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Tenets of a Holistic Approach to Drinking Water-Associated Pathogen Research, Management, and Communication

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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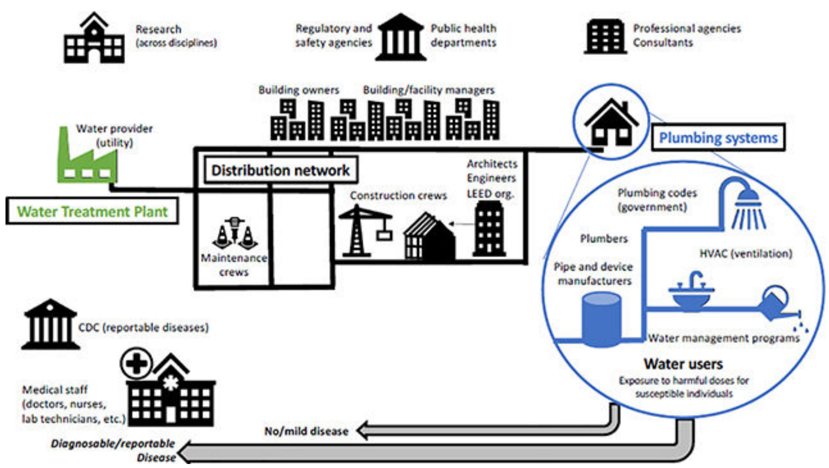
Abstract

In recent years, drinking water-associated pathogens that can cause infections in immunocompromised or otherwise susceptible individuals (henceforth referred to as DWPI), sometimes referred to as opportunistic pathogens or opportunistic premise plumbing pathogens, have received considerable attention. DWPI research has largely been conducted by experts focusing on specific microorganisms or within silos of expertise. The resulting mitigation approaches optimized for a single microorganism may have unintended consequences and trade-offs for other DWPI or other interests (e.g., energy costs and conservation). For example, the ecological and epidemiological issues characteristic of *Legionella pneumophila* diverge from those relevant for *Mycobacterium avium* and other nontuberculous mycobacteria. Recent advances in understanding DWPI as part of a complex microbial ecosystem inhabiting drinking water systems continues to reveal additional challenges: namely, how can all microorganisms of concern be managed simultaneously? In order to protect public health, we must take a more holistic approach in all aspects of the field, including basic research, monitoring methods, risk-based mitigation techniques, and policy. A holistic approach will (i) target multiple microorganisms simultaneously, (ii) involve experts across several disciplines, and (iii) communicate results across disciplines and more broadly, proactively addressing source water-to-customer system management.

Graphical Abstract



Keywords

Pathogens; drinking water; building plumbing; Opportunistic premise plumbing pathogens (OPPs); *Legionella* ; nontuberculous mycobacteria

1. INTRODUCTION

Since the identification of Legionnaires' Disease following the 1976 American Legion convention, the field of research on drinking water-associated pathogens that predominantly cause infections in immunocompromised individuals (DWPI) has exploded. New DWPI continue to be identified (*e.g.,* a 2018 *Balamuthia mandrillaria* infection tentatively linked to tap water (Piper et al., 2018)) at the same time that our understanding of the diverse ecological system within pipes is expanding (Douterelo et al., 2019; Hull et al., 2019). Today, infections associated with DWPI are estimated to cost the US economy $2.39 billion annually (Collier et al., 2021), and the incidence of several DWPI continues to increase (Adjemian et al., 2012; Billinger et al., 2009; CDC, 2011; Collier et al., 2021; Marras et al., 2007; Prevots et al., 2010; Prussin et al., 2017).

