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<http://dx.doi.org/10.1289/ehp.1408692>

Received: 14 May 2014

Accepted: 17 March 2015

Advance Publication: 20 March 2015

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

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Acknowledgments: A multidisciplinary workshop expert panel authored this review based on the proceedings of the WRF-sponsored Workshop. The workshop was organized and co-chaired by A.P, M.E. and JOF III and sponsored by a grant from the Water Research Foundation (Project 4379).

Disclaimers: The views expressed in this report are those of the individual authors and do not necessarily reflect the views and policies of the U.S. Environmental Protection Agency (EDH) or the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry (MJA). Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Competing financial interests: The authors declare they have no actual or potential competing financial interests.

Abstract

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 pre-existing risk factors and frequently require hospitalization.

Objectives: 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. As such 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: As broadly effective community-level engineering interventions for the control of OPPPs have yet to be identified, and as 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.

Introduction

As community water systems supply water to over 95 % of the approximately 300 million people in the United States, there is a need for a firm understanding of their precise contribution 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. As such, 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 means 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. While longer 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. 2013).

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. Those pathogens were not normal inhabitants of water, but were contaminants, and generally are not capable of reproduction in the water supply. Operationally, one can identify the point source for such “waterborne pathogens” by moving up stream 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; Haralo and Edberg 1997; Hsu et al. 1984; Lin et al. 1998; Squier et al., 2000; 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 (Donlon and Costerton, 2002; Høiby et al., 2010; Murga et al., 2001; Simoes et al., 2010; 2010; Steed and Falkinham, 2006; Suman et al., 2008).

In March 25-27, 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 two-day Workshop assembled over 50 experts in drinking water and water borne pathogens to (1) review the state of knowledge of OPPPs, (2) identify gaps in knowledge of OPPPs, and (3) 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; (1) Pontiac Fever and (2) either community-acquired or (3) 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 (CDC, 2011b; Yoder et al., 2008). In the United States, the most recent estimated number of hospitalized cases, based on a population based study (1997), is 8000-18,000 (CDC 2011b). Moreover, the number of reported Legionellosis cases increased 3.5-fold between 2000 and 2011 (Table 1); (CDC, 2011b, CDC, 2013b; Yoder et al. 2008). Legionellosis is an acute illness and, in its most severe form, is generally re-

sponsive to timely and appropriate antimicrobial therapy (Bruin et al. 2012; Niederman et al. 2001).

In addition to identification of 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 2002; Boccia 2006; CDC, 2013a; Neil and Berkelmann 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, 2013a; 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 Berkelmann 2008). As it has been shown that *L. pneumophila* is present in drinking water distribution systems and household water (Arnou 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 amongst 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 (Arnold et al. 1985; Borella et al. 2005; Hung et al. 1993; Kusnetsov 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; Sabrina et al. 2002; Stout et al. 1988). 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 ground water) 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, as 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 U.S. (Kool 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 2011). Recent publications have demonstrated the presence of ‘non-culturable’ cells of *L. pneumophila* and methods for their resuscitation to ensure that colony counts are not underestimated (Ducret 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 to those linked to building water distribution systems.

Legionella are not completely eliminated from drinking water by standard water treatment practices. For example, *Legionella* are comparatively more resistant to chlorine than *Escherichia coli* (Garcia and Pelaz 2008; Hosein 2005; Kim 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 of 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 (Arnou et al. 1985; Donlon and Costerton 2002; Lin et al. 1998; Murga et al. 2001). One important factor appears to be water temperature. Buildings with circulating 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 (Arnou et al. 1985; Darelid 2002). The microorganism is readily found in biofilm and de-

tritus 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. 2014; 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 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 faucets can be sources of infection (Stout and Muder 2004) 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. 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—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* Epidemiology**

M. avium infections are known to originate from environmental sources (Falkinham 1996). It is one amongst 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 et al., 2001; Falkinham, 2011).

