Epidemiology and Ecology of Opportunistic Premise Plumbing Pathogens: *Legionella pneumophila*, *Mycobacterium avium*, and *Pseudomonas aeruginosa*  

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**Background**: *Legionella pneumophila*, *Mycobacterium avium*, and *Pseudomonas aeruginosa* are opportunistic premise plumbing pathogens (OPPPs) that persist and grow in household plumbing, habitats they share with humans. Infections caused by these OPPPs involve individuals with preexisting risk factors and frequently require hospitalization.

**Objectives**: The objectives of this report are to alert professionals of the impact of OPPPs, the fact that 30% of the population may be exposed to OPPPs, and the need to develop means to reduce OPPP exposure. We herein present a review of the epidemiology and ecology of these three bacterial OPPPs, specifically to identify common and unique features.

**Methods**: A Water Research Foundation–sponsored workshop gathered experts from across the United States to review the characteristics of OPPPs, identify problems, and develop a list of research priorities to address critical knowledge gaps with respect to increasing OPPP-associated disease.

**Discussion**: OPPPs share the common characteristics of disinfectant resistance and growth in biofilms in water distribution systems or premise plumbing. Thus, they share a number of habitats with humans (e.g., showers) that can lead to exposure and infection. The frequency of OPPP-infected individuals is rising and will likely continue to rise as the number of at-risk individuals is increasing. Improved reporting of OPPP disease and increased understanding of the genetic, physiologic, and structural characteristics governing the persistence and growth of OPPPs in drinking water distribution systems and premise plumbing is needed.

**Conclusions**: Because broadly effective community-level engineering interventions for the control of OPPPs have yet to be identified, and because the number of at-risk individuals will continue to rise, it is likely that OPPP-related infections will continue to increase. However, it is possible that individuals can take measures (e.g., raise hot water heater temperatures and filter water) to reduce home exposures.

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**Introduction**

Because community water systems supply water to > 95% of the approximately 300 million people in the United States, there is a need for a firm understanding of the precise contribution of these water systems to the spread of waterborne disease (Craun et al. 2010; Kozicki et al. 2012). Community water systems deliver water to premise plumbing, which is the portion of the water distribution system beyond the property line and includes households, office buildings, and hospitals. Therefore, premise plumbing serves as the interface of exposure of people to the microbes inhabiting their water supply.

Several unique features of premise plumbing can increase risk of microbial infection. High surface-to-volume ratio, intermittent stagnation, low disinfectant residual, and warming cycles can stimulate growth of waterborne pathogens. High surface-to-volume ratios of premise plumbing systems mean that there is a large area conducive for biofilm formation. Biofilms can be attractive habitats for pathogens and offer protection from disinfectants. Opportunistic pathogens have been found to grow in shower heads, faucets, along pipe walls, and in water heaters. The long residence time of water in premise plumbing enhances biofilm formation, including growth of resident pathogens. Although greater water ages are thought to enhance attenuation of traditional enteric pathogens, opportunistic pathogens can adapt and grow at low oxygen levels characteristic of stagnation in premise plumbing.

Herein, we review the epidemiology and ecology of opportunistic premise plumbing pathogens (OPPPs), focusing on three of the most commonly tracked bacterial agents: *Legionella pneumophila*, *Mycobacterium avium*, and *Pseudomonas aeruginosa*. Infections by all three have been linked to human exposure via premise plumbing (Table 1). It has been estimated that the costs of the estimated 29,636 cases of OPPP disease per year is approximately $850 million (Collier et al. 2012).

Until recently [e.g., 1976 (Legionella), 1980 (Mycobacterium), 1997 (Pseudomonas aeruginosa)], there was no consideration of pathogenic microorganisms that were natural inhabitants of water and drinking water systems. In the past, the term “waterborne pathogens” referred to those agents in human or animal waste that entered water as contaminants with ingestion of drinking water, the principle route of exposure and infection. Classically, these included polio virus, *Shigella*, *Salmonella*, and other enteric bacteria, all examples of pathogens with a fecal–oral route of infection.

These pathogens are not normal inhabitants of water but contaminants, and generally are not capable of reproduction in the water supply. Operationally, one can identify the point source for each “waterborne pathogens” by moving upstream as their numbers increase, as they get closer to the point source. In contrast, the numbers of OPPPs increase as the distance from the treatment plant increases because they can multiply in pipes and plumbing systems (Falkinham et al. 2001; Hardalo and Edberg 1997; Hsu et al. 1984; Lin et al. 1998; Squier et al. 2006; States et al. 1987). Further, OPPP numbers do not correlate with fecal coliform numbers (Falkinham et al. 2001).

Increasingly, the role of biofilms within water distribution systems and premise plumbing is recognized as important for the establishment and maintenance of the chronic colonization associated with *L. pneumophila*, *M. avium*, and *P. aeruginosa*. Biofilms and amoebic host organisms can protect these pathogens from effective treatment with...
disinfectants, such as chlorine (Donlan and Costerton 2002; Holby et al. 2010; Murga et al. 2001; Simões et al. 2010; Steed and Falkinham 2006; Suman et al. 2008).

On 25–27 March 2012, a Water Research Foundation-sponsored expert workshop, “Research Needs for Opportunistic Pathogens in Premise Plumbing,” was held at the Virginia Tech Northern Virginia Center, Falls Church, Virginia, to document the widespread prevalence and impact of OPPPs on humans and to review their epidemiology and ecology and identify common features contributing to the infective process. The 2-day workshop assembled > 50 experts in drinking water and waterborne pathogens to a) review the state of knowledge of OPPPs, b) identify gaps in knowledge of OPPPs, and c) identify and prioritize research objectives (Pruden et al. 2013). This literature review serves to summarize the state of the knowledge with respect to epidemiology and ecology of key bacterial OPPPs, which may better inform the development of approaches to reduce human exposure and infection. Key knowledge gaps identified at the expert workshop that should be prioritized for future research are also described.

Legionella Epidemiology

There are three major presentations of Legionella spp. infection: a) Pontiac fever and b) either community-acquired or c) outbreak-associated Legionnaires’ disease. Pontiac fever is an influenza-like, mild illness that is spontaneously resolved without therapy and is caused by a variety of Legionella spp. from either water (Castor et al. 2005) or soil (Cramp et al. 2010). The focus here will be on L. pneumophila, the causative agent of serious, life-threatening pneumonia (“Legionnaires’ disease”), often requiring hospitalization [Centers for Disease Control and Prevention (CDC) 2011b; Yoder et al. 2008]. In the United States, the most recent estimated number of hospitalized cases, based on a population-based study in 2000–2009, is 8,000–18,000 (CDC 2011b). Moreover, the number of reported legionellosis cases increased 3.5-fold between 2000 and 2011 (Table 1); (CDC 2011b, 2013a; Yoder et al. 2008). Legionellosis is an acute illness and, in its most severe form, is generally responsive to timely and appropriate antimicrobial therapy (Bruin et al. 2012; Niederman et al. 2001).

In addition to air-conditioning systems and cooling towers as sources (Nguyen et al. 2006; Sabria et al. 2006), it is now understood that drinking water is an important source of L. pneumophila (Benin et al. 2002; Boccia 2006; CDC 2013b; Neil and Berkelman 2008; Phares et al. 2007; Yoder et al. 2008). L. pneumophila recently became the single most common cause of reported disease outbreaks involving drinking water (CDC 2013b; Craun et al. 2010; Yoder et al. 2008), in part because of improved detection and diagnosis and also likely because it is the first OPPP-associated disease that must be reported to the National Notifiable Diseases Surveillance System (http://wwwn.cdc.gov/nndss/).

Community-acquired L. pneumophila pneumonia. The prevalence of community-acquired and healthcare-associated legionellosis are both increasing. One-quarter (25%) of Legionella spp. infections are healthcare associated (Neil and Berkelman 2008). Because L. pneumophila is present in drinking water distribution systems and household water (Arnow et al. 1985; Bollin et al. 1985; Borella et al. 2005; Donohue et al. 2014), persons at risk for Legionnaires’ disease should take precautions. Risk factors for Legionnaires’ disease include reduced immune competence, smoking, alcoholism, and older age (CDC 2011b). Case reports are highest in summer and in the mid-Atlantic region of the United States (CDC 2011b).

Outbreak-associated L. pneumophila pneumonia. Legionnaires’ disease was first described as an outbreak of pneumonia among attendees of an American Legion convention in Philadelphia, Pennsylvania, in 1976 (Fraser et al. 1977). In the absence of evidence of person-to-person transmission, it was hypothesized that the infective agent originated from the environment (Fraser et al. 1977). Since that first report, outbreaks of L. pneumophila disease have been linked to water sources in hospitals, hotels, cruise ships, industrial facilities, and multiple and single family residences (Arnow et al. 1985; Borella et al. 2005; Hung et al. 1993; Kusnetsov et al. 2003; O’Loughlin et al. 2007; Polverino et al. 2010). The British Communicable Disease Surveillance Centre reported that 19 of 20 hospital outbreaks of Legionnaires’ disease in the United Kingdom from 1980 to 1992 were primarily attributed to hospital water systems (Joseph et al. 1994). Legionella spp. in hospital drinking water samples have been linked to patient isolates by DNA-fingerprinting methods (Den Boer et al. 2008; Kozak-Muiznieks et al. 2014; Ragull et al. 2007; Sabrì et al. 2001; Stout et al. 1985).

Consequently, Legionnaires’ disease should be considered for all pneumonia cases with prior hospital exposure, particularly the elderly, smokers, immunosuppressed, and those with chronic lung disease.

Legionella Ecology

The natural habitat for Legionella appears to be aquatic bodies including rivers, streams, and thermally polluted waters (Brooks et al. 2004; Colbourne and Dennis 1989; Hsu et al. 1984; Lin et al. 1998; Stout et al. 1985). Legionella bacteria have been detected in all segments of water distribution—from the source water (rivers and groundwater) to the tap. Natural aquatic bodies contain only small numbers of Legionella. The presence of Legionella in a water distribution system is not necessarily an indication that the system is poorly maintained because this bacterium may be a normal constituent of the microbial population of water distribution systems. It has been estimated that Legionella are found in approximately 50% of large building water systems and 10–30% of home water systems in the United States (Kool et al. 1999; Stout and Yu 2011), and detection methods are becoming increasingly sensitive. Depending on the study and methods, a range of 12–70% of hospital water systems are estimated to be colonized with Legionella (Stout and Yu 1997). A recent publication demonstrated the presence of “nonculturable” cells of L. pneumophila as well as methods for their resuscitation, to ensure that colony counts are not underestimated (Ducrut et al. 2014). In the first national study to use more sensitive molecular techniques, Legionella genetic material was detected in 50% of cold water samples (Donohue et al. 2014).

Cooling towers and, to a lesser degree, evaporative condensers were implicated in the earlier outbreaks prior to recognition of potable water as a reservoir (Bentham 2000; Nguyen et al. 2006). The emphasis of cooling towers in the dissemination of Legionella has been challenged (Stout and Muder 2004). Reports of cooling towers as reservoirs for legionellosis have dwindled in comparison with those linked to building water distribution systems.

### Table 1. Estimated OPPP disease occurrence.

| Disease (ICD-10 code) | Measurement | Source |
|-----------------------|-------------|--------|
| Disease prevalence/100,000 | 0.39 (2000) to 1.36 (2011) | CDC 2011b, 2013a |
| NTM, USA (031.0) | 3.5 (1994–1996) to 4.9 (2004–2006) | Prevots et al. 2010 |
| Men | 4.5 (1994–1996) to 7.5 (2004–2006) | Prevots et al. 2010 |
| NTM, Ontario (031.0) | 29.3 (1998–2002) to 41.3 (2008–2010) | Marras et al. 2013 |
| Isolation prevalence/year | 2,025 (1986) to 5,205 (2000) | Gaynes et al. 2005 |
| P. aeruginosa (J15.1) | > 3,000 global cases (2004) | Schuster and Visvesvara 2004 |
| N. fowleri | 111 cases (1962–2008) | Yoder et al. 2008 |

Abbreviations: ICD-10, International Classification of Diseases, 10th Revision; NTM, nontuberculous mycobacteria.
Legionella are not completely eliminated from drinking water by standard water treatment processes. For example, Legionella are comparatively more resistant to chlorine than Escherichia coli (Garcia and Pelaz 2008; Hosein et al. 2003; Kim et al. 2002; Zhang et al. 2007). Legionella are also known to be sheltered within encysted amoebae; indeed, after phagocytosis by amoebae, whose cells are relatively chlorine-resistant, Legionella can survive up to 50 ppm chlorine (Kilvington and Price 1990). Legionella growth and proliferation occur in engineered habitats, especially water distribution systems, which provide favorable water temperatures (25–42°C), surfaces for biofilm formation, and nutrients (Arnow et al. 1985; Donlan and Costerton 2002; Lin et al. 1998; Murga et al. 2001).

One important factor appears to be water temperature. Buildings with recirculating hot water distribution systems colonized with L. pneumophila were significantly more likely to have lower hot water heater temperatures (<60°C) than systems that were not colonized (Arnow et al. 1985; Danelid et al. 2002). The microorganism is readily found in biofilm and detritus at the bottom of hot water tanks. Bacteria, protozoa, and amoebae also colonize water pipe surfaces, some of which have been shown to promote Legionella replication (Buse et al. 2014b; Kilvington and Price 1990). Legionella and other microorganisms attach to surfaces and form biofilms on pipes throughout the water distribution system. Cold-water sources, such as ice from ice machines and water from fountains with stable biofilm-colonized surfaces, have also been implicated as a source of infection (Hoebe et al. 1998; O’Loughlin et al. 2007; Stout et al. 1985).

Sources of Legionella Exposure and Transmission

Multiple modes have been identified for transmission of Legionella to humans; there is evidence for aerosolization, aspiration, or even instillation into the lung during respiratory tract manipulation. Because one of the first environmental isolations of L. pneumophila was from a showerhead (Stout and Yu 2003), it has been widely thought that aerosols from showers may be an important means for dissemination of this microorganism. However, as Legionella are prevalent in home water systems, any shower or faucet can be a source of infection (Stout and Muder 2002), and detectable airborne Legionella aerosols have been detected in proximity to faucets (Bollin et al. 1985).

Aspiration of contaminated water or oropharyngeal secretions appears to be the major mode of transmission in the hospital setting (Blatt et al. 1993; Yu 1993). Colonization of oropharyngeal flora by L. pneumophila is a theoretical possibility, and the evidence for aspiration has accumulated. Nasogastric tube placement has been shown to be a significant risk factor for healthcare-associated legionellosis in intubated patients; microaspiration of contaminated water was the presumed mode of entry (Blatt et al. 1993). It is possible that ingestion of water also can play a role. During the original 1976 outbreak, consumption of water and possible aspiration at the implicated hotel was associated with acquisition of disease (Fraser et al. 1977)—an association that has been generally overlooked.

Healthcare personnel frequently use tap water to rinse respiratory apparatus and tubing used for ventilators. If the tap water contains L. pneumophila, the bacteria could possibly be instilled directly into the lung of a patient (Tablan et al. 2004). In numerous studies, the risk of Legionnaires’ disease was significantly greater for patients who underwent endotracheal tube placement more often or had a significantly longer duration of intubation than for patients who had other causes of pneumonia. Use of sterile water for all nasogastric suspensions, for humidifiers in breathing circuits of mechanical ventilators, and for flushing tubes has been recommended to prevent Legionella infection (Tablan et al. 2004).

Mycobacterium avium

M. avium infections are known to originate from environmental sources (Falkinham 1996). It is one among 175 species of the genus Mycobacterium that do not belong to the Mycobacterium tuberculosis complex (Tortoli 2003) and thus are called nontuberculous mycobacteria (NTM). Our focus is on M. avium as the causal agent of the majority of NTM infections in the United States (Falkinham 1996), and it is the most prevalent Mycobacterium in drinking water (Falkinham 2011; Falkinham et al. 2001).

There are three major presentations of M. avium infection: a) bacteremia in HIV-infected individuals, b) cervical lymphadenitis in young children, and c) community-acquired M. avium infection in adults. There have been few reports of M. avium disease outbreaks, and these tend to be associated with contamination of solutions and instruments in hospitals (Wallace et al. 1998). Reports have also linked isolation of M. avium from bronchoscopies, ice, whirlpool tubs, pools, footbaths, and prepared cleaning and irrigation solutions (Gubler et al. 1992; Kahana et al. 1997; Winthrop et al. 2002). M. avium bacteremia among HIV-infected and immunosuppressed persons emerged during the 1980s (Horsburgh and Selik 1989). At one time, approximately 50% of late-stage AIDS patients had M. avium bacteremia (Horsburgh and Selik 1989), but with the implementation of highly active antiretroviral therapy, the number of HIV-infected patients with M. avium infections has fallen dramatically. Although there are no national statistics documenting numbers of M. avium–associated cervical lymphadenitis in children, there has been no published evidence of this manifestation disappearing or increasing (Wolinsky 1995).

In a comprehensive, prospective study of cervical lymphadenitis published in 1995 by a physician evaluating incident cases in the United States over the period of 1958–1990, only 105 cases were found (Wolinsky 1995). The age of the infected children (median age 3 years) suggests that exposure to water or soil containing M. avium, coupled with gum trauma due to erupting teeth, led to M. avium infection of the lymph nodes of the head and neck.

Community-acquired M. avium infection.

Currently, the majority of M. avium cases are community acquired. As disease caused by M. avium is not nationally notifiable in the United States (CDC 2011a), population-based studies are uncommon, and the public health burden of M. avium disease is difficult to measure. Two recent population-based studies in the United States have described rates of M. avium disease as increasing among older persons and women (Table 1) (Prevots et al. 2010; Winthrop et al. 2010). Estimated prevalence of M. avium lung disease varies by study, location, age, and susceptible population; prevalence increases with age and is highest among patients with AIDS, at 647 cases/100,000 persons (Marras and Daley 2002; Prevots et al. 2010).

Among studies that evaluated numbers of M. avium isolates recovered from clinical specimens, rates of positive cultures appear to be increasing. These include reports from the United States (du Moulin et al. 1985; Prevots et al. 2010), Japan (Tsukamura et al. 1988), Canada (Al Houqani et al. 2011; Marras et al. 2013), Taiwan (Chen et al. 2011), South Korea (Ryoo et al. 2008), and China (Wang et al. 2010). The increase in M. avium isolation and disease frequency has also coincided with a shift in the observed epidemiology, from disease occurring principally in older men with reduced lung function due to smoking or occupational dust exposure, to tall, slender, and older women (Prince et al. 1989). Among studies where age is reported, older age was associated with a higher prevalence of disease. However, factors (e.g., improved detection) other than age alone may be associated with increased rates reported per year in many studies, even in countries with aging populations (Al-Houqani et al. 2012).

Both pulmonary and extrapulmonary M. avium infections have been described
Factors include temperature, water flow, nutrients, pipe material and condition, residual disinfectant, free-living phagocytic amoebae, mycobacteriophages, and other bacteria. Reports implicate some of these risk factors, but none alone predict M. avium concentration at the point of use. Concentrations of M. avium in water were significantly correlated with organic carbon concentrations (Falkinham et al. 2001), hot water plumbing lines (du Moulin et al. 1988; Falkinham 2011), and plastic pipe material (Schulze-Röbbecke et al. 1992), although Norton et al. (2004) reported significant M. avium concentrations in water independent of pipe material. M. avium has been isolated from multiple environmental sources, including from water and biofilm (Schulze-Röbbecke et al. 1992; Tsintzou et al. 2000). Reports of M. avium in water distribution and treatment plants suggest that biofilms that form in the distribution system act as an important niche for the survival of M. avium (Falkinham et al. 2001; Feazel et al. 2009; Hilborn et al. 2006). Further, M. avium, like L. pneumophila and P. aeruginosa, is an amoeba-resisting microorganism, able to grow and survive in amoebae (Cirillo et al. 1997; Thomas and Ashbolt 2011). M. avium is approximately 500 times more resistant to chlorine than Escherichia coli (Taylor et al. 2000) and 40 times more tolerant to chlorine than P. aeruginosa (Grobe et al. 2001). Further, M. avium survives and multiplies in distribution systems despite ambient chlorine residual concentrations (Falkinham et al. 2001). M. avium grown in water is more chlorine resistant than the same strains grown in culture medium, and most strains are more resistant to chloramine than to free chlorine (Taylor et al. 2000).

Drinking water is a known environmental source of NTM and has been extensively studied in an attempt to characterize the risk of human exposure to NTM. Quantitative interpretation of the results of these studies is problematic because isolation methods vary and because decontamination steps to prevent the growth of more rapidly growing microorganisms are known to reduce concentrations of M. avium in water samples (Thomson et al. 2008). Unfortunately, there are no selective or differential media for the cultivation of NTM from samples containing other microorganisms. Therefore, observed and reported occurrence in water samples should be interpreted as conservative estimates of true occurrence and abundance. M. avium isolation from drinking water has been documented at points of use within both public and private buildings (Falkinham et al. 2008; Hilborn et al. 2006; Perkins et al. 2009).

Sources of M. avium Exposure and Transmission

Water is a well-documented source of M. avium exposure. M. avium isolation from hospital water supplies is of particular concern due to the potential for exposure of immuno-suppressed patients (Baird et al. 2011; du Moulin et al. 1988). The persistence of a single clone of M. avium (up to 18 months) in hospital (von Reyn et al. 1994) and distributed municipal drinking water (Hilborn et al. 2006) as a potential chronic source of human exposure is a major challenge. It is important to point out that identification of identical M. avium clones in patients and their household plumbing does not necessarily indicate that the tap water is the original source, especially if the patients collected the samples. It could be that patients continually reinfect their own taps.

Filters have been recommended as a means to reduce M. avium exposure from water. However, some types of filters, that is, granular activated charcoal (GAC) filters, have been shown to be colonized by M. avium and support their growth (Hollinger et al. 2014; Rodgers et al. 1999; Williams et al. 2011). Consequently, the GAC filter becomes a source of M. avium and likely other OPPPs.

Epidemiology of Pseudomonas aeruginosa

There are four major presentations of P. aeruginosa infection (Table 1): a) bacteremia in immunocompromised individuals, b) pneumonia in cystic fibrosis (CF) patients, c) community-acquired ear and pneumonia infections, and d) hospital-acquired outbreaks, principally associated with contaminated solutions or medical devices used in general patients or those in intensive care units (ICUs) (Fujitani et al. 2011). In all four presentations, water containing P. aeruginosa is the source of infection.

Community-acquired pneumonia. Very few data are available about the occurrence of P. aeruginosa disease outside of the healthcare setting. Much of what is known about infections in the community setting is from reports in the published literature (e.g., case reports, outbreak investigations). Infections produced by P. aeruginosa are not nationally notifiable, so the burden of disease is difficult to assess. The primary infections (ear and skin) acquired in the community involve the use of swimming pools, hot tubs, and whirlpools where there has been a failure to maintain the equipment or to maintain sufficient residual disinfectant (Hlavsa et al. 2014). P. aeruginosa is rarely carried by healthy individuals (2–10% of individuals, likely in the ear) but can be recovered from 50–60% of hospitalized patients (Cholley et al. 2008). P. aeruginosa is a major cause of otitis externa (“swimmer’s ear”), with a magnitude of 2.4 million cases per year and an estimated outpatient cost of approximately $500 million (CDC 2011a).