Many microorganisms could be considered DWPI. While there is no perfect term for these organisms, there are several criteria for grouping them under this umbrella. Here we focus on bacteria and amoebae that are (1) adapted to grow in drinking water systems, particularly within building water systems and water fixtures (*e.g.,* cold and hot water lines, heaters, faucets, fountains) and (2) frequently cause disease in susceptible populations (e.g., at risk individuals, older people, people with immune compromise and/or lung disease). We consider that the characteristics of susceptible populations vary and that infections sometimes occur in individuals that appear to be healthy. While present naturally in the environment (surface water, soil), DWPI typically are not present in high concentrations in finished water leaving the drinking water treatment plant, but multiply within the distribution network and especially in building water systems, often within biofilms. While all DWPI are capable of causing disease, the risk of becoming infected is influenced by various factors including the microorganism's virulence, exposure dose, and the susceptibility of the exposed population. Here, we focus on DWPI that typically pose a lesser threat to healthy individuals compared to those with underlying health conditions or susceptibilities. Moreover, common routes of exposure to DWPI are typically through non-ingestion (*e.g.,* aspiration or inhalation of aerosols generated from showering or other exposures) rather than ingestion (drinking) routes. Although fecal pathogens are occasionally found in drinking water systems, we exclude traditional fecal-oral pathogens, which cause gastrointestinal disease after ingestion, as these microorganisms tend not to proliferate or regrow in municipally-treated drinking water (Ashbolt, 2015). Some so-called "true" or "frank" pathogens can also grow within drinking water systems and cause disease through non-ingestion routes of exposure (*e.g., Naegleria fowleri*), although this is less common. The entire list of organisms we consider to be DWPI can be found in Table 1. Much of our discussion focuses on three exemplary bacterial groups that represent a significant disease burden (Collier et al., 2021), have distinct physiologies, and have the most research available: *Legionella pneumophila*, *Mycobacterium avium* and other nontuberculous mycobacteria (NTM), and *Pseudomonas aeruginosa*.

To combat DWPI disease, emerging guidance documents attempt to synthesize research knowledge into risk management strategies(Julien et al., 2020a; Singh et al., 2020). In 2015, the first industry standard for drinking water system management in buildings was released (ASHRAE, 2015; Rhoads et al., 2014) and many resources are now available to help develop what are generally known as water safety plans (ASHRAE, 2020a, 2018; CDC, 2021a). Mitigation efforts are often targeted at healthcare facilities, as many DWPI infections are nosocomial (healthcare- or hospital-acquired) due, in part to the high density of vulnerable individuals. The Veterans Affairs Office first issued a directive concerning *Legionella* spp.

prevention in healthcare facilities in 2014 (VHA, 2014); updated in 2021 (VHA, 2021), and all hospitals receiving Medicare/Medicaid funding are now required to have building water management plans ((CMS, 2017), updated in 2018 (CMS, 2018)). Unfortunately, the content of these plans is not specifically mandated (Ambrose et al., 2021).

Regulations are a step in the right direction, but have shortcomings (Bope et al., 2018) and vary globally, as illustrated by *L. pneumophila* standards and guidelines (NASEM, 2019; Van Kenhove et al., 2019b). For example, although the ASHRAE standard was a substantial forward in taking some action, it does not address residential (home) systems, despite the epidemiological link between building drinking water systems and infection by DWPI (Lande et al., 2019; Pedro-Botet et al., 2002; Stout et al., 1992; Yu et al., 2004). In fact, 96% of Legionnaires' disease cases in the U.S. are of unknown etiology, and it is suspected that sporadic residential exposure is a substantial source (CDC, 2011). It is difficult to measure the success of interventions, especially given the consistent increase in incidence of Legionnaires' disease (i.e., 5.5 times increase in incidence of reported cases in the U.S. from 2000 to 2017 (CDC, 2018)), with reporting potentially due to factors such as increased awareness and testing in addition to an actual increase in disease incidence or prevalence. Moreover, environmental testing data (i.e., water samples) are not mandated to be centrally reported in the U.S. (CDC, 2019a), even for *Legionella* spp.. While the maximum contaminant level goal (MCLG) for *Legionella* spp. has long been zero (US EPA, 2015), regular direct monitoring in drinking water systems is not required in the U.S. In contrast, other countries (e.g., Germany) have required environmental monitoring for both *L. pneumophila* and *P. aeruginosa* (NASEM, 2019). Perhaps because *Legionella* spp. is associated with the most reported data and strictest regulations, available guidance has focused on *Legionella* spp., but guidance may be at the expense of other DWPI in certain situations. For example, NTM is thought to be at least as important to the US disease burden as *Legionella* spp.(Collier et al., 2021; Daley and Salfinger, 2016). A complicating factor but also a potential motivation for holistic approaches is that clinically meaningful antibiotic resistance (AR) often complicates treatment for infections by DWPI such as *Legionella* spp. (De Giglio et al., 2015; Shadoud et al., 2015), NTM (van Ingen et al., 2012), and *P. aeruginosa* (Smith et al., 2016).

1.1 Justification and Scope

In considering the fifty-plus years of research progress and mitigation efforts for DWPIs, it has become clear that a more holistic approach is needed to unite and strengthen efforts taking place across numerous fