (Act and HlyA), a heat-stable enterotoxin (Ast) and a heat-labile enterotoxin (Alt) (Erova et al., 2008 Janda and Abbott, 2010). Despite such findings, the role and relative significance of each remain uncertain, as studies have also found these factors distributed among numerous clinical and environmental strains in different combinations (Erova et al., 2008 von Graevenitz, 2007; Castilho et al., 2009; Janda and Abbott, 2010). It has been proposed that only certain subsets of *Aeromonas* strains have the ability to cause disease (Janda and Abbott, 2010).

Aeromonas is not a reportable organism in North America or in most countries worldwide (Janda and Abbott, 2010; PHAC, 2010). Of the case reports or outbreaks of *Aeromonas*-related illness encountered in the literature, most have been tied to food, hospitals, travel or non-water environments, or their causes are unknown. At present, no epidemiological evidence has been provided linking an *Aeromonas* outbreak to ingestion, inhalation or skin contact with treated drinking water supplies (U.S. EPA, 2006; von Graevenitz, 2007; Janda and Abbott, 2010).

As *Aeromonas*-related gastrointestinal illness is mild and self-limiting, treatment for infection is generally not necessary. However, for other presentations of infection, antibiotic therapy is usually implemented. Aeromonads are resistant to ampicillin and a variety of other β-lactam antibiotics, including penicillin and some cephalosporins (Percival et al., 2004; Janda and Abbott, 2010).

B.2.3.3 Treatment technology

As mentioned previously, aeromonads are ubiquitous in many water environments. Consequently, they will be present in most source waters used for drinking water production. Nonetheless, existing evidence indicates that current treatment and disinfection methods can effectively remove *Aeromonas* from drinking water. Data from pilot-scale (Harrington et al., 2003; Xagoraraki et al., 2004) and full-scale (Chauret et al., 2001; El-Taweel and Shaban, 2001; Yu et al., 2008) investigations have demonstrated that well-operated conventional filtration systems (i.e., coagulation, flocculation and sedimentation) are capable of *Aeromonas* removals of up to 4 log. In a pilot-scale conventional treatment study, Xagoraraki et al. (2004) observed that reducing filter effluent turbidity to less than 0.2 NTU resulted in *A. hydrophila* removals of > 3 log to just under 4 log (median: 3.5 log). Yu et al. (2008) investigated the effectiveness of different water treatment processes in removing *Aeromonas* as measured using both culture-based and real-time polymerase chain reaction (PCR) detection methods. Conventional filtration (three full-scale plants) resulted in removals of culturable *Aeromonas* ranging from > 0.3 log to 4 log (Yu et al., 2008). The authors further reported that no culturable *Aeromonas* could be detected after sedimentation. Log removals as measured by real-time PCR detection correlated well with, but were routinely lower than, those demonstrated by the culture-based detection method (Yu et al. 2008).

For slow sand filtration, the authors examined one pilot-scale and two full-scale plants, reporting log removals of > 1 log (> 1 log for the pilot-scale plant and > 1.8 log for the full-scale plants). Culturable *Aeromonas* was not detected in samples collected post-filtration (Yu et al., 2008). Meheus and Peeters (1989) reported similar results for slow sand filtration, observing *Aeromonas* removals of 98–100%.

With membrane filtration, a full-scale plant included in the Yu et al. (2008) study demonstrated a capability of removing culturable *Aeromonas* by > 3.8 log.

Aeromonads are susceptible to inactivation by disinfectants commonly used in drinking water treatment, such as chlorine, monochloramine, chlorine dioxide, ozone and UV (Knøchel, 1991; Medema et al., 1991; Sisti et al., 1998; U.S. EPA, 2002, 2006). For chlorination, Sisti et al.

(1998) reported *Aeromonas* T_{95} values of 5 minutes at a free chlorine concentration of 0.6 mg/L and 68 minutes at a free chlorine concentration of 0.05 mg/L in a laboratory-scale chlorination experiment. The authors also found *Aeromonas* (clinical strains) to be more susceptible to chlorine than *E. coli* (clinical strains). Free chlorine concentrations of 0.14 mg/L (10°C) and > 0.5 mg/L (20–37°C) were sufficient to produce a 5 log inactivation of clinical and nosocomial strains of *Aeromonas* within 5 minutes in an experiment conducted by Chamorey et al. (1999). In contrast, de Oliveira Scoaris et al. (2008) observed that the majority of *Aeromonas* strains (water and culture collection strains) were not killed after 1 minute of exposure to free chlorine at 1.2 mg/L.

Chauret et al. (2001) conducted a study at both full scale and pilot scale simultaneously to assess the presence of *Aeromonas* in source water and at various sites within the treatment plant and distribution system and to assess biofilm formation. The authors noted no detectable *Aeromonas* in treated water immediately after secondary disinfection with chloramine (dose range: 2–3 mg/L), despite observing counts ranging from < 1 to 490 CFU/100 mL after chlorine disinfection (pre-filtration) and post–granular activated carbon filtration.

With chlorine dioxide, Medema et al. (1991) reported CT_{99} values of 0.04–0.14 mg·min/L for a drinking water strain of *A. hydrophila*. In the same study, a naturally occurring *Aeromonas* population (predominantly *A. sobria*) was observed to be slightly more sensitive, with a reported CT_{99} of 0.1 mg·min/L.

For UV disinfection, data produced by the U.S. EPA (2002) suggested the capability for a 1 and 2 log inactivation of *A. hydrophila* at doses of 3 and 8 mWs/cm^2, respectively (equivalent to 3 and 8 mJ/cm^2)—doses significantly less than those commonly employed in water treatment.

In the distribution system, maintaining an adequate disinfectant residual should provide control of *Aeromonas* in the finished water. The potential exists for *Aeromonas* to regrow in the distribution system, however. During a year-long survey of a major drinking water distribution system in Scotland, Gavriel et al. (1998) reported that although *Aeromonas* was not detected in water samples collected downstream from chlorination prior to the distribution network, it could occasionally be recovered from distribution samples, even at locations maintaining a substantial chlorine residual (> 0.2 mg/L). Similarly, other studies have demonstrated that *Aeromonas* could be detected in municipal distribution systems at locations having temperatures below 14°C and chlorine residuals above 0.2 mg/L (Chauret et al., 2001; Pablos et al., 2009).

Elimination of *Aeromonas* in the distribution system once the organisms become established in biofilms can be difficult (Holmes and Nicolls, 1995; Gavriel et al., 1998; Långmark et al., 2007). Aeromonads sequestered in biofilms resist disinfection and persist for long periods (U.S. EPA, 2006). Elements important for helping to control *Aeromonas* growth include limiting the number of organisms entering the distribution system through effective treatment, maintaining low water temperatures, providing appropriate free chlorine residuals, limiting the levels of organic carbon compounds and proper maintenance of the distribution system (WHO, 2010).

B.2.3.4 Assessment

Some studies have been undertaken to determine whether the indicators currently used in the drinking water industry, including *E. coli*, total coliforms and HPC, can be used as surrogates for the presence of *Aeromonas*. Several studies have showed no evidence of a relationship between *Aeromonas* incidence and coliforms, *E. coli* or HPC (Holmes et al., 1996; Gavriel et al., 1998; Fernández et al., 2000; Pablos et al., 2009). Although no direct correlation exists between *Aeromonas* populations and total HPC, the organisms do make up a portion of HPC bacteria

found in water and are detected by HPC tests (Pablos et al., 2009). The Netherlands has established drinking water standards for *A. hydrophila*, consisting of a median value (over a 1-year period) of 20 CFU/100 mL in water leaving the treatment plant and a 90th percentile value (over a 1-year period) of 200 CFU/100 mL in distribution system water (van der Kooij, 2003; Pablos et al., 2009). These values have been based on an assessment of achievability and are motivated by a precautionary approach, rather than on the public health significance of their occurrence in drinking water (WHO, 2002).

Aeromonas is not considered to be an indicator of faecal contamination or treatment failure (U.S. EPA, 2002). The organisms have been proposed as a possible supplemental indicator of drinking water quality by relating to the presence of biofilm. Therefore, if there are significant increases in *Aeromonas* concentrations in a drinking water supply, this indicates a general deterioration of bacteriological quality.

When looking at the overall public health significance of *A. hydrophila* in drinking water, further epidemiological studies are needed for a better understanding of the relationship between *Aeromonas* illness and the presence of these organisms in drinking water. Based on the current evidence, treated drinking water likely represents a very low risk. It has been proposed that in comparison with other pathogens that can potentially be acquired through drinking water, *Aeromonas* is at the low end of the scale in terms of relative risk (Rusin et al., 1997; Janda and Abbott, 2010). Nevertheless, it is advisable to minimize *Aeromonas* levels in drinking water supplies as much as is practical until its public health significance has been fully investigated.

B.2.4 *Helicobacter pylori*

Helicobacter pylori is a recognized human pathogen that can colonize the stomach. The understanding of how this organism is spread is still quite limited; however, it is believed that there are a few routes of transmission, including through drinking water (Percival and Thomas, 2009). The majority of people infected with *H. pylori* are asymptomatic, and they may live their entire lives with the organism. However, more serious disorders, such as peptic ulcers or stomach cancer, can develop in a small percentage of cases.

Helicobacter are Gram-negative, motile, small curved rods that are closely related to *Campylobacter*. The organisms have two distinct forms, a spiral rod shape and a shorter coccoid form, which is taken on under conditions of stress. To date, the coccoid form has been found to be non-culturable. The genus *Helicobacter* contains at least 25 species, as determined by deoxyribonucleic acid (DNA) sequencing, of which *H. pylori* is the species of relevance for the water industry. Other *Helicobacter* species have been detected in humans that have been associated with gastric illness; however, these are not considered to be as prevalent as *H. pylori*.

B.2.4.1 Sources and exposure

The primary reservoir identified for *H. pylori* is the human stomach (Dunn et al., 1997; Brown, 2000). It has been suggested that some animals (i.e., cats, dogs, sheep, primate monkeys) can be infected by *H. pylori*, but the consensus at present is that they do not play a significant role as reservoirs in transmitting this organism to humans (Baele et al., 2009; Haesebrouck et al., 2009). Although *H. pylori* has been cultured from human faeces, its isolation from water using culture methods has not been successful to date (Percival and Thomas, 2009). It is believed that the spiral culturable form rapidly transforms into a viable, non-culturable state (coccoid form) in the water environment. This is thought to be a response to environmental stresses, including changes in temperature, nutrient availability and osmolarity (Adams et al., 2003; Percival and Thomas, 2009.

Exact details on the transmission of *H. pylori* remain unclear (Bellack et al., 2006). Based on epidemiological findings, a higher risk of *H. pylori* infection exists among persons of low economic status living in crowded conditions or unhygienic environments (Brown, 2000; Gomes and De Martinis, 2004). Transfer mechanisms that have been proposed include gastric–oral, oral–oral and faecal–oral (Percival and Thomas, 2009). Overall, it is speculated that person-to-person transfer is the most likely route of transmission (Brown, 2000). The fact that it has not yet been possible to culture viable *Helicobacter* from the water environment has raised questions regarding the possibility of waterborne transmission. Nevertheless, there has been significant evidence provided in support of water as an important source of infection. Molecular techniques (PCR, fluorescent *in situ* DNA hybridization) have been used to confirm the presence of *H. pylori* in natural waters (Hegarty et al., 1999; Sasaki et al., 1999; Horiuchi et al., 2001; Benson et al., 2004; Moreno et al., 2007). As well, in the laboratory, *H. pylori* has been shown to survive for periods ranging from days up to weeks in sterile river water, stream water, saline solution and distilled water at a wide variety of pH values and at temperatures ranging from 4°C to 25°C (West et al., 1992; Shahamat et al., 1993; Adams et al., 2003; Azevedo et al., 2008). As with *Legionella* and mycobacteria, evidence has been supplied that biofilms and free-living waterborne amoebae may provide environmental niches where *H. pylori* can persist (Park et al., 2001; Winiecka-Krusnell et al., 2002; Watson et al., 2004; Bragança et al., 2007).

Waterborne transmission has been suggested as an important source of infection in developing countries (Bellack et al., 2006). Supporting evidence has come from epidemiological studies showing that individuals consuming untreated or contaminated waters had a high risk of infection (Klein et al., 1991; Goodman et al., 1996; McKeown et al., 1999; Herbarth et al., 2001; Brown et al., 2002; Rolle-Kampczyk et al., 2004). There has been less evidence supporting the importance of waterborne transmission in developed countries (Percival and Thomas, 2009) owing to the difficulty in isolating *H. pylori* from drinking water with culturable methods. These difficulties in isolating the bacteria are due to changes in morphology, growth and metabolism when *H. pylori* is exposed to varying environments (Bode et al., 1993). However, the detection of *H. pylori* in drinking water distribution systems using molecular techniques suggests that it can still play an important role (Baker and Hegarty, 2001; Watson et al., 2004; Gião et al., 2008; Percival and Thomas, 2009). Additional research is required to provide further insight into the persistence, viability and associated risk of *H. pylori* in drinking water systems.

The infectious dose necessary for colonization of humans is not known. Results of challenge studies suggest that it is less than 10^4 cells and related to stomach pH (Solnick et al., 2001; Graham et al., 2004). However, given the high percentage of infected individuals among the population and the evidence from cases of accidental infection (e.g., from laboratory work, use of improperly maintained endoscopes), the dose could be much lower (Langenberg et al., 1990; Matysiak-Budnik et al., 1995).

B.2.4.2 Health effects

Human infection with *H. pylori* leads to gastritis, or inflammation of the stomach lining (Dunn et al., 1997; Kusters et al., 2006). The organism colonizes the human stomach, stimulating the immune system and inflammatory cells, and it is this response that brings about gastritis. In the majority of *H. pylori* infections, there are no obvious signs of disease (Kusters et al., 2006). It has been well established that infections with *H. pylori* are generally acquired during childhood, with a lower frequency of infection in adults (Ernst and Gold, 2000; Allaker et al., 2002). Further, infection, once established, is considered to be lifelong unless treatment is pursued (Blaser, 1992; Kusters et al., 2006). *Helicobacter pylori* is the primary cause of peptic ulcers

(Kuipers et al., 1995). It has been estimated that 85–95% of ulcers are the result of infection with this organism (Kuipers et al., 1995). Carriage of *H. pylori* has also been recognized as an important risk factor for the development of gastric cancer (i.e., gastric lymphoma and adenocarcinoma) (Dunn et al., 1997; Pinto-Santini and Salama, 2005). Broad estimates of the risk of infected persons developing these advanced diseases have been put at 10–20% for peptic ulcers and 1–2% for gastric cancer (Ernst and Gold, 2000; Kusters et al., 2006).

Infection with *H. pylori* is treatable (Scott et al., 1998; Vakil and Megraud, 2007), and data from animal and human infection studies suggest that an *H. pylori* vaccine is possible (Graham et al., 2004; Del Guidice et al., 2009). This area of research is currently being explored.

B.2.4.3 Treatment technology

Similar to other bacteria, a proportion of the *H. pylori* present in the source water will be removed using physical methods, such as conventional filtration (i.e., coagulation, flocculation and sedimentation). *Helicobacter pylori* is also susceptible to disinfectants commonly used in drinking water treatment (e.g., chlorine, UV, ozone and monochloramine).

Literature regarding the disinfection of *H. pylori* is limited when compared with that available for other waterborne bacterial pathogens. Investigations are difficult because the cells of *H. pylori* become viable but non-culturable in the environment, and this form cannot be detected easily by regular culture methods (Moreno et al., 2007). With chlorination, data provided from the few reported studies suggest log reductions of culturable *H. pylori* cells ranging from 0.3 log at a chlorine concentration of 0.1 mg/L for 1 minute (Baker et al., 2002) to > 4 log at a chlorine concentration of 0.5 mg/L for 80 seconds (Johnson et al., 1997) to approximately 7 log at a chlorine concentration of 1 mg/L for 5 minutes (Moreno et al., 2007). Moreno et al. (2007) conducted research using a combination of direct viable count and fluorescent *in situ* DNA hybridization methods specifically to study the effects of chlorination on *H. pylori* cell viability. The researchers demonstrated that viable *H. pylori* cells could be detected after 3 hours of exposure to a chlorine concentration of 1.0 mg/L, but not after 24 hours of exposure.

The current body of research suggests that the CT provided by a conventional water treatment plant is sufficient to inactivate *H. pylori* in the finished water. However, if *H. pylori* does enter the distribution system, potentially through a break in treatment or infiltration into the system, disinfectant residuals maintained in the distribution system are probably insufficient for inactivation (Baker et al., 2002). Disinfectant CT_{99} values for *H. pylori* reported by Baker et al. (2002) were 0.24 mg/L min for ozone, 0.299 mg/L min for chlorine and 9.5 mg/L min for monochloramine. In terms of response to disinfection, compared with *E. coli*, Baker et al. (2002) reported that *H. pylori* was statistically more resistant to chlorine and ozone, but not to monochloramine. Other authors have similarly reported *H. pylori* having greater resistance to chlorine compared with *E. coli* (Johnson et al., 1997; Moreno et al., 2007). For UV disinfection, Hayes et al. (2006) reported a greater than 4 log inactivation of culturable *H. pylori* cells at fluences of less than 8 mJ/cm^2.

Association with biofilms has also been shown to protect *H. pylori* from disinfectants, similar to other bacterial pathogens. Gião et al. (2010) observed that *H. pylori* cells (measured by peptide nucleic acid probe) remained viable for at least 26 days following exposure to chlorine at 0.2 and 1.2 mg/L. Also, in contrast to findings provided by other researchers, the authors observed that *H. pylori* cells in suspension did not lose culturability after 30 minutes of exposure to chlorine at an initial concentration of 1.2 mg/L (Gião et al., 2010). Successful distribution system control of *Helicobacter* would similarly be aided by management steps to reduce the formation of biofilm and the presence of free-living amoebae in this environment.

B.2.4.4 Assessment

Overall, the predominant transmission route for *H. pylori* seems to be situation dependent, with person-to-person transmission playing a key role in many circumstances. Water and food appear to be of lesser direct importance, but they can still play a significant role in situations with improper sanitation and lax hygiene

Much is still unknown regarding the ecology and behaviour of *H. pylori* in water systems. However, sufficient information has been provided to suggest that *H. pylori* can be regarded as a potential human pathogen with the potential for waterborne transmission. Illness associated with *H. pylori* infection is of a mild or benign nature in the majority of cases, and outbreaks of illness have not been linked to the presence of *H. pylori* in drinking water supplies. Further research is needed to provide clarity on such topics as its presence in source waters, its susceptibility to treatment and disinfection, and its overall significance for drinking water systems in Canada.

B.3 Issues of emerging interest

B.3.1 Disinfection and antibiotic-resistant organisms

Disinfectants and antibiotics exert action on bacteria through very different mechanisms. Antibiotics characteristically act against specific target sites within the bacteria, interfering with a particular component of an essential process or pathway. In contrast, disinfectants act in a general manner against multiple targets that are fundamental components of the bacterial cell (e.g., proteins and DNA/ribonucleic acid [RNA]). Free chlorine, chloramine, chlorine dioxide and ozone are all very strong oxidizers ,which inactivate bacterial cells by destroying the activity of cell proteins that can be involved with cell structure or metabolism. UV light inactivates bacterial cells by altering the DNA in such a way that the cell can no longer multiply. Because of the fundamental differences in the way in which these two types of antibacterial strategies operate, antibiotic-resistant bacteria are not expected to show increased resistance to the action of drinking water disinfectants.

Antibiotic-resistant pathogens have the ability to change and become less susceptible to drugs. Bacterial resistance to antibiotics can be brought about in a variety of ways; for example, cells may not allow penetration of the antibiotic, they may lack the required target site or they may possess enzymes that can modify or destroy the antibiotic. Repeated exposure of bacteria to antibacterial agents and access of bacteria to increasingly large pools of antibiotic-resistant genes in mixed bacterial populations are the primary driving forces for emerging antibacterial resistance.

There are numerous types of antibiotics, which can be categorized into different classes based on their structure or mode of action. Bacteria having a particular resistance mechanism may be unaffected by antibiotics of a similar class or that target the same site. These same bacteria may be vulnerable to different antibiotics or may possess mechanisms that make them resistant to multiple classes of antibiotics. The growing problem with antibacterial resistance is diminution of the effectiveness of antibacterial agents, resulting in antibiotic-resistant pathogens that are more virulent than their susceptible counterparts, causing more prolonged or severe illnesses.

Very few data have been generated to date regarding the effects of disinfectants on antibiotic-resistant bacteria in drinking water. Some early work found that a greater proportion of

HPC bacteria in treated water are antibiotic-resistant bacteria, compared with those in untreated water (Armstrong et al., 1981, 1982). Templeton et al. (2009) conducted an investigation on the susceptibility of ampicillin- and trimethoprim-resistant strains of *E. coli* to free chlorine and UV disinfection. The authors observed no differences in UV inactivation between antibiotic-resistant and antibiotic-sensitive *E. coli* under the doses and contact times tested. The trimethoprim-resistant *E. coli* strain did show slightly greater resistance to free chlorine compared with the antibiotic-sensitive *E. coli*; however, the authors concluded that the difference was likely to be negligible under chlorine doses and contact times typically observed in routine drinking water treatment. It was further concluded that these disinfectants did not likely select for ampicillin or trimethoprim resistance during drinking water treatment. No drinking water studies were found pertaining to the inactivation rates for other disinfectants, such as ozone or chlorine dioxide, against antibiotic-resistant bacteria.

At present, there is little evidence to indicate that the use of disinfectants in drinking water systems favours the selection of antibiotic-resistant bacteria in any way (Templeton et al., 2009). However, one study by Xi et al. (2009) suggested that water treatment could increase the antibiotic resistance of surviving bacteria or induce antibiotic resistance gene transfer. Additional study in this area is needed. The evidence at present, although limited, suggests that antibiotic resistance in bacteria is not an important factor in chlorine and UV treatment effectiveness at doses and contact times typically applied in drinking water treatment systems.

B.3.2 Showerheads

Shower use can provide a source of exposure to microorganisms through aerosolization, as the inside of a showerhead provides a moist, warm, dark environment that is frequently replenished with low-level nutrient sources.

Inhalation of aerosols from showerhead water has been implicated in respiratory disease (Falkinham et al., 2008; Feazel et al., 2009). Although opportunistic pathogens have been cultured from showerheads, little is known about either the prevalence or the nature of the microorganisms that can be aerosolized during showering. To determine the composition of showerhead biofilms and waters, Feazel et al. (2009) analysed ribosomal RNA gene sequences of biofilms from 45 showerheads from nine sites in the United States. The authors found that sequences representative of non-tuberculous mycobacteria and other opportunistic pathogens were highly enriched in many showerhead biofilms. They concluded that showerheads may present a significant potential exposure to aerosolized microorganisms and that the health risk associated with showerheads needs further investigation, particularly for individuals with compromised immune or respiratory systems.

B.4 Residential-scale treatment

B.4.1 Residential-scale and private drinking water systems

Residential-scale[2] treatment is also applicable to small drinking water systems. This would include both privately owned systems and systems with a minimal or no distribution

[2] For the purposes of this document, a residential-scale water supply system is defined as a system, with a minimal or no distribution system, that provides water to the public from a facility not connected to a municipal supply. Examples of such facilities include schools, personal care homes, day care centres, hospitals, community wells, hotels and restaurants. The definition of a residential-scale supply may vary between jurisdictions.

system that provide water to the public from a facility not connected to a municipal supply (previously referred to as semi-public systems).

The presence of *E. coli* in a residential-scale or private drinking water system indicates that the source or the system has likely been affected by recent faecal contamination; as a result, the water should be deemed as unsafe to drink. The absence of *E. coli* during routine verification should be an adequate indication of the sufficient removal and inactivation of enteric bacterial pathogens. Where applicable, testing frequencies for residential-scale systems will be determined by the responsible authority and should include times when the risk of contamination is greatest—for example, in early spring after the thaw, after an extended dry spell or following heavy rains. For owners of private supplies, existing wells should be tested two to three times per year and during these same periods. New or rehabilitated wells should also be tested before use to confirm microbiological safety.

Non-faecal bacterial pathogens that occur naturally in the water environment can be found in groundwater, although typically at a lower frequency and in lower numbers than in surface waters. The levels of organisms necessary to cause disease in healthy individuals are uncertain, although limited study has suggested that reasonably elevated numbers beyond those typically found in source waters are required. These organisms are most likely to be found in distribution system biofilms and can survive and grow there to reach significant populations. In smaller systems, distribution system biofilms are less of a concern than in municipal systems, because distribution systems are smaller or non-existent and the retention time for the finished water is shorter.

Various options are available for treating source waters to provide high-quality pathogen-free drinking water. These include filtration and disinfection with chlorine-based compounds or alternative technologies, such as UV light. These technologies are similar to the municipal treatment barriers, but on a smaller scale.

Private homeowners should also be aware that domestic hot water systems can be contaminated with *Legionella*; as a result, water heaters should be kept at a suitable temperature (at least 60ºC) to protect against the potential growth of this organism. The National Plumbing Code of Canada includes requirements for a minimum water temperature of 60°C in hot water storage tanks to address the growth of *Legionella* (NRCC, 2010). Homeowners should also take appropriate safety measures to reduce the risk of scalding at the tap. These measures include installing thermostatic or pressure-balanced mixing valves to control the water temperature at the tap (Bartram et al., 2007; Bentham et al., 2007). This strategy may also be useful in reducing other microorganisms in hot water heaters, as many of them do not survive at this higher temperature (LeChevallier and Au, 2004; AWWA, 2006).

Larger plumbing systems could use additional control measures. These measures include temperature control; control of water system design and construction to prevent the accumulation of biofilms, sediments or deposits; and nutrient control strategies (Bartram et al., 2007; Bentham et al., 2007).

B.4.2 Use of residential-scale treatment devices

The information on treatment, disinfection and inactivation of microorganisms in this document is relevant primarily to municipal-scale systems. Municipal treatment of drinking water is designed to reduce microbial contaminants to levels below those typically shown to be associated with disease. The use of residential-scale treatment devices on municipally treated water is generally not necessary, but is primarily based on individual choice. In cases where small systems or individual households obtain drinking water from private wells or surface water

supplies such as lakes, treatment devices can be used as an additional barrier for reducing pathogen concentrations in drinking water.

Health Canada does not recommend specific brands of drinking water treatment devices, but it strongly recommends that consumers use devices that have been certified by an accredited certification body as meeting the appropriate NSF International/American National Standards Institute drinking water treatment unit standards. These standards have been designed to safeguard drinking water by helping to ensure the material safety and performance of products that come into contact with drinking water. Certification organizations provide assurance that a product conforms to applicable standards and must be accredited by the Standards Council of Canada.

Point-of-use systems (installed at the faucet) and point-of-entry systems (installed where water enters the home) are of interest for use in treatment and disinfection of drinking water in small, rural or remote communities, particularly those using a groundwater source. The most common types of treatment device that are generally able to inactivate waterborne pathogens (including bacteria) use UV disinfection. Although membrane filtration (reverse osmosis) may be able to reduce pathogens, certified devices are generally intended for use on water supplies deemed microbiologically safe. Before a treatment device is installed, the water should be tested to determine its general water chemistry. Periodic testing by an accredited laboratory should be conducted on both the water entering the treatment device and the finished water to verify that the treatment device is effective. Devices can lose removal capacity through usage and time and need to be maintained and/or replaced. Consumers should verify the expected longevity of the components (e.g., UV lamp, membrane) in their treatment device as per the manufacturer's recommendations and service the device when required. Homeowners should ensure that the selection and installation of treatment devices comply with applicable local regulations.

Part C. References and acronyms

C.1 References

Abu Kwaik, Y., Venkataraman, C., Harb, O.S. and Gao, L.-Y. (1998). Signal transduction in the protozoan host *Hartmannella vermiformis* upon attachment and invasion by *Legionella micdadei.* Appl. Environ. Microbiol., 64(9): 3134–3139.

Adams, B.L., Bates, T.C. and Oliver, J.D. (2003). Survival of *Helicobacter pylori* in a natural freshwater environment. Appl. Environ. Microbiol., 69: 7462–7466.

Alamanos, Y., Maipa, V., Levidiotou, S. and Gessouli, E. (2000). A community waterborne outbreak of gastro-enteritis attributed to *Shigella sonnei*. Epidemiol. Infect., 125: 499–503.

Alary, M. and Joly, J.R. (1991). Risk factors for contamination of domestic hot water systems by legionellae. Appl. Environ. Microbiol., 57(8): 2360–2367.

Allaker, R.P., Young, K.A., Hardie, J.M., Domizio, P and Meadows, N.J. (2002). Prevalence of *Helicobacter pylori* at oral and gastrointestinal sites in children: evidence for possible oral-to-oral transmission. J. Med. Microbiol., 51: 312–317.

Angulo, F.J., Tippen, S., Sharp, D.J., Payne, B.J., Collier, C., Hill, J.E., Barrett, T.J., Clark, R.M., Geldreich, E.E., Donnell, H.D., Jr. and Swerdlow, D.L. (1997). A community waterborne outbreak of salmonellosis and the effectiveness of a boil water order. Am. J. Public Health, 87: 580–584.

APHA, AWWA and WEF (2005). Standard methods for the examination of water and wastewater. 21st edition. American Public Health Association, American Water Works Association and Water Environment Federation, Washington, DC.

Archuleta, R., Mullens, P. and Primm, T.P. (2002). The relationship of temperature to desiccation and starvation tolerance of the *Mycobacterium avium* complex. Arch. Microbiol., 178: 311–314.

Armstrong, J.L., Shigeno, D.S., Calomiris, J.J. and Seidler, R. (1981). Antibiotic-resistant bacteria in drinking water. Appl. Environ. Microbiol., 42: 277–283.

Armstrong, J.L., Calomiris, J.J. and Seidler, R.J. (1982). Selection of antibiotic-resistant standard plate count bacteria during water treatment. Appl. Environ. Microbiol., 44: 308–316.

Armstrong, T.W. and Haas, C.N. (2008). Legionnaires' disease: evaluation of a quantitative microbial risk assessment model. J. Water Health, 6(2): 149–166.

Arnell, B., Bennett, J., Chehey, R. and Greenblatt, J. (1996). *Shigella sonnei* outbreak associated with contaminated drinking water—Island Park, Idaho, August 1995. MMWR Morb. Mortal. Wkly. Rep., 45(11): 229–231.

Aronson, T., Holtzman, A., Glover, N., Boian, M., Froman, S., Berlin, O.G.W., Hill, H. and Stelma, G., Jr. (1999). Comparison of large restriction fragments of *Mycobacterium avium* isolates recovered from AIDS and non-AIDS patients with those of isolates from potable water. J. Clin. Microbiol., 37(4): 1008–1012.

ASHRAE (2000). ASHRAE Guideline 12-2000: Minimizing the risk of legionellosis associated with building water systems. American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., Atlanta, GA. Available at: http://spxcooling.com/pdf/guide12.pdf

Auger, P., Pouliot, B., De Grace, M., Milot, C., Lafortune, M. and Bergeron, Z. (1981). Epidemic of bacillary dysentery. Can. Med. Assoc. J., 125: 733–736.

AWWA (2005). AWWA Standard: Disinfecting Water Mains (ANSI/AWWA C651-05). American Water Works Association, Denver, Colorado.

AWWA (2006). AWWA manual of water supply practices—M48: Waterborne pathogens. 2nd edition. American Water Works Association, Denver, Colorado.

Azevedo, N.F., Almeida, C., Fernandes, I., Cerqueira, L., Dias, S., Keevil, C.W. and Vieira, M.J. (2008). Survival of gastric and enterohepatic *Helicobacter* spp. in water: implications for transmission. Appl. Environ. Microbiol., 74: 1805–1811.

Baele, M., Pasmans, F., Flahou, B., Chiers, K., Ducatelle, R. and Haesebrouck, F. (2009). Non-*Helicobacter pylori* helicobacters detected in the stomach of humans comprise several naturally occurring *Helicobacter* species in animals. FEMS Immunol. Med. Microbiol., 55: 306–313.

Baker, K.H. and Hegarty, J.P. (2001). Presence of *Helicobacter pylori* in drinking water is associated with clinical infection. Scand. J. Infect. Dis., 33: 744–746.

Baker, K.H., Hegarty, J.P., Redmond, B., Reed, N.A. and Herson, D.S. (2002). Effect of oxidizing disinfectants (chlorine, monochloramine, and ozone) on *Helicobacter pylori*. Appl. Environ. Microbiol., 68: 981–984.

Bartram, J., Bentham, R., Briand, E., Callan, P., Crespi, S., Lee, J.V. and Surman-Lee, S. (2007). Chapter 3: Approaches to risk management. In: *Legionella* and the prevention of legionellosis. Bartram, J., Chartier, Y., Lee, J.V., Pond, K. and Surman-Lee, S. (eds.). World Health Organization, Geneva, Switzerland. pp. 39–56. Available at: www.who.int/water_sanitation_health/emerging/legionella.pdf

Bellack, N.R., Koehoorn, M.W., MacNab, Y.C. and Morshed, M.G. (2006). A conceptual model of water's role as a reservoir in *Helicobacter pylori* transmission: a review of the evidence. Epidemiol. Infect., 134: 439–449.

Benin, A.L., Benson, R.F. and Besser, R.E. (2002). Trends in legionnaires disease, 1980–1998: declining mortality and new patterns of diagnosis. Clin. Infect. Dis., 35(9): 1039–1046.

Benson, J.A., Fode-Vaughan, K.A. and Collins, M.L.P. (2004). Detection of *Helicobacter pylori* in water by direct PCR. Lett. Appl. Microbiol., 39: 221–225.

Bentham, R., Surman-Lee, S., Lee, J.V., Briand, E. and Van de Kooj, D. (2007). Chapter 4: Potable water and in-building distribution systems. In: *Legionella* and the prevention of legionellosis. Bartram, J., Chartier, Y., Lee, J.V., Pond, K. and Surman-Lee, S. (eds.). World Health Organization, Geneva, Switzerland. pp. 57–68. Available at: www.who.int/water_sanitation_health/emerging/legionella.pdf

Black, R.E., Levine, M.M., Clements, M.L., Hughes, T.P. and Blaser, M.J. (1988). Experimental *Campylobacter jejuni* infection in humans. J. Infect. Dis., 157(3): 472–479.

Blanc, D.S., Carrara, P., Zanetti, G. and Francioli, P. (2005). Water disinfection with ozone, copper and silver ions, and temperature increase to control *Legionella*: seven years of experience in a university teaching hospital. J. Hosp. Infect., 60(1): 69–72.

Blaser, M.J., Wells, J.H., Powers, B. and Wang, W.L. (1980). Survival of *Campylobacter fetus* subsp. *jejuni* in biological milieus. J. Clin. Microbiol., 11: 309–313.

Blaser, M.J. (1992). *Helicobacter pylori*: its role in disease. Clin. Infect. Dis. 15, 386–393.

Bode, G., Mauch, F. and Malfertheiner, P. (1993). The coccoid forms of *Helicobacter pylori*. Criteria for their viability. Epidemiol. Infect., 111: 483–490.

Borchardt, M.A., Stemper, M.E. and Standridge, J.H. (2003). *Aeromonas* isolates from human diarrheic stool and groundwater compared by pulsed-field gel electrophoresis. Emerg. Infect. Dis., 9: 224–228.

Borella, P., Montagna, M.T., Romano-Spica, V., Stampi, S., Stancanelli, G., Triassi, M., Neglia, R., Marchesi, I., Fantuzzi, G., Tatòt, D., Napoli, C., Quaranta, G., Laurenti, P., Leoni, E., De Luca, G., Ossi, C., Moro, M. and D'Alcalà, G.R. (2004). *Legionella* infection risk from domestic hot water. Emerg. Infect. Dis., 10(3): 457–464.

Borella, P., Guerrieri, E., Marchesi, I., Bondi, M. and Messi, P. (2005). Water ecology of *Legionella* and protozoan: environmental and public health perspectives. Biotechnol. Annu. Rev., 11: 355–380.

Boring, J.R., III, Martin, W.T. and Elliott, L.M. (1971). Isolation of *Salmonella typhimurium* from municipal water, Riverside, California, 1965. Am. J. Epidemiol., 93: 49–54.

Bragança, S.M., Azevedo, N.F., Simöes, L.C., Keevil, C.W. and Vieira, M.J. (2007). Use of fluorescent *in situ* hybridisation for the visualisation of *Helicobacter pylori* in real drinking water biofilms. Water Sci. Technol., 55: 387–393.

Brandi, G., Sisti, M., Giardini, F., Schiavano, G.F. and Albano, A. (1999). Survival ability of cytotoxic strains of motile *Aeromonas* spp. in different types of water. Lett. Appl. Microbiol., 29: 211–215.

Brooks, T., Osicki, R.A., Springthorpe, V.S., Sattar, S.A., Filion, L., Abrial, D. and Riffard, S. (2004). Detection and identification of *Legionella* species from groundwaters. J. Toxicol. Environ. Health A, 67: 1845–1859.

Brown, L.M. (2000). *Helicobacter pylori*: epidemiology and routes of transmission. Epidemiol. Rev., 22: 283–297.

Brown, L.M., Thomas, T.L., Ma, J., Chang, Y., You, W., Liu, W., Zhang, L., Pee, D. and Gail, M.H. (2002). *Helicobacter pylori* infection in rural China: demographic, lifestyle and environmental factors. Int. J. Epidemiol., 31: 638–646.

Bruce-Grey-Owen Sound Health Unit (2000). The investigative report on the Walkerton outbreak of waterborne gastroenteritis. May–June, Owen Sound, Ontario. Available at: www.publichealthgreybruce.on.ca

Burke, V., Gracey, M. and Robinson, J. (1983). The microbiology of childhood gastroenteritis: *Aeromonas* species and other infective agents. J. Infect. Dis., 148: 68–74.

Burnsed, L.J., Hicks, L.A., Smithee, L.M.K., Fields, B.S., Bradley, K.K., Pascoe, N., Richards, S.M., Mallonee, S., Littrell, L., Benson, R.F., Moore, M.R., Bos, J., Lee, A., Rayno, K., Coffman, B., Grubbs, M., Eberly, D., Minor, V., Botchlet, R., Johnson, S., Lytle, M., McDermott, M., Mathewson, J., Evans, T., Madewell, T., Campbell, S., Bowman, L., Myers, S., Wagner, J., Jones, R., Stone, P., Lin, H., Ibrahim, S., Duszynski, T., Beard, B., Howe, A., Burkholder, S., Ramey, P., Overholt, K., Weber, A., Azene, N., Comstock, D., Mallonee, J., Garrison, P., Powell, R., Stevenson, K., Orr, C., Kostiuk, E., Jordan, L. and Hawkins, L. (2007). A large, travel-associated outbreak of legionellosis among hotel guests: utility of the urine antigen assay in confirming Pontiac fever. Clin. Infect. Dis., 44(2): 222–228.

Cachafeiro, S.P., Naveira, I.M. and García, I.G. (2007). Is copper–silver ionisation safe and effective in controlling *Legionella*? J. Hosp. Infect., 67: 209–216.

Cangelosi, G., Clark-Curtiss, J., Behr, M., Bull, T. and Stinear, T. (2004). Chapter 4: Biology of waterborne pathogenic mycobacteria. In: Pathogenic mycobacteria in water, a guide to public health consequences, monitoring and management. Pedley, S., Bartram, J., Rees, G., Dufour, A. and Cotruvo, J.A. (eds.). Published by IWA Publishing, London, United Kingdom, on behalf of the World Health Organization, Geneva, Switzerland. pp. 39–54.

Cappelluti, E., Fraire, A.E. and Schaefer, O.P. (2003). A case of "hot tub lung" due to *Mycobacterium avium* complex in an immunocompetent host. Arch. Intern. Med., 163(7): 845–848.

Carter, A.M., Pacha, R.E., Clark, G.W. and Williams, E.A. (1987). Seasonal occurrence of *Campylobacter* spp. in surface waters and their correlation with standard indicator bacteria. Appl. Environ. Microbiol., 53: 523–526.

Castilho, M.C.B., Castro, T.L.A., Araújo, V.S., Trajano, R.S., Santos, P.A., Pimenta, P.M.C., Lucheze, K., Melo, J.T.B., Gonçalves, A.M., Nogueira, R.T., Luna, M.G. and Freitas-Almeida, A.C. (2009). High frequency of hemolytic and cytotoxic activity in *Aeromonas* spp. isolated from clinical, food and environmental in Rio de Janeiro, Brazil. Antonie Van Leeuwenhoek, 96(1): 53–61.

Chamorey, E., Forel, M. and Drancourt, M. (1999). An in-vitro evaluation of the activity of chlorine against environmental and nosocomial isolates of *Aeromonas hydrophila*. J. Hosp. Infect., 41: 45–49.

Chang, J.C.H., Ossoff, S.F. and Lobe, D.C. (1985). UV inactivation of pathogenic and indicator microorganisms. Appl. Environ. Microbiol., 49(6): 1361–1365.

Chauret, C., Volk, C., Creason, R., Jarosh, J., Robinson, J. and Warnes, C. (2001). Detection of *Aeromonas hydrophila* in a drinking-water distribution system: a field and pilot study. Can. J. Microbiol., 47: 782–786.

Chauret, C., Smith, C. and Baribeau, H. (2008). Inactivation of *Nitrosomonas europaea* and pathogenic *Escherichia coli* by chlorine and monochloramine. J. Water Health, 6(3): 315–322.

Chen, K.T., Chen, C.J. and Chiu, J.P. (2001). A school waterborne outbreak involving both *Shigella sonnei* and *Entamoeba histolytica*. J. Environ. Health, 64: 9–13.

Cirillo, J.D., Falkow, S., Tompkins, L.S. and Bermudez, L.E. (1997). Interaction of *Mycobacterium avium* with environmental amoebae enhances virulence. Infect. Immun., 65: 3759–3767.

Clark, C.G., Price, L., Ahmed, R., Woodward, D.L., Melito, P.L., Rodgers, F.G., Jamieson, F., Ciebin, B., Li, A. and Ellis, A. (2003). Characterization of waterborne outbreak–associated *Campylobacter jejuni*, Walkerton, Ontario. Emerg. Infect. Dis., 9: 1232–1241.

Clark, W.F., Sontrop, J.M., Macnab, J.J., Salvadori, M., Moist, L., Suri, R. and Garg, A.X. (2010). Long term risk for hypertension, renal impairment, and cardiovascular disease after gastroenteritis from drinking water contaminated with *Escherichia coli* O157:H7: a prospective cohort study. BMJ, 341:c6020. Available at: www.bmj.com/content/341/bmj.c6020

Cooper, I.R. and Hanlon, G.W. (2010). Resistance of *Legionella pneumophila* serotype 1 biofilms to chlorine-based disinfection. J. Hosp. Infect., 74: 152–159.

Costa, J., Tiago, I., Da Costa, M.S. and Veríssimo, A. (2005). Presence and persistence of *Legionella* spp. in groundwater. Appl. Environ. Microbiol., 71(2): 663–671.

Covert, T.C., Rodgers, M.R., Reyes, A.L. and Stelma, G.N., Jr. (1999). Occurrence of nontuberculous mycobacteria in environmental samples. Appl. Environ. Microbiol., 65: 2492–2496.

Cowgill, K.O., Lucas, C.E., Benson, R.F., Chamany, S., Brown, E.W., Fields, B.S. and Feikin, D.R. (2005). Recurrence of legionnaires disease at a hotel in the United States Virgin Islands over a 20-year period. Clin. Infect. Dis., 40: 1205–1207.

Craun, M.F., Craun, G.F., Calderon, R.L. and Beach, M.J. (2006). Waterborne outbreaks reported in the United States. J. Water Health, 4(Suppl. 2): 19–30.

Crow, H.E., King, C.T., Smith, C.E., Corpe, R.F. and Stergus, I. (1957). A limited clinical, pathologic, and epidemiologic study of patients. Am. Rev. Tuberc., 75: 199–222.

Cunliffe, D. (2007). Chapter 10: Regulatory aspects. In: *Legionella* and the prevention of legionellosis. Bartram, J., Chartier, Y., Lee, J.V., Pond, K. and Surman-Lee, S. (eds.). World Health Organization, Geneva, Switzerland. pp. 161–174. Available at: www.who.int/water_sanitation_health/emerging/legionella.pdf

Daley, C.D. and Griffith, D.E. (2010). Pulmonary non-tuberculous mycobacterial infections. Int. J. Tuberc. Lung Dis., 14: 665–671.

Declerck, P., Behets, J., De Keersmaecker, B. and Ollevier, F. (2007). Receptor-mediated uptake of *Legionella pneumophila* by *Acanthamoeba castellanii* and *Naegleria lovaniensis.* J. Appl. Microbiol., 103(6): 2697–2703.

Declerck, P., Behets, J., Margineanu, A., van Hoef, V., De Keersmaecker, B. and Ollevier, F. (2009). Replication of *Legionella pneumophila* in biofilms of water distribution pipes. Microbiol. Res., 164(6): 593–603.

Delaedt, Y., Daneels, A., Declerck, P., Behets, J., Ryckeboer, J., Peters, E. and Ollevier, F. (2008). The impact of electrochemical disinfection on *Escherichia coli* and *Legionella pneumophila* in tap water. Microbiol. Res., 163(2): 192–199.

Del Giudice, G., Malfertheiner, P. and Rappuoli, R. (2009). Development of vaccines against *Helicobacter pylori*. Expert Rev. Vaccines, 8: 1037–1049.

De Oliveira Scoaris, D., Bizerra, F.C., Yamada-Ogatta, S.F., De Abreu Filho, B.A., Ueda-Nakamura, T., Nakamura, C.V. and Dias Filho, B.P. (2008). The occurrence of *Aeromonas* spp. in the bottled mineral water, well water and tap water from the municipal supplies. Braz. Arch. Biol. Technol., 51: 1049–1055.

Diederen, B.M.W. (2008). *Legionella* spp. and Legionnaires' disease. J. Infect., 56: 1–12.

Dominique, E.L., Tydall, R.L., Mayberry, W.R. and Pancorbo, O.C. (1988). Effects of three oxidizing biocides on *Legionella pneumophila* serogroup 1. Appl. Environ. Microbiol., 54: 741–747.

Doyle, R.M. and Heuzenroeder, M.W. (2002). A mutation in an ompR-like gene on a *Legionella longbeachae* serogroup1 plasmid attenuates virulence. Int. J. Med. Microbiol., 292: 227–239.

Dufour, A.P. and Jakubowski, W. (1982). Drinking water and Legionnaires' disease. J. Am. Water Works Assoc., 74: 631–637.

Dufresne, S.F., Locas, M.C., Duchesne, A., Restieri, C., Ismaïl, J., Lefebvre, B., Labbé, A.C., Dion, R., Plante, M. and Laverdière, M. (2012). Sporadic Legionnaires' disease: the role of domestic electric hot-water tanks. Epidemiol. Infect., 140(1): 172–181.

du Moulin, G.C., Stottmeier, K.D., Pelletier, P.A., Tsang, A.Y. and Hedley-Whyte, J. (1988). Concentration of *Mycobacterium avium* by hospital hot water systems. JAMA, 11: 1599–1601.

Dunn, B.E., Cohen, H. and Blaser, M.J. (1997). *Helicobacter pylori*. Clin. Microbiol. Rev., 10: 720–741.

Dutka, B.J., Walsh, K., Ewan, P., El-Shaarawi, A. and Tobin, R.S. (1984). Incidence of *Legionella* organisms in selected Ontario (Canada) cities. Sci. Total Environ., 39: 237–249.

Eden, K.V., Rosenburg, M.L., Stoopler, M., Wood, B.T., Highsmith, A.K., Skaliy, P., Wells, J.G. and Feeley, J.C. (1977). Waterborne gastrointestinal illness at a ski resort. Public Health Rep., 92: 245–250.

El-Taweel, G.E. and Shaban, A.M. (2001). Microbiological quality of drinking water at eight water treatment plants. Int. J. Environ. Health Res., 11: 285–290.

Emekdas, G., Aslan, G., Tezcan, S., Serin, M.S., Yildiz, C., Ozturhan, H. and Durmaz, R. (2006). Detection of the frequency, antimicrobial susceptibility, and genotypic discrimination of *Aeromonas* strains isolated from municipally treated tap water samples by cultivation and AP-PCR. Int. J. Food Microbiol., 107: 310–314.

Ernst, P.B. and Gold, B.D. (2000). The disease spectrum of *Helicobacter pylori*: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. Annu. Rev. Microbiol., 54: 615–640.

Erova, T.E., Kosykh, V.G., Fadl, A.A., Sha, J., Horneman, A.J. and Chopra, A.K. (2008). Cold shock exoribonuclease R (VacB) is involved in *Aeromonas hydrophila* pathogenesis. J. Bacteriol., 190: 3467–3474.

Falkinham, J.O., III (1996). Epidemiology of infection by nontuberculous mycobacteria. Clin. Microbiol. Rev., 9:177–215.

Falkinham, J.O. (2004). Chapter 3: Environmental sources of *Mycobacterium avium* linked to routes of exposure. In: Pathogenic mycobacteria in water, a guide to public health consequences, monitoring and management. Pedley, S., Bartram, J., Rees, G., Dufour, A. and J.A. Cotruvo

(eds.). Published by IWA Publishing, London, United Kingdom, on behalf of the World Health Organization, Geneva, Switzerland. pp. 26–38.

Falkinham, J.O., III, Norton, C.D. and LeChevallier, M.W. (2001). Factors influencing numbers of *Mycobacterium avium*, *Mycobacterium intracellulare*, and other mycobacteria in drinking water distribution systems. Appl. Environ. Microbiol., 67: 1225–1231.

Falkinham, J.O., III, Iseman, M.D., de Haas, P. and van Soolingen, D. (2008). *Mycobacterium avium* in a shower linked to pulmonary disease. J. Water Health, 6(2): 209–213.

Feazel, L.M., Baumgartner, L.K., Peterson, K.L., Frank, D.N., Harris, J.K. and Pace, N.R. (2009). Opportunistic pathogens enriched in showerhead biofilms. Proc. Natl. Acad. Sci. U. S. A., 106: 16393–16398.

Fernández, M.C., Giampaolo, B.N., Ibañez, S.B., Guagliardo, M.V., Esnaola, M.M., Conca, L., Valdivia, P., Stagnaro, S.M., Chiale, C. and Frade, H. (2000). *Aeromonas hydrophila* and its relation with drinking water indicators of microbiological quality in Argentina Genetica, 108: 35–40.

Field, S.K., Fisher, D. and Cowie, R.L. (2004). *Mycobacterium avium* complex pulmonary disease in patient without HIV infection. Chest, 126: 566–581.

Fields, B.S. (1996). The molecular ecology of legionellae. Trends Microbiol., 4: 286–290.

Fields, B.S., Benson, R.F. and Besser, R.E. (2002). *Legionella* and legionnaires' disease: 25 years of investigation. Clin. Microbiol. Rev., 15(3): 506–526.

Fliermans, C.B., Cherry, W.B. and Orrison, L.H. (1981). Ecological distribution of *Legionella pneumophila*. Appl. Environ. Microbiol., 41(1): 9–16.

Gavriel, A.A., Landre, J.P.B. and Lamb, A.J. (1998). Incidence of mesophilic *Aeromonas* within a public drinking water supply in north-east Scotland. J. Appl. Microbiol., 84: 383–392.

Gião, M.S., Azevedo, N.F., Wilks, S.A., Vieira, M.J. and Keevil, C.W. (2008). Persistence of *Helicobacter pylori* in heterotrophic drinking-water biofilms. Appl. Environ. Microbiol., 74: 5898–5904.

Gião, M.S., Azevedo, N.F., Wilks, S.A., Vieira, M.J. and Keevil, C.W. (2010). Effect of chlorine on incorporation of *Helicobacter pylori* into drinking water biofilms. Appl. Environ. Microbiol., 76: 1669–1673.

Glover, N., Holtzman, A., Aronson, T., Froman, S., Berlin, O.G.W., Dominguez, P., Kunkel, K.A., Overturf, G., Stelma, G., Jr., Smith, C. and Yakrus, M. (1994). The isolation and identification of *Mycobacterium avium* complex (MAC) recovered from Los Angeles potable water, a possible source of infection in AIDS patients. Int. J. Environ. Health Res., 4: 63–72.

Gold, W.L. and Salit, I.E. (1993). *Aeromonas hydrophila* infections of skin and soft tissue: report of 11 cases and review. Clin. Infect. Dis., 16: 69–74.

Gomes, B.C. and De Martinis, E.C.P. (2004). The significance of *Helicobacter pylori* in water, food and environmental samples. Food Control, 15: 397–403.

Goodman, K.J., Correa, P., Tenganá Aux, H.J., Ramirez, H., DeLany, J.P., Pepinosa, O.G., Quiñones, M.L. and Parra, T.C. (1996). *Helicobacter pylori* infection in the Colombian Andes: a population-based study of transmission pathways. Am. J. Epidemiol., 144: 290–299.

Graham, D.Y., Opekun, A.R., Osato, M.S., El-Zimaity, H.M.T., Lee, C.K., Yamaoka, Y., Qureshi, W.A., Cadoz, M. and Monath, T.P. (2004). Challenge model for *Helicobacter pylori* infection in human volunteers. Gut, 53: 1235–1243.

Grange, J.M. (1991). Environmental mycobacteria and human disease. Lepr. Rev., 62: 353–361.

Greub, G. and Raoult, D. (2004). Microorganisms resistant to free-living amoebae. Clin. Microbiol. Rev., 17: 413–433.

Gyles, C.L. (2007). Shiga toxin–producing *Escherichia coli*: an overview. J. Anim. Sci., 85(Suppl. 13):E45–E62.

Haesebrouck, F., Pasmans, F., Flahou, B., Chiers, K., Baele, M., Meyns, T., Decostere, A. and Ducatelle, R. (2009). Gastric helicobacters in domestic animals and nonhuman primates and their significance for human health. Clin. Microbiol. Rev., 22: 202–223.

Hara-Kudo, Y. and Takatori, K. (2011). Contamination level and ingestion dose of foodborne pathogens associated with infections. Epidemiol. Infect., 139: 1505–1510.

Harrington, G.W., Xagoraraki, I., Assavasilavasukul, P. and Standridge, J.H. (2003). Effect of filtration conditions on removal of emerging waterborne pathogens. J. Am. Water Works Assoc., 95(12): 95–104.

Havelaar, A.H., Versteegh, J.F.M. and During, M. (1990). The presence of *Aeromonas* in drinking water supplies in the Netherlands. Zentralbl. Hyg. Umweltmed., 190: 236–256.

Hayes, S.L., White, K.M. and Rodgers, M.R. (2006). Assessment of the effectiveness of low-pressure UV light for inactivation of *Helicobacter pylori*. Appl. Environ. Microbiol., 72: 3763–3765.

Hayes, S.L., Sivaganesan, M., White, K.M. and Pfaller, S.L. (2008). Assessing the effectiveness of low-pressure ultraviolet light for inactivating *Mycobacterium avium* complex (MAC) micro-organisms. Lett. Appl. Microbiol., 47: 386–392.

Health Canada (2008). Guidelines for Canadian drinking water quality: guideline technical document—Chlorite and chlorate. Water Quality and Health Bureau, Healthy Environments

and Consumer Safety Branch, Ottawa, Ontario. Available at: www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/chlorite-chlorate/index-eng.php

Health Canada (2012). Guidelines for Canadian drinking water quality: guideline technical document—*Escherichia coli*. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. Available at: www.hc-sc.gc.ca/waterquality

Hegarty, J.P., Dowd, M.T. and Baker, K.H. (1999). Occurrence of *Helicobacter pylori* in surface water in the United States. J. Appl. Microbiol., 87: 697–701.

Herbarth, O., Krumbiegel, P., Fritz, G.J., Richter, M., Schlink, U., Müller, D.M. and Richter, T. (2001). *Helicobacter pylori* prevalences and risk factors among school beginners in a German urban center and rural county. Environ. Health Perspect., 109: 573–577.

Hershey, J., Burrus, B., Marcussen, V., Notter, J., Watson, K., Wolford, R., Shaffner, R.E., III, Barrett, E., Woolard, D., Branch, L., Hackler, R., Rouse, B., Gibson, L., Jenkins, S., Rullan, J., Miller, G., Jr. and Curran, S. (1997). Legionnaires disease associated with a whirlpool spa display—Virginia, September–October, 1996. MMWR Morb. Mortal. Wkly. Rep., 46: 83–86.

Hilborn, E.D., Covert, T.C., Yakrus, M.A., Harris, S.I., Donnelly, S.F., Rice, E.W., Toney, S., Bailey, S.A. and Stelma, G.N., Jr. (2006). Persistence of nontuberculous mycobacteria in a drinking water system after addition of filtration treatment. Appl. Environ. Microbiol., 72: 5864–5869.

Hiransuthikul, N., Tantisiriwat, W., Lertutsahakul, K., Vibhagool, A. and Boonma, P. (2005). Skin and soft-tissue infections among tsunami survivors in southern Thailand. Clin. Infect. Dis., 41(10): e93–96.

Hoebe, C.J.P., Cluitmans, J.J.M. and Wagenvoort, J.H.T. (1998). Two fatal cases of nosocomial *Legionella pneumophila* pneumonia associated with a contaminated cold water supply. Eur. J. Clin. Micriobiol. Infect. Dis., 17: 740–749.

Holmes, P. and Nicolls, L.M. (1995). Aeromonads in drinking-water supplies: their occurrence and significance. J. Chartered Inst. Water Environ. Manage., 9: 464–469.

Holmes, P., Nicolls, L.M. and Sartory, D.P. (1996). The ecology of mesophilic *Aeromonas* in aquatic environment. In: The genus *Aeromonas*. Austin, B., Altwegg, M., Gosling, P. and Joseph, S.W. (eds.). John Wiley & Sons, New York, New York. pp. 39–76.

Horiuchi, T., Ohkusa, T., Watanabe, M., Kobayashi, D., Miwa, H. and Eishi, Y. (2001). *Helicobacter pylori* DNA in drinking water in Japan. Microbiol. Immunol., 45: 515–519.

Hörman, A., Rimhanen-Finne, R., Maunula, L., Von Bonsdorff, C.-H., Torvela, N., Heikinheimo, A. and Hänninen, M.-L. (2004). *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., Noroviruses, and indicator organisms in surface water in southwestern Finland, 2000–2001. Appl. Environ. Microbiol., 70(1): 87–95.

Hunter, P.R. (1997). Waterborne disease—Epidemiology and ecology. John Wiley & Sons, Chichester, United Kingdom.

ISO (2004). Water quality—Detection and enumeration of *Legionella*—Part 2: Direct membrane filtration method for waters with low bacterial counts. International Organization of Standardization. ISO 11731-2:2004.

ISO (2005). Water quality—Detection and enumeration of thermotolerant *Campylobacter* species. International Organization of Standardization. ISO 17995:2005.

Jacangelo, J.G., Patania, N.L., Trussell, R.R., Hass, C.N. and Gerba, C. (2002). Inactivation of waterborne emerging pathogens by selected disinfectants. AWWA Research Foundation and the American Water Works Association, Denver, Colorado, pp. 1–145.

Jackson, S.G., Goodbrand, R.B., Johnson, R.P., Odorico, V.G., Alves, D., Rahn, K., Wilson, J.B., Welch, M.K. and Khakhria, R. (1998). *Escherichia coli* O157:H7 diarrhoea associated with well water and infected cattle on an Ontario farm. Epidemiol. Infect., 120(1): 17–20.

Janda, M.J. and Abbott, S.L. (1998). Evolving concepts regarding the genus *Aeromonas*: An expanding panorama of species, disease presentations, and unanswered questions. Clin. Infec. Dis. 27:332-344.

Janda, M.J. and Abbott, S.L. (2010). The genus *Aeromonas*: taxonomy, pathogenicity, and infection. Clin. Microbiol. Rev., 23: 35–73.

Johnson, C., Rice, E. and Reasoner, D. (1997). Inactivation of *Helicobacter pylori* by chlorination. Appl. Environ. Microbiol., 63: 4969–4970.

Joly, J. (1985). *Legionella* and domestic water heaters in the Quebec City area. Can. Med. Assoc. J., 132(2): 160.

Kahana, L.M., Kay, J.M., Yakrus, M.A. and Waserman, S. (1997). *Mycobacterium avium* complex infection in an immunocompetent young adult related to hot tub exposure. Chest, 111: 242–245.

Kersters, I., Huys, G., Van Duffel, H., Vancanneyt, M., Kersters, K. and Verstraete, W. (1996). Survival potential of *Aeromonas hydrophila* in freshwaters and nutrient-poor waters in comparison with other bacteria. J. Appl. Bacteriol., 80: 266–276.

Khajanchi, B.K., Fadl, A.A., Borchardt, M.A., Berg, R.L., Horneman, A.J., Stemper, M.E., Joseph, S.W., Moyer, N.P., Sha, J. and Chopra, A.K. (2010). Distribution of virulence factors and molecular fingerprinting of *Aeromonas* species isolates from water and clinical samples: suggestive evidence of water-to-human transmission. Appl. Environ. Microbiol., 76: 2313–2325.

Kilvington, S. and Price, J. (1990). Survival of *Legionella pneumophila* within cysts of *Acanthamoeba polyphaga* following chlorine exposure. J. Appl. Bacteriol., 68(5): 519–525.

Kim, B.R., Anderson, J.E., Mueller, S.A., Gaines, W.A. and Kendall, A.M. (2002). Literature review—Efficacy of various disinfectants against *Legionella* in water systems. Water Res., 36: 4433–4444.

Kirschner, R.A., Jr., Parker, B.C. and Falkinham, J.O., III (1992). Epidemiology of infection by nontuberculous mycobacteria: *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum* in acid, brown-water swamps of the southeastern United States and their association with environmental variables. Am. Rev. Respir. Dis., 145: 271–275.

Kirschner, R.A., Jr., Parker, B.C. and Falkinham, J.O., III (1999). Humic and fulvic acids stimulate the growth of *Mycobacterium avium*. FEMS Microbiol. Ecol., 30: 327–332.

Klein, P.D., Gilman, R., Leon-Barua, R., Ramirez-Ramos, A., Recaverren, S., Spira, W., Wanatabe, J., Graham, D.Y., Gaillour, A., Opekun, A.R. and O'Brian Smith, E. (1991). Water source as risk factor for *Helicobacter pylori* infection in Peruvian children. Lancet, 337: 1503–1506.

Knøchel, S. (1991). Chlorine resistance of motile *Aeromonas* spp. Water Sci. Technol., 24: 327–330.

Koivunen, J. and Heinonen-Tanski, H. (2005). Inactivation of enteric microorganisms with chemical disinfectants, UV irradiation and combined chemical/UV treatments. Water Res., 39(8): 1519–1526.

Kool, J.L., Carpenter, J.C. and Fields, B.S. (1999). Effect of monochloramine disinfection of municipal drinking water on risk of nosocomial Legionnaires' disease. Lancet, 353: 272–277.

Kothary, M.H. and Babu, U.S. (2001). Infective dose of foodborne pathogens in volunteers: a review. J. Food Saf., 21(1): 49–73.

Kramer, M.H.J. and Ford, T.E. (1994). Legionellosis: ecological factors of an environmentally "new" disease. Zentralbl. Hyg. Umweltmed., 195: 470–482.

Kühn, I., Allestam, G., Huys, G., Janssen, P., Kersters, K., Krovacek, K. and Stenström, T.-A. (1997). Diversity, persistence, and virulence of *Aeromonas* strains isolated from drinking water distribution systems in Sweden. Appl. Environ. Microbiol., 63: 2708–2715.

Kuipers, E.J., Thijs, J.C. and Festen, H.P.M. (1995). The prevalence of *Helicobacter pylori* in peptic ulcer disease. Aliment. Pharmacol. Ther., 9(Suppl. 2): 59–69.

Kusnetsov, J., Iivanainen, E., Elomaa, N., Zacheus, O. and Martikainen, P.J. (2001). Copper and silver ions more effective against legionellae than against mycobacteria in a hospital warm water system. Water Res., 35: 4217–4225.

Kusters, J.G., Van Vliet, A.H.M. and Kuipers, E.J. (2006). Pathogenesis of *Helicobacter pylori* infection. Clin. Microbiol. Rev., 19: 449–490.

LaBombardi, V.J., O'Brien, A.M. and Kislak, J.W. (2002). Pseudo-outbreak of *Mycobacterium fortuitum* due to contaminated ice machines. Am. J. Infect. Control, 30: 184–186.

Lafrance, G., Lafrance, R., Roy, G.L., Ouellete, D. and Bourdeau, R. (1986). Outbreak of enteric infection following a field trip—Ontario. Can. Dis. Wkly. Rep., 12: 171–172.

Langenberg, W., Rauws, E.A.J., Oudbier, J.H. and Tytgat, G.N.J. (1990). Patient-to-patient transmission of *Campylobacter pylori* infection by fiberoptic gastroduodenoscopy and biopsy. J. Infect. Dis., 161: 507–511.

Långmark, J., Storey, M.V., Ashbolt, N.J. and Stenström, T.-A. (2007). The effects of UV disinfection on distribution pipe biofilm growth and pathogen incidence within the greater Stockholm area, Sweden. Water Res., 41: 3327–3336.

Lau, H.Y. and Ashbolt, N.J. (2009). The role of biofilms and protozoa in *Legionella* pathogenesis: implications for drinking water. J. Appl. Microbiol., 107: 368–378.

LeChevallier, M.W. (2004). Chapter 11: Control, treatment and disinfection of *Mycobacterium avium* complex in drinking water. In: Pathogenic mycobacteria in water, a guide to public health consequences, monitoring and management. Pedley, S., Bartram, J., Rees, G., Dufour, A. and Cotruvo, J.A. (eds.). Published by IWA Publishing, London, United Kingdom, on behalf of the World Health Organization, Geneva, Switzerland. pp. 143–168.

LeChevallier, M.W. and Au, K-K. (2004). Water treatment and pathogen control—Process efficiency in achieving safe drinking water. World Health Organization, Geneva, Switerland.

LeChevallier, M.W., Seidler, R.J. and Evans, T.M. (1980). Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. Appl. Environ. Microbiol., 40: 922–930.

LeChevallier, M.W., Abbaszadegan, M., Camper, A.K., Hurst, C.J., Izaguirre, G., Marshall, M.M., Naumovitz, D., Payment, P., Rice, E.W., Rose, J., Schaub, S., Slifko, T.R., Smith, D.B., Smith, H.V., Sterling, C.R. and Stewart, M. (1999). Committee report: Emerging pathogens—bacteria. J. Am. Water Works Assoc., 91(9): 101–109.

Le Dantec, C., Duguet, J.-P., Montiel, A., Dumoutier, N., Dubrou, S. and Vincent, V. (2002a). Chlorine disinfection of atypical mycobacteria isolated from a water distribution system. Appl. Environ. Microbiol., 68: 1025–1032.

Le Dantec, C., Duguet, J.-P., Montiel, A., Dumoutier, N., Dubrou, S. and Vincent, V. (2002b). Occurrence of mycobacteria in water treatment lines and in water distribution systems. Appl. Environ. Microbiol., 68: 5318–5325.

Loret, J.F., Robert, S., Thomas, V., Cooper, A.J., McCoy, W.F. and Lévi, Y. (2005). Comparison of disinfectants for biofilm, protozoa and *Legionella* control. J. Water Health, 3: 423–433.

Lück, P.C., Schneider, T., Wagner, J., Walther, I., Reif, U., Weber, S. and Weist, K. (2008). Community-acquired Legionnaires' disease caused by *Legionella pneumophila* serogroup 10 linked to the private home. J. Med. Microbiol., 57: 240–243.

Lumb, R., Stapledon, R., Scroop, A., Bond, P., Cunliffe, D., Goodwin, A., Doyle, R. and Bastian, I. (2004). Investigation of spa pools associated with lung disorders caused by *Mycobacterium avium* complex in immunocompetent adults. Appl. Environ. Microbiol., 70: 4906–4910.

Lund, V. (1996). Evaluation of *E. coli* as an indicator for the presence of *Campylobacter jejuni* and *Yersinia enterocolitica* in chlorinated and untreated oligotrophic lake water. Water Res., 30(6): 1528–1534.

Maalej, S., Gdoura, R., Dukan, S., Hammami, A. and Bouain, A. (2004). Maintenance of pathogenicity during entry into and resuscitation from viable but nonculturable state in *Aeromonas hydrophila* exposed to natural seawater at low temperature. J. Appl. Microbiol., 97: 557–565.

Mangione, E.J., Huitt, G., Lenaway, D., Beebe, D., Bailey, A., Figoski, M., Rau, M.P., Albrecht, K.D. and Yakrus, M.A. (2001). Nontuberculous mycobacterial disease following hot tub exposure. Emerg. Infect. Dis., 7(6): 1039–1042.

Manresa, M.J., Villa, A.V., Giralt, A.G. and González-Enseñat, M.A. (2009) *Aeromonas hydrophila* Folliculitis Associated with an Inflatable Swimming Pool: Mimicking *Pseudomonas aeruginosa* Infection. Ped. Derma. 26:601-603.

Marras, T.K. and Daley, C.L. (2002). Epidemiology of human pulmonary infection with nontuberculous mycobacteria. Clin. Chest Med., 23: 553–567.

Marras, T.K., Wallace, R.J., Jr., Koth, L.L., Stulbarg, M.S., Cowl, C.T. and Daley, C.L. (2005). Hypersensitivity pneumonitis reaction to *Mycobacterium avium* in household water. Chest, 127: 664–671.

Marras, T.K., Chedore, P., Ying, A.M. and Jamieson, F. (2007). Isolation prevalence of pulmonary non-tuberculous mycobacteria in Ontario, 1997–2003. Thorax, 62: 661–666.

Marrie, T., Green, P., Burbridge, S., Bezanson, G., Neale, S., Hoffman, P.S. and Haldane, D. (1994). Legionellaceae in the potable water of Nova Scotia hospitals and Halifax residences. Epidemiol. Infect., 112: 143–150.

Mary, P., Chihib, N.E., Charafeddine, O., Defives, C. and Hornez, J.P. (2002). Starvation survival and viable but nonculturable states in *Aeromonas hydrophila*. Microb. Ecol., 43: 250–258.

Massa, S., Armuzzi, R., Tosques, M., Canganella, F. and Trovatelli, L.D. (1999). Susceptibility to chlorine of *Aeromonas hydrophila* strains [note]. J. Appl. Microbiol., 86: 169–173.

Mathys, W., Stanke, J., Harmuth, M. and Junge-Mathys, E. (2008). Occurrence of *Legionella* in hot water systems of single-family residences in suburbs of two German cities with special reference to solar and district heating. Int. J. Hyg. Environ. Health, 211(1–2): 179–185.

Matysiak-Budnik, T., Briet, F., Heyman, M. and Megraud, F. (1995). Laboratory-acquired *Helicobacter pylori* infection [letter]. Lancet, 346: 1489–1490.

McEvoy, M., Batchelor, N., Hamilton, G., MacDonald, A., Faiers, M., Sills, A., Lee, J. and Harrison, T. (2000). A cluster of cases of legionnaires' disease associated with exposure to a spa pool on display. Commun. Dis. Public Health, 3: 43–45.

McFeters, G.A., Bissonnette, G.K., Jezeski, J.J., Thomson, C.A. and Stuart, D.G. (1974). Comparative survival of indicator bacteria and enteric pathogens in well water. Appl. Microbiol., 27: 823–829.

McKeown, I., Orr, P., Macdonald, S., Kabani, A., Brown, R., Coghlan, G., Dawood, M., Embil, J., Sargent, M., Smart, G. and Bernstein, C.N. (1999). *Helicobacter pylori* in the Canadian Arctic: seroprevalence and detection in community water samples. Am. J. Gastroenterol., 94: 1823–1829.

McNeil, C.A., Out, K., Pagan, R.T., McMyre, P., Black, W.A. and Mathias, R.G. (1981). Possible waterborne *Campylobacter* outbreak—British Columbia. Can. Dis. Wkly. Rep., 7: 223–227.

Medema, G.J., Wondergem, E., Van Dijk-Looyaard, A.M. and Havelaar, A.H. (1991). Effectivity of chlorine dioxide to control *Aeromonas* in drinking water distribution systems. Water Sci. Technol., 24: 325–326.

Meheus, J. and Peeters, P. (1989). Preventive and corrective actions to cope with *Aeromonas* growth in water treatment. Water Supply, 7: 10–14.

Mentzing, L.O. (1981). Waterborne outbreaks of *Campylobacter* enteritis in central Sweden. Lancet, 318(8242): 352–354.

Merino, S., Rubires, X., Knochel, S. and Tomas, J.M. (1995). Emerging pathogens: *Aeromonas* spp. Int. J. Food Microbiol., 28: 157–168.

Mitchell, D.O. and Starzyk, M.J. (1975). Survival of *Salmonella* and other indicator microorganisms. Can. J. Microbiol., 21: 1420–1421.

Moe, C.L. (1997). Waterborne transmission of infectious agents. In: Manual of environmental microbiology. Hurst, C.J., Knudsen, G.R., McInerney, M.J., Stetzenbach, L.D. and Walter, M.V. (eds.). ASM Press, Washington, DC.

Montecalvo, M.A., Forester, G., Tsang, A.Y., du Moulin, G. and Wormser, G.P. (1994). Colonisation of potable water with *Mycobacterium avium* complex in homes of HIV-infected patients [letter]. Lancet, 343: 1639.

Moreno, Y., Piqueres, P., Alonso, J.L., Jiménez, A., González, A. and Ferrús, M.A. (2007). Survival and viability of *Helicobacter pylori* after inoculation into chlorinated drinking water. Water Res., 41: 3490–3496.

Morgan, D.R., Johnson, P.C., DuPont, H.L., Satterwhite, T.K. and Wood, L.V. (1985). Lack of correlation between known virulence properties of *Aeromonas hydrophila* and enteropathogenicity for humans. Infect. Immun., 50: 62–65.

Muniesa, M., Jofre, J., García-Aljaro, C. and Blanch, A.R. (2006). Occurrence of *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli* in the environment. Environ. Sci. Technol., 40(23): 7141–7149.

Nichols, G., Ford, T., Bartram, J., Dufour, A. and Portaels, F. (2004). Introduction. In: Pathogenic mycobacteria in water, a guide to public health consequences, monitoring and management. S. Pedley, J. Bartram, G. Rees, A. Dufour and J.A. Cotruvo (eds.). Published by IWA Publishing, London, United Kingdom, on behalf of the World Health Organization, Geneva, Switzerland. pp. 1–14.

Norton, C.D., LeChevallier, M.W. and Falkinham, J.O., III (2004). Survival of *Mycobacterium avium* in a model distribution system. Water Res., 38: 1457–1466.

Novello, A. (1999). Outbreak of *Escherichia coli* O157:H7 and *Campylobacter* among attendees of the Washington County Fair—New York, 1999. MMWR Morb. Mortal. Wkly. Rep., 48: 803–804.

NRCC (2010). National Plumbing Code of Canada. Canadian Commission on Building and Fire Codes, National Research Council of Canada, Ottawa, Canada. Available at: www.nationalcodes.nrc.gc.ca/eng/npc/index.shtml

Oliver, B., Gross, E.A., Talan, D.A., Moran, G.J. and Pinner, R. (2005). Legionnaires disease associated with potable water in a hotel—Ocean City, Maryland, October 2003 to February 2004. Ann. Emerg. Med., 46: 288–290.

Olsen, S.J., Miller, G., Breuer, T., Kennedy, M., Higgins, C., Walford, J., McKee, G., Fox, K., Bibb, W. and Mead, P. (2002). A waterborne outbreak of *Escherichia coli* O157:H7 infections and hemolytic uremic syndrome: implications for rural water systems. Emerg. Infect. Dis., 8: 370–375.

Pablos, M., Rodríguez-Calleja, J.M., Santos, J.A., Otero, A. and García-López, M.L. (2009). Occurrence of motile *Aeromonas* in municipal drinking water and distribution of genes encoding virulence factors. Int. J. Food Microbiol., 135: 158–164.

Pagnier, I., Merchat, M. and La Scola, B. (2009). Potentially pathogenic amoeba-associated microorganisms in cooling towers and their control. Future Microbiol., 4: 615–629.

Palmer, C.J., Tsai, Y-L., Paszko-Kolva, C., Mayer, C. and Sangermano, L.R. (1993). Detection of *Legionella* species in sewage and ocean water by polymerase chain reaction, direct fluorescent-antibody, and plate culture methods. Appl. Environ. Microbiol., 59: 3618–3624.

Park, S.R., Mackay, W.G. and Reid, D.C. (2001). *Helicobacter* sp. recovered from drinking water biofilm sampled from a water distribution system. Water Res., 35: 1624–1626.

Payment, P., Gamache, F. and Paquette, G. (1988). Microbiological and virological analysis of water from two water filtration plants and their distribution systems. Can. J. Microbiol., 34: 1304–1309.

Pazzaglia, G., Escalante, J.R., Sack, R.B., Rocca, C. and Benavides, V. (1990). Transient intestinal colonization by multiple phenotypes of *Aeromonas* species during the first week of life. J. Clin. Microbiol., 28(8):1842–1846.

Pazzaglia, G., Sack, R.B., Salazar, E., Yi, A., Chea, E., Leon-Barua, R., Guerrero, C.E. and Palomino, J. (1991). High frequency of coinfecting enteropathogens in *Aeromonas*-associated diarrhea of hospitalized Peruvian infants. J. Clin. Microbiol., 29: 1151–1156.

Percival, S.L. and Thomas, J.G. (2009). Transmission of *Helicobacter pylori* and the role of water and biofilms. J. Water Health, 7: 469–477.

Percival, S.L., Chalmers, R.L., Embrey, M., Hunter, P.R., Sellwood, J. and Wyn-Jones, P. (2004). Microbiology of waterborne diseases. Elsevier Academic Press, San Diego, California.

Peters, M., Müller, C., Rüsch-Gerdes, S., Seidel, C., Göbel, U., Pohle, H.D. and Ruf, B. (1995). Isolation of atypical mycobacteria from tap water in hospitals and homes: is this a possible source of disseminated MAC infection in AIDS patients? J. Infect., 31: 39–44.

PHAC (2006). Notifiable Disease Incidence by Year, 1989-2004, Public Health Agency of Canada, Ottawa, Ontario. Available at: http://dsol-smed.phac-aspc.gc.ca/dsol-smed/ndis/c_time-eng.php

PHAC (2010). Notifiable diseases on-line. Public Health Agency of Canada, Ottawa, Ontario. Available at: http://www.phac-aspc.gc.ca/bid-bmi/dsd-dsm/duns-eng.php

Phillips, M.S. and von Reyn, C.F. (2001). Nosocomial infections due to nontuberculous mycobacteria. Clin. Infect. Dis., 33: 1363–1374.

Pinto-Santini, D. and Salama, N.R. (2005). The biology of *Helicobacter pylori* infection, a major risk factor for gastric adenocarcinoma. Cancer Epidemiol. Biomarkers Prev., 14: 1853–1858.

Pond, K. (2005). Water recreation and disease—Plausibility of associated infections: acute effects, sequelae and mortality. IWA Publishing, London, United Kingdom.

Presley, S.M., Rainwater, T.R., Austin, G.P., Platt, S.G., Zak, J.C., Cobb, G.P., Marsland, E.J., Tian, K., Zhang, B., Anderson, T.A., Cox, S.B., Abel, M.T., Leftwich, B.D., Huddleston, J.R.,

Jeter, R.M. and Kendall, R.J. (2006). Assessment of pathogens and toxicants in New Orleans, LA following Hurricane Katrina. Environ. Sci. Technol., 40: 468–474.

Pryor, M., Springthorpe, S., Riffard, S., Brooks,T., Huo, Y., Davis, G. and Sattar, S.A. (2004). Investigation of opportunistic pathogens in municipal drinking water under different supply and treatment regimes. Water Sci. Technol., 50(1): 83–90.

Rahman, M., Abd, H., Romling, U., Sandstrom, G. and Möllby, R. (2008). *Aeromonas–Acanthamoeba* interaction and early shift to a viable but nonculturable state of *Aeromonas* by *Acanthamoeba*. J. Appl. Microbiol., 104: 1449–1457.

Reimer, A.R., Au, S., Schindle, S. and Bernard, K.A. (2010). *Legionella pneumophila* monoclonal antibody subgroups and DNA sequence types isolated in Canada between 1981 and 2009: laboratory component of national surveillance. Eur. J. Clin. Microbiol. Infect. Dis., 29: 191–205.

Reingold, A.L., Thomason, B.M., Brake, B.J., Thacker, L., Wilkinson, H.W. and Kuritsky, J.N. (1984). *Legionella* pneumonia in the United States: the distribution of serogroups and species causing human illness. J. Infect. Dis., 149: 819.

Rice, E.W. (1999). *Escherichia coli*. In: AWWA manual M48: Waterborne pathogens. American Water Works Association, Denver, CO. pp. 75–78.

Rickman, O.B., Ryu, J.H., Fidler, M.E. and Kalra, S. (2002). Hypersensitivity pneumonitis associated with *Mycobacterium avium* complex and hot tub use. Mayo Clin. Proc., 77(11): 1233–1237.

Rogers, J., Dowsett, A.B., Dennis, P.J., Lee, J.V. and Keevil, C.W. (1994). Influence of plumbing materials on biofilm formation and growth of *Legionella pneumophila* in potable water systems. Appl. Environ. Microbiol., 60(6): 1842–1851.

Roig, J., Sabria, M. and Pedro-Botet, M.L. (2003). *Legionella* spp.: community acquired and nosocomial infections. Curr. Opin. Infect. Dis., 16: 145–151.

Rolle-Kampczyk, U.E., Fritz, G.J., Diez, U., Lehmann, I., Richter, M. and Herbarth, O. (2004). Well water—one source of *Helicobacter pylori* colonization. Int. J. Hyg. Environ. Health, 207: 363–368.

Rose, L.J., Rice, E.W., Hodges, L., Peterson, A. and Arduino, M.J. (2007). Monochloramine inactivation of bacterial select agents. Appl. Environ. Microbiol., 73(10): 3437–3439.

Rowbotham, T.J. (1986). Current views on the relationships between amoebae, legionellae and man. Isr. J. Med. Sci., 22: 678–689.

Rowbotham, T.J. (1998). Isolation of *Legionella pneumophila* serogroup 1 from human feces with use of amebic cocultures. Clin. Infect. Dis., 26: 502–503.

Rusin, P.A., Rose, J.B., Haas, C.N. and Gerba, C.P. (1997). Risk assessment of opportunistic bacterial pathogens in drinking water. Rev. Environ. Contam. Toxicol., 152: 57–83.

Sacks, J.J., Spencer, L., Baldy, L., Berta, S., Patton, C.M., White, M.C., Bigler, W.J. and Witte, J.J. (1986). Epidemic campylobacteriosis associated with a community water supply. Am. J. Public Health, 76: 424–428.

Sasaki, K., Tajiri, Y., Sata, M., Fujii, Y., Matsubara, F., Zhao, M., Shimizu, S., Toyonaga, A., and Tanikawa, K. (1999). *Helicobacter pylori* in the natural environment. Scand. J. Infect. Dis., 31: 275–280.

Schuster, C.J., Ellis, A.G., Robertson, W.J., Charron, D.F., Aramini, J.J., Marshall, B.J. and Medeiros, D.T. (2005). Infectious disease outbreaks related to drinking water in Canada, 1974–2001. Can. J. Public Health, 96(4): 254–255.

Scott, D., Weeks, D., Melchers, K. and Sachs, G. (1998). The life and death of *Helicobacter pylori*. Gut, 43(Suppl. 1): S56–S60.

Sebakova, H., Kozisek, F., Mudra, R., Kaustova, J., Fiedorova, M., Hanslikova, D., Nachtmannova, H., Kubina, J., Vraspir, P. and Sasek, J. (2008). Incidence of nontuberculous mycobacteria in four hot water systems using various types of disinfection. Can. J. Microbiol., 54: 891–898.

September, S.M., Brözel, V.S. and Venter, S.N. (2004). Diversity of nontuberculoid *Mycobacterium* species in biofilms of urban and semiurban drinking water distribution systems. Appl. Environ. Microbiol., 70: 7571–7573.

September, S.M., Els, F.A., Venter, S.N. and Brözel, V.S. (2007). Prevalence of bacterial pathogens in biofilms of drinking water distribution systems. J. Water Health, 5: 219–227.

Shahamat, M., Mai, U., Paszko-Kolva, C., Kessel, M. and Colwell, R.R. (1993). Use of autoradiography to assess viability of *Helicobacter pylori* in water. Appl. Environ. Microbiol., 59: 1231–1235.

Sherman, P.M., Ossa, J.C. and Wine, E. (2010). Bacterial infections: new and emerging enteric pathogens. Curr. Opin. Gastroenterol., 26(1): 1–4.

Sidari, F.P., III, Stout, J.E., Vanbriesen, J.M., Bowman, A.M., Grubb, D., Neuner, A., Wagener, M.M. and Yu, V.L. (2004). Keeping *Legionella* out of water systems. J. Am. Water Works Assoc., 96(1): 111–119.

Sisti, M., Albano, A. and Brandi, G. (1998). Bactericidal effect of chlorine on motile *Aeromonas* spp. in drinking water supplies and influence of temperature on disinfection efficacy. Lett. Appl. Microbiol., 26: 347–351.

Smith, A., Reacher, M., Smerdon, W., Adak, G.K., Nichols, G. and Chalmers, R.M. (2006). Outbreaks of waterborne infectious intestinal disease in England and Wales, 1992–2003. Epidemiol. Infect., 134(6): 1141–1149.

Sniadack, D.E., Ostroff, S.D., Karlix, M.A., Smithwick, R.W., Schwartz, B., Spaucer, M.A., Silcox, V.A. and Good, R.C. (1992). A nosocomial pseudo-outbreak of *Mycobacterium xenopi* due to a contaminated potable water supply: lessons in prevention. Infect. Control Hosp. Epidemiol., 14: 636–641.

Sobsey, M.D. (1989). Inactivation of health-related microorganisms in water by disinfection processes. Water Sci. Technol., 21(3): 179–195.

Solnick, J.V., Hansen, L.M., Canfield, D.R. and Parsonnet, J. (2001). Determination of the infectious dose of *Helicobacter pylori* during primary and secondary infection in rhesus monkeys (*Macaca mulatta*). Infect. Immun., 69: 6887–6892.

Sommer, R., Lhotsky, M., Haider, T. and Cabaj, A. (2000). UV inactivation, liquid-holding recovery, and photoreactivation of *Escherichia coli* O157 and other pathogenic *Escherichia coli* strains in water. J. Food Protect., 63(8): 1015–1020.

Sood, A., Sreedhar, R., Kulkarni, P. and Nawoor, A.R. (2007). Hypersensitivity pneumonitis-like granulomatous lung disease with nontuberculous mycobacteria from exposure to hot water aerosols. Environ. Health Perspect., 115: 262–266.

Steed, K.A. and Falkinham, J.O., III (2006). Effect of growth in biofilms on chlorine susceptibility of *Mycobacterium avium* and *Mycobacterium intracellulare*. Appl. Environ. Microbiol., 72: 4007–4011.

Steinert, M., Birkness, K., White, E., Fields, B. and Quinn, F. (1998). *Mycobacterium avium* bacilli grow saprozoically in coculture with *Acanthamoeba polyphaga* and survive within cyst walls. Appl. Environ. Microbiol., 64(6): 2256–2261.

Storey, M.V., Winiecka-Krusnell, J., Ashbolt, N.J. and Stenström, T.-A. (2004). The efficacy of heat and chlorine treatment against thermotolerant acanthamoebae and legionellae. Scand. J. Infect. Dis., 36(9): 656–662.

Stout, J.E. and Yu, V.L. (1997). Legionellosis. N. Engl. J. Med., 337(10): 682–687.

Stout J.E., Lin, Y.S.E., Goetz, A. M. and Muder R.R. (1998). Controlling *Legionella* in hospital water systems: experience with the superheat-and-flush method and copper–silver ionization. Infect Control Hosp Epidemiol 19:911-914.

Stout, J.E. and Yu, V.L. (2003). Experiences of the first 16 hospitals using copper–silver ionization for *Legionella* control: implications for the evaluation of other disinfection modalities. Infect. Control Hospital Epidemiol., 24: 563–568.

Stout, J.E., Yu, V.L., Muraca, P., Joly, J., Troup, N. and Tompkins, L.S. (1992a). Potable water as a cause of sporadic cases of community-acquired legionnaires' disease. N. Engl. J. Med., 326: 151–155.

Stout, J.E., Yu, V.L., Yee, Y.C., Vaccarello, S., Diven, W. and Lee, T.C. (1992b). *Legionella pneumophila* in residential water supplies: environmental surveillance with clinical assessment for Legionnaires' disease. Epidemiol. Infect., 109(1): 49–57.

Straus, W.L., Plouffe, J.F., File, T.M., Jr., Lipman, H.B., Hackman, B.H., Salstrom, S.-J., Benson, R.F. and Breiman, R.F. (1996). Risk factors for domestic acquisition of legionnaires disease. Arch. Intern. Med., 156(15): 1685–1692.

Swerdlow, D.L., Woodruff, B.A., Brady, R.C., Griffin, P.M., Tippen, S., Donnell, H.D., Jr., Geldreich, E., Payne, B.J., Meyer, A., Jr., Wells, J.G., Greene, K.D., Bright, M., Bean, N.H. and Blake, P.A. (1992). A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. Ann. Intern. Med., 117(10): 812–819.

Taylor, D.N., McDermott, K.T., Little, J.R., Wells, J.G. and Blaser, M.J. (1983). *Campylobacter* enteritis from untreated water in the Rocky Mountains. Ann. Intern. Med., 99: 38–40.

Taylor, R., Sloan, D., Cooper, T., Morton, B. and Hunter, I. (2000). A waterborne outbreak of *Salmonella saintpaul*. Commun. Dis. Intell., 24: 336–340.

Taylor, R.H., Falkinham, J.O., III, Norton, C.D. and LeChevallier, M.W. (2000). Chlorine, chloramine, chlorine dioxide, and ozone susceptibility of *Mycobacterium avium*. Appl. Environ. Microbiol., 66: 1702–1705.

Temmerman, R., Vervaeren, H., Noseda, B., Boon, N. and Verstraete, W. (2006). Necrotrophic growth of *Legionella pneumophila*. Appl. Environ. Microbiol., 72(6): 4323–4328.

Templeton, M.R., Oddy, F., Leung, W.-K. and Rogers, M. (2009). Chlorine and UV disinfection of ampicillin-resistant and trimethoprim-resistant *Escherichia coli.* Can. J. Civ. Eng., 36(5): 889–894.

Thomas, V., Bouchez, T., Nicolas, V., Robert, S., Loret, J.F. and Lévi, Y. (2004). Amoebae in domestic water systems: resistance to disinfection treatments and implication in *Legionella* persistence. J. Appl. Microbiol., 97: 950–963.

Thompson, J.S. and Gravel, M.J. (1986). Family outbreak of gastroenteritis due to *Yersinia enterocolitica* serotype 0:3 from well water. Can. J. Microbiol., 32: 700–701.

Tobin, R.S., Ewan, P., Walsh, K. and Dutka, B. (1986). A survey of *Legionella pneumophila* in water in 12 Canadian cities. Water Res., 20: 495–501.

Tobin-D'Angelo, M.J., Blass, M.A., Del Rio, C., Halvosa, J.S., Blumberg, H.M. and Horsburgh, C.R., Jr. (2004). Hospital water as a source of *Mycobacterium avium* complex isolates in respiratory specimens. J. Infect. Dis., 189: 98–104.

Tsintzou, A., Vantarakis, A., Pagonopoulou, O., Athanassiadou, A. and Papapetropoulou, M. (2000). Environmental mycobacteria in drinking water before and after replacement of the water distribution network. Water Air Soil Pollut., 120: 273–282.

U.S. EPA (1999a). *Legionella*: human health criteria document. Office of Science and Technology, Office of Water, United States Environmental Protection Agency, Washington, DC. November (EPA 822-R-99-011).

U.S. EPA (1999b). Alternative disinfectants and oxidants guidance manual. Office of Water, United States Environmental Protection Agency, Washington, DC. April (EPA 815-R-99-014).

U.S. EPA (2002). Treatability screening for CCL microbial contaminants. Office of Water, United States Environmental Protection Agency, Washington, DC (EPA/822/R/01/002).

U.S. EPA (2006). *Aeromonas*: human health criteria document. Office of Science and Technology, Office of Water, United States Environmental Protection Agency, Washington, DC. March (EPA 68-C-02-026).

U.S. EPA (2009). Drinking water contaminant candidate list 3—Final. United States Environmental Protection Agency, Washington, DC. Fed. Regist., 74(194): 51850–51862.

Vaerewijck, M.J.M., Huys, G., Palomino, J.C., Swings, J. and Portaels, F. (2005). Mycobacteria in drinking water distribution systems: ecology and significance for human health. FEMS Microbiol. Rev., 29: 911–934.

Vakil, N. and Megraud, F. (2007). Eradication therapy for *Helicobacter pylori*. Gastroenterology, 133: 985–1001.

Van der Kooij, D. (2003). Chapter 11: Managing re-growth in drinking water distribution systems. In: Heterotrophic plate counts and drinking-water safety—The significance of HPCs for water quality and human health. Bartram, J., Cotruvo, J., Exner, M., Fricker, C. and Glasmacher, A. (eds.). Published by IWA Publishing, London, United Kingdom, on behalf of the World Health Organization, Geneva, Switzerland. pp. 199–232.

Vicuña-Reyes, J.P., Luh, J. and Mariñas, B.J. (2008). Inactivation of *Mycobacterium avium* with chlorine dioxide. Water Res., 42: 1531–1538.

Vogt, R.L., Sours, H.E., Barrett, T., Feldman, R.A., Dickson, R.J. and Witherell, L. (1982). *Campylobacter* enteritis associated with contaminated water. Ann. Intern. Med., 96: 292–296.

Von Graevenitz, A. (2007). The role of *Aeromonas* in diarrhea: a review. Infection, 35: 59–64.

Von Reyn, C.F., Waddell, R.D., Eaton, T., Arbeit, R.D., Maslow, J.N., Barber, T.W., Brindle, R.J., Gilks, C.F., Lumio, J., Lähdevirta, J., Ranki, A., Dawson, D. and Falkinham, J.O., III (1993). Isolation of *Mycobacterium avium* complex from water in the United States, Finland, Zaire, and Kenya. J. Clin. Microbiol., 31: 3227–3230.

Von Reyn, C.F., Maslow, J.N., Barber, T.W., Falkinham, J.O., III and Arbeit, R.D. (1994). Persistent colonisation of potable water as a source of *Mycobacterium avium* infection in AIDS. Lancet, 343: 1137–1141.

Wang, W.L.L., Powers, B.W., Blaser, M.J. and Leuchtefeld, N.W. (1982). Laboratory studies of disinfectants against *Campylobacter jejuni*. In: Proceedings of the Annual Meeting of the American Society of Microbiology. American Society of Microbiology, Washington, DC.

Wang, Y., Claeys, L., Van Der Ha, D., Verstraete, W. and Boon, N. (2010). Effects of chemically and electrochemically dosed chlorine on *Escherichia coli* and *Legionella beliardensis* assessed by flow cytometry. Appl. Microbiol. Biotechnol., 87: 331–341.

Watson, C.L., Owen, R.J., Said, B., Lai, S., Lee, J.V., Surman-Lee, S. and Nichols, G. (2004). Detection of *Helicobacter pylori* by PCR but not culture in water and biofilm samples from drinking water distribution systems in England. J. Appl. Microbiol., 97: 690–698.

Weintraub, J.M., Flannery, B., Vugia, D.J., Gelling, L.B., Salerno, J.J., Conroy, M.J., Stevens, V.A., Rose, C.E., Besser, R.E., Fields, B.S. and Moore, M.R. (2008). *Legionella* reduction after conversion to monochloramine for residual disinfection. J. Am. Water Works Assoc., 100(4): 129–139.

Wendt, S.L., George, K.L., Parker, B.C., Gruft, H. and Falkinham, J.O., III (1980). Epidemiology of infection by nontuberculous mycobacteria: isolation of potentially pathogenic mycobacteria from aerosols. Am. Rev. Respir. Dis., 122: 259–263.

West, A.P., Millar, M.R. and Tompkins, D.S. (1992). Effect of physical environment on survival of *Helicobacter pylori*. J. of Clin. Pathol. 45:228-231.

White, F.M. and Pedersen, A.T. (1976). Epidemic shigellosis on a worktrain in Labrador. Can. Med. Assoc. J., 115: 647–649.

WHO (2002). Guidelines for drinking-water quality. 2nd edition. Addendum: Microbiological agents in drinking water. World Health Organization, Geneva, Switzerland.

WHO (2010). Chapter 11: Microbial fact sheets. In: Guidelines for drinking-water quality. 3rd edition. World Health Organization, Geneva, Switzerland.

Winiecka-Krusnell, J., Wreiber, K., Von Euler, A., Engstrand, L. and Linder, E. (2002). Free-living amoebae promote growth and survival of *Helicobacter pylori*. Scand. J. Infect. Dis., 34: 253–256.

Wojcicka, L., Hofmann, R., Baxter, C., Andrews, R.C., Auvray, I., Lière, J., Miller, T., Chauret, C. and Baribeau, H. (2007). Inactivation of environmental and reference strains of heterotrophic bacteria and *Escherichia coli* O157:H7 by free chlorine and monochloramine. J. Water Supply: Res. Technol. – Aqua, 56(2): 137–150.

Xagoraraki, I., Harrington, G.W., Assavasilavasukul, P. and Standridge, J.H. (2004). Removal of emerging waterborne pathogens and pathogen indicators by pilot-scale conventional treatment. J. Am. Water Works Assoc., 96(5): 102–113.

Xi, C., Zhang, Y., Marrs, C., Ye, W., Simon, C., Foxman, B. and Nriagu, J. (2009). Prevalence of antibiotic resistance in drinking water treatment and distribution systems. Appl. Environ. Microbiol, 75(17): 5714–5718.

Yoder, J., Roberts, V., Craun, G.F., Hill, V., Hicks, L.A., Alexander, N.T., Radke, V., Calderon, R.L., Hlavsa, M.C., Beach, M.J. and Roy, S.L. (2008). Surveillance for waterborne disease and outbreaks associated with drinking water and water not intended for drinking—United States, 2005–2006. MMWR Surveill. Summ., 57: 39–62.

Yu, C.P., Farrell, S.K., Robinson, B. and Chu, K.H. (2008). Development and application of real-time PCR assays for quantifying total and aerolysin gene–containing *Aeromonas* in source, intermediate, and finished drinking water. Environ. Sci. Technol., 42: 1191–1200.

Zimmer-Thomas, J.L., Slawson, R.M. and Huck, P.M. (2007). A comparison of DNA repair and survival of *Escherichia coli* O157:H7 following exposure to both low- and medium-pressure UV irradiation. J. Water Health, 5(3): 407–415.

C.2 List of acronyms

AIDS	acquired immunodeficiency syndrome
CFU	colony-forming unit
CT	concentration × time
DAEC	diffuse adherent *E. coli*
DNA	deoxyribonucleic acid
EAEC	enteroaggregative *E. coli*
EHEC	enterohaemorrhagic *E. coli*
EIEC	enteroinvasive *E. coli*
EPA	Environmental Protection Agency (U.S.)
EPEC	enteropathogenic *E. coli*
ETEC	enterotoxigenic *E. coli*
HIV	human immunodeficiency virus
HPC	heterotrophic plate count
Mac	*Mycobacterium avium* complex
PCR	polymerase chain reaction
RNA	ribonucleic acid
UV	ultraviolet