Hospital-acquired infections. In a meta-analysis of 43 water-associated outbreaks in
Opportunistic premise plumbing pathogens

It is expected that the number of people susceptible to infection with OPPPs will grow in the United States. Cystic fibrosis patients, transplant recipients, and immunosuppressed patients are living longer lives, resulting in more time to become colonized or infected. For example, the U.S. population is aging, and it is estimated that the proportion of individuals > 60 years of age will increase from 16.1% in 2000 to 24.8% in 2025 (United Nations, Population Division 2002). Such an increase in individuals > 60 years of age, coupled with higher rates of infection by OPPPs in that age group, strongly suggests that the prevalence of OPPP-related disease will rise. Our public water systems are deteriorating and in desperate need of maintenance and replacement; thus, there are increased opportunities for intrusion of soil-associated OPPPs and for biofilm formation, which favors their growth. At the same time, exposure occurs via premise plumbing, and knowledge of the interaction between the chemistry and microbiology of municipal water and premise plumbing is needed to inform risk management strategies.

Common Features of OPPPs

There are several traits shared by the three bacterial OPPPs that select for their presence and persistence in premise plumbing. Those traits include disinfectant resistance, biofilm formation, survival at high temperatures, and growth in free-living phagocytic amoebae. The concentrations of water treatment disinfectants (e.g., chlorine, chloramine) required to kill 99.9% of the bacterial OPPPs are higher than those needed to obtain a 3-log reduction in Giardia lamblia cysts (U.S. Environmental Protection Agency 2010), the standard used for water disinfection. The presence of residual disinfectant provides these resistant OPPPs a competitive advantage (Grobe et al. 2001; Seyfried and Fraser 1980; Taylor et al. 2000). One factor likely leading to the occurrence and persistence of the bacterial OPPPs in premise plumbing and distributions systems is their ability to adhere to surfaces and form biofilms. Bacteria in biofilms are also more resistant to disinfectants (Simões et al. 2010). Residence in biofilms also makes bacterial OPPPs more accessible to free-living, phagocytic amoebae [e.g., Acanthamoeba and Vermamoeba (formerly called Hartmanella (Smirnov et al. 2005)], which can actually enhance their proliferation in drinking water (Thomas and Ashbolt 2011). All three bacterial OPPPs belong to the category of amoeba-resistant microorganisms: they are not necessarily killed by amoebae following phagocytosis but can actually survive and grow. Finally, because these OPPS have relative resistance to high temperatures that are encountered in hot conditions.
water pipes (e.g., 35–45°C), their numbers actually increase in hot water heaters and premise plumbing.

**Remediation and Control of OPPPs**

Currently, there are no documented broadly effective community-level engineering control strategies for OPPPs in municipal drinking water or premise plumbing. Identification and use of methods to effectively and economically control OPPPs is in its infancy. Preventative measures could be imposed by water utilities, healthcare operators, and homeowners (Table 2). Although a simple increase in disinfectant concentrations is possible, it would likely be counterproductive in that it would create taste and odor problems, and additional chlorination may result in potentially carcinogenic disinfection by-products. In addition, increasing the disinfection concentrations might be ineffective because the OPPPs are very resistant to disinfectants, particularly those encased within amoebae cysts (Hardalo and Edberg 1997).

Participants of the workshop, “Research Needs for Opportunistic Pathogens in Premise Plumbing,” took the view that novel approaches are needed: for example, manipulation of the chemistry or microbiome of drinking water distribution systems and premise plumbing. A recent review identified specific challenges for in-building control of *Legionella*, much of which is driven by limitations of water chemistry, scaling, and corrosion control (Rhoads et al. 2014). Even if possible measures are identified, there may be wide variance in outcomes because of differences in types of disinfectant, organic carbon levels, pipe composition, system design, water chemistry, and even bacterial species and strains. At the level (and responsibility) of the water provider, reduction of turbidity and organic carbon levels and the employment of biofilm-discouraging pipes (e.g., antimicrobial coated or impregnated) might prove useful (Table 2). For the hydrophobic *M. avium* and other mycobacteria, turbidity reductions may be reported, the full impact of disease caused by OPPPs. Because disease caused by *M. avium* and *P. aeruginosa* is not required to be reported, the full impact of disease caused by OPPPs (infectious dose) and disease. Two factors make such risk–analysis calculation difficult: The OPPPs vary widely in virulence (even within single species) and individuals vary widely in susceptibility.