There are three major presentations of *M. avium* infection: (1) bacteremia in HIV-infected individuals; (2) cervical lymphadenitis in young children; and (3) 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 bronchoscopes, 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 report published in 1995 by a physician who had seen mostly all cases of cervical lymphadenitis 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 *M. avium* disease rates as increasing among older persons and women (Table 1, Prevots et al. 2010; Winthrop et al. 2010). Estimated prevalence of *M. avium* lung disease vary by study, location, age, and susceptible population, and increasing with age and highest among patients with AIDS at 647 cases/100,000 persons (Marras and Daly 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 (Bodle et al. 2008; Maras and Daley 2002; Winthrop et al. 2002). Extrapulmonary sites of *M. avium* infection include skin and soft tissue, catheter-related bacteremia, exit-site infection, surgical site infections and infections such as peritonitis, lymphadenitis, keratitis, and osteomyelitis (Piersimoni and Scarparo 2009). *M. avium* disease typically involves sub acute to chronic infections that are difficult to treat and are frequently resistant to antimicrobials (Brown-Elliott et al. 2012; Griffith et al., 2007; Philley and Griffith 2013). Susceptibility to *M. avium* disease is poorly characterized; however, multiple host risk factors have been associated with *M. avium* infection. Competent cell-mediated (innate) immunity is an essential first line of defense against mycobacterial infections, defects in these pathways or in T-cell function, may be a risk factor for infection (Collins 1989; Holland 2007). Chronic lung disease such as chronic obstructive pulmonary disease, bronchiectasis (Fowler et al. 2006; Maugein et al. 2005), silicosis (Armen and Morrow 1956; Bailey et al. 1974), cystic fibrosis (Olivier et al. 2003); alveolar proteinosis (Witty et al. 1994), pneumoconiosis (Fujita et al. 2004) and previous tuberculosis (Sonnenberg et al. 2000) have all been identified as risk factors for NTM-associated pulmonary disease. Other risk factors for

NTM pulmonary disease include: connective tissue disorders and abnormal cystic fibrosis genotypes (Iseman et al. 1991; Kim et al. 2008).

Multiple reports have linked *M. avium* disease with exposure to drinking water using molecular typing methods. Closely related *M. avium* isolates have been recovered from hospitalized patients and their water (Tobin-D'Angelo et al. 2004; von Reyn et al. 1994), from residents and their household water (Falkinham et al. 2008; Falkinham 2011) and from a resident and his/her municipal water (Hilborn et al. 2008). Strains of *M. avium* isolated from humans have been shown to be competent biofilm formers (Carter et al. 2003; Mullis and Falkinham 2013; Williams et al. 2009). An *in vitro* study suggests that a *M. avium* strain's ability to form biofilm is associated with pathogenicity and invasion of bronchial epithelial cells (Yamazaki et al. 2006).

***Mycobacterium avium* Ecology**

Many factors appear to interact in complex and poorly characterized ways to support or inhibit *M. avium* growth in water pipes. Factors include: temperature, water flow, nutrients, pipe material and condition, residual disinfectant, free-living phagocytic amoebae, mycobacteriophage, 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), with hot water plumbing lines (du Moulin et al. 1988; Falkinham 2011), and plastic pipe material (Schultze-Robbecke 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 (Schultze-Robbecke et al. 1992; Tsintzou et al. 2000). Reports of *M. avium* in water distri-

bution 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 compared 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 as isolation methods vary and 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 or 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 immunosuppressed pa-

tients (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 re-infect their own taps.

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

Epidemiology of *Pseudomonas aeruginosa*

There are four major presentations of *P. aeruginosa* infection (Table 1): (1) bacteremia in immunocompromised, (2) pneumonia in cystic fibrosis (CF) patients, (3) community-acquired ear and pneumonia infections, and (4) hospital-acquired outbreaks, principally associated with contaminated solutions or medical devices amongst general patients or those in intensive care units (Fujitani et al., 2011). In all four presentations, water containing *P. aeruginosa* is the source of infection.

Community-Acquired Pneumonia

Very little 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 from case reports and outbreak investigations. Infections produced by *P. aeruginosa* are also 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 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 (“Swimmers’ ear”) with a magnitude of 2.4 million cases per year and an estimated outpatient cost of ~ \$ 500 million (CDC, 2011a).

Hospital-Acquired Infections

In a meta-analysis of 43 water-associated outbreaks in hospitals covering 35 years (1966-2001), it was estimated that there were approximately 1,400 nosocomial pneumonia deaths per year in the United States caused by *P. aeruginosa* (Anaissie et al, 2002). Among Gram-negative healthcare-associated pathogens, *P. aeruginosa* is the second most frequent pathogen causing ventilator-associated pneumonia, and the third or fourth most frequent pathogen causing septicemia, urinary tract infections, and surgical wound infections (Trautmann et al. 2009). A 10-year molecular epidemiological study attempted to determine the respective roles of exogenous and endogenous flora and time on infection and the effect of *P. aeruginosa* infection in ICU patients (Cuttelod et al. 2011). Isolates fell into three types: (1) identical patient and faucet isolates, (2) identical patient isolates, but none in faucets, and (3) unrelated patient and faucet isolates. Higher levels of faucet contamination with *P. aeruginosa* were correlated with higher numbers of cases in group 1; namely 34 per 1,000 patient admissions (Cuttelod et al. 2011). The number of type (3) or “endogenous” cases was considerably lower and stable over time (Cuttelod et al. 2011). Two studies of *Pseudomonas aeruginosa* transmission amongst ICU patients established that patient isolates contaminated faucets using either pulsed field gel electrophoresis (PFGE) or

arbitrary-primed PCR (AP-PCR) (Reuter et al., 2002; Rogues et al., 2007). This information should be considered when hypothesizing transmission pathways involving other OPPPs; for example, are *M. avium*-infected patients the source of identical clones of *M. avium* in household plumbing?

***P. aeruginosa* in Cystic Fibrosis**

Cystic fibrosis patients become colonized early in life with *P. aeruginosa*, and the prevalence of colonization increases with age (Rajan and Saiman 2002). There are 30,000 cystic fibrosis patients in the United States and 1,000 new patients appear annually (Aaron et al., 2010). *P. aeruginosa* is a major pathogen of CF patients; 60-80 % of CF patients are infected (Aaron et al., 2010). Although a proportion of CF patients are infected with *P. aeruginosa* from other patients (cohabitating hospital wards), the major source of infection is water (Geddes, 2008; Hayes et al., 2010).

***Pseudomonas aeruginosa* Ecology**

Pseudomonas aeruginosa is a ubiquitous Gram-negative bacillus commonly associated with soil and water with minimal nutritional requirements that enable it to survive in many different environments and especially known for possessing a characteristically large genome with vast metabolic capabilities (Klockgether et al. 2011) and also as a model biofilm-forming organism (Masak et al. 2014). In addition to the ability of pseudomonads to grow on a wide variety of organic compounds, *P. aeruginosa* is resistant to chlorine and other disinfectants used in water treatment (Grobe et al., 2001; Seyfried and Fraser, 1980). *P. aeruginosa* has been isolated from water (Favero et al. 1971; Mena and Gerba 2009), antimicrobial soaps (Bertrand et al. 2000; Laniini et al. 2011), and disinfectant solutions and chlorine-based sanitizing solutions (Russell 1999). *P. aeruginosa* is widely detected in a variety of aquatic environments, including tap water, and

are notoriously subject to multiple antibiotic resistance and can therefore their infections can be very difficult to treat (Vaz-Moreira et al. 2012).

Sources of *P. aeruginosa* Exposure and Transmission

The role of tap water as a source of *P. aeruginosa* disease has been established in a number of published studies (Crivaro et al. 2009; Trautmann et al. 2001; 2005; 2009). The modes of transmission have included direct contact with water and aerosols, aspiration, indirect transfer from moist environmental surfaces, and via healthcare worker hands (Döring et al. 1993; Hollyoak et al. 1995). A number of such studies used molecular markers to demonstrate relatedness of tap water and patient isolates. Tap water samples from sinks within in the intensive care (ICUs) have been found to contain *P. aeruginosa* strains that were identical by molecular typing to those obtained from infected and colonized patients (Crivaro et al. 2009). Tap water faucets were colonized with the same *P. aeruginosa* strain for more than 2 years, even though *P. aeruginosa* was not recovered from the mains supplying the sinks (Reuter et al. 2002). Tap water and outlets appear to be the reservoir for *P. aeruginosa* within healthcare facilities (Trautmann et al. 2001; 2005; 2009). However, in a surveillance study hospitalized patients, most were colonized before admission (Cholley et al. 2008). *P. aeruginosa* in tap water was shown to be the source of infection in 1 of 14 patients based on the identity of water and patient isolates (Cholley et al. 2008).

Future of OPPPs

Information available for disease incidences for each bacterial OPPPs focused on in this review are listed in Table 1, with two amoebal OPPPs also indicated for comparison as emerging and serious health threats. In particular, *Naegleria fowleri*, a brain-eating amoeba that prefers warm aquatic environments, was recently detected in premise plumbing and linked to high-profile

deaths in the southern U.S., with investigations ongoing (Bartrand et al. 2014). Overall, it is expected that the number of people susceptible to infection with OPPPs will grow in the U.S.

Cystic fibrosis, transplant recipient 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 over 60 years will increase from 16.1 % in 2000 to 24.8 % in 2025 (UN Population Division, 2002). Such an increase in individuals over 60 years coupled with higher rates of infection by OPPPs in that age group strongly suggests that the prevalence of OPPP disease will rise. Our public water systems are also deteriorating and in desperate need of maintenance and replacement, with 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 the municipal water and the premise plumbing is needed in order to inform risk management strategies.

Common Features of OPPPs

There are a number of traits shared by the three bacterial OPPPs that selects for their presence and persistence in premise plumbing. Those traits include: disinfectant-resistance, biofilm-formation, survival to high temperatures, and growth in free-living phagocytic amoebae. The concentrations of disinfectants used to treat water (e.g., chlorine, chloramine) required to kill 99.9 % of the bacterial OPPPs are greater than that needed to result in a 3 log reduction in *Giardia lamblia* cysts [See 40 CFR 141.72 (a) (4) & (b) (3), U.S. EPA 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* (nee *Hartmanella*; Smirov 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 amoebae-resisting-microorganisms; they are not necessarily killed by amoebae following phagocytosis but can actually survive and grow. Finally, relative resistance to high temperatures that are encountered in hot water pipes (e.g., 35°- 45° C) means that the numbers of these OPPPs actually increase in the hot water heater 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: (1) water utilities, (2) healthcare operators, and (3) homeowners (Table 2). Although a simple increase in disinfectant concentration 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 byproducts. Also, it might be ineffective, as the OPPPs are very resistant to disinfectants, particularly if they are encased within amoebae cysts (Haralo and Edberg 1997). The Workshop participants took the view that novel approaches are needed; for example through manipulation of the chemistry or microbiome of drinking water distribution systems and premise plumbing. A recent review identifies 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 do reduce numbers (Falkinham et al. 2001); most likely as the cells enter the treatment plant adhered to soil particulates. Organic carbon reduction might also be expected to reduce OPPP concentrations, as all three of the species discussed herein are heterotrophs, requiring organic carbon for growth. Although speculative, it might be possible to reduce OPPP biofilm numbers by pre-treating distribution and plumbing pipes with agents (e.g., antimicrobials or surfactants) that reduce adherence and biofilm-growth.

Individuals or building managers can also employ measures to reduce OPPP numbers in premise plumbing (Rhoads et al. 2014) (Table 2). First, temporary high disinfectant concentrations or high temperature water can be applied to a building plumbing system to kill viable OPPPs. Although that has been performed successfully, the OPPPs at least occasionally return, sometimes in higher numbers than were present before the temporary treatment, likely due to necrophilic growth (Temmerman et al. 2006). Point-of-use microbiological filters have been used in some healthcare settings to prevent exposure of patients to OPPPs or to prevent contamination of solutions that may come in contact with patients (e.g., dye and disinfecting solutions). However, those filters can, over time, become sources of OPPPs (Rodgers et al. 1999).

Research Needs for OPPPs

One outcome of the 2012 Expert Workshop sponsored by the Water Research Foundation was a listing of research gaps in our understanding and knowledge of the bacterial OPPPs (Pruden et al.,

2013). Those research gaps specifically focused on epidemiology and ecology of OPPPs are listed in Appendix 1 and are ordered by priority as judged by the experts participating in the workshop. Of single importance above all research needs identified at the workshop was the determination of the prevalence, incidence, and trends of disease caused by OPPPs. As disease caused by *M. avium* and *P. aeruginosa* is not required to be reported, the full impact of disease caused by these OPPPs can only be estimated; and those are under-estimates. Second, it will be important to determine the relationship between OPPP densities (infectious dose) and disease. Two factors make such risk-analysis calculation difficult is that the OPPPs vary widely in virulence (even within single species) and individuals vary widely in susceptibility.

Although the focus of the Workshop and this review is on three OPPPs, it is to be understood that 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. Such studies might provide understanding of the distribution system microbiome to predict when colonization by OPPPs would be favored. This idea was described in detail in a recent review (Wang et al. 2013). Putting such an idea into practice might not only require study of the role of other bacteria, but 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 methods should include the use of ultraviolet irradiation, an agent that not only results in killing, but also mutations. Some studies also indicate that overuse of chlorination can also enhance selection of antibiotic-resistant pathogens (Karumathi et al. 2014; Shrivastava et al. 2004). As the OPPPs belong to the category of amoebae-resisting microorganisms, a thorough investigation of the role of free-living, phagocytic amoebae 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). As such, the interaction between OPPPs and free-living phagocytic amoebae and disinfectant regulation needs careful examination (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 microbial ecologic controls could effectively reduce human exposure to OPPPs.

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Table 1. Estimated OPPP Disease Occurrence.

Disease (ICD-Code)	Measurement	Source
	Disease Prevalence/100,000	
Legionnaires' (A48.1)	0.39 (2000) to 1.36 (2011)	CDC (2011b; 2013b)
NTM ^a -US (J31.0)		
Men	3.5 (94-96) to 4.9 (04-06)	Prevots et al. (2010)
Women	4.5 (94-96) to 7.5 (04-06)	Prevots et al. (2010)
NTM ^a -Ontario (031.0)	29.3 (98-02) to 41.3 (06-10)	Marras et al. (2013)
	Isolation Prevalence/year	
<i>P. aeruginosa</i> (J15.1)	2,025 (1986) to 5,205 (2000)	Gaynes et al. (2005)
<i>Acanthamoeba</i>	> 3,000 global cases (2004)	Schuster and Visvesvara (2004)
<i>N. fowleri</i>	111 cases (1962-2008)	Yoder et al. (2008)

^aNTM = Nontuberculous Mycobacteria

Table 2. Possible Measures to Reduce OPPP Numbers in Premise Plumbing.

Measures to Be Taken	Outcome Measure
Water Provider Actions:	
Reduction of Turbidity	Turbidity Measurement
Reduction of Biologically Available Carbon	AOC or BDOC Measurement
Biofilm-Discouraging Pipes	Biofilm Mass and Microbial Number Reduction
Building Manager or Homeowner Actions:	
Raise Hot Water Heater Temperature	Temperature in Hot Water Heater or Tap
Employ Point-of-Use Filters at Taps and Showers	Frequency of POU Filters
Increase Oxygen Levels	Frequency of Installed Aerators Plumbing Water Oxygen Levels

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

1. Determine the prevalence, incidence, and trends of disease caused by OPPPs
2. Determine the dose-response relationship between OPPP numbers and disease
3. Determine whether the microbiome of a distribution system is a determinant of premise plumbing microbiome
4. Determine the role of free-living phagocytic amoebae species on the prevalence, persistence, growth, and survival of OPPPs
5. Determine whether microbial ecologic controls could reduce exposure to OPPPs
6. Determine the contributions of bacterial, viral, and eukaryotic microorganisms to the persistence of OPPPs
7. Determine the impact of disinfection methods, including ultraviolet irradiation, on the emergence of antibiotic-resistant OPPPs