Table 2. Possible measures to reduce OPPP numbers in premise plumbing.

| Measures to be taken                        | Outcome measure                  |
|--------------------------------------------|----------------------------------|
| Water provider action                      | Turbidity measurement            |
| Reduction of turbidity                     | AOC or BDOC measurement          |
| Reduction of biologically available carbon | Biofilm mass and microbial number reduction |
| Biofilm-discouraging pipes                 | Temperature in hot water heater or tap |
| Building manager or homeowner action       | Frequency of POU filters          |
| Employ point-of-use filters at taps and showers | Frequency of installed aerators |
| Increase oxygen levels                     | Plumbing water oxygen levels      |

Abbreviations: AOC, assimilable organic carbon; BDOC, biodegradable dissolved organic carbon.

Although the focus of the workshop and this review is on three OPPPs, there are other opportunistic pathogens in premise plumbing. As mentioned above, amoebal OPPPs, such as *N. fowleri* and *Acanthamoeba*, are of growing concern both as pathogens and as hosts for bacterial OPPPs. Other important bacterial OPPPs include *Acinetobacter* spp., *Stenotrophomonas* spp., *Brevundimonas* spp., *Sphingomonas* spp., and *Chryseobacterium* spp. (Baron et al. 2014). In addition, the slow-growing, lipid-rich, mycobacterial-like *Segniliparus* spp. isolated from cystic fibrosis patients (Butler et al. 2007) are also waterborne OPPPs.

One novel research concept that was identified by the workshop was the possibility that the microbiome of a water distribution system or premise plumbing could be manipulated to influence the presence of OPPPs. Studies of that type might provide understanding of the distribution system microbiome to aid in predicting when colonization by OPPPs would be favored. This idea was reviewed recently by Wang et al. (2013). Putting such an idea into practice would not only require study of the roles of other bacteria but also better understanding the role of bacterial viruses (bacteriophage) and amoebae. Evidence that a switch from chlorine disinfection to chloramine resulted in an absence of *Legionella* but an increase in *M. avium* (Baron et al. 2014; Pryor et al. 2004; Williams et al. 2005) illustrates the complexity of the challenge presented by OPPPs and deserves further exploration and research. Studies of the impact of disinfection

**Appendix 1: Recommended Research Projects Related to Epidemiology and Ecology of OPPPs**

- Determine the prevalence, incidence, and trends of disease caused by OPPPs
- Determine whether the microbiome of a distribution system is a determinant of the premise plumbing microbiome
- Determine the role of free-living phagocytic amoeba species on the prevalence, persistence, growth, and survival of OPPPs
- Determine whether microbial ecologic controls could reduce exposure to OPPPs
- Determine the contributions of bacterial, viral, and eukaryotic microorganisms to the persistence of OPPPs
- Determine the impact of disinfection methods, including ultraviolet irradiation, on the emergence of antibiotic-resistant OPPPs.
methods should include the use of ultraviolet irradiation, an agent that not only results in killing but also in mutagenesis. Some studies also indicate that overuse of chlorination can also enhance selection of antibiotic-resistant pathogens (Karumathil et al. 2014; Shrivastava et al. 2004). Because the OPPPs belong to the category of amoeba-resisting microorganisms, a thorough investigation of the role of free-living, phagocytic amoeba in supporting the persistence of OPPPs in premise plumbing is warranted. There is a dynamic, changing relationship between OPPPs and amoebae over time (Buse et al. 2014a). Thus, the interaction between OPPPs and amoebae during infection (Buse et al. 2014b; Revetta et al. 2013) to provide a guide for remedial measures. As discussed above concerning remediation and control of OPPPs, it will be important to determine whether microbially ecologic controls could effectively reduce human exposure to OPPPs.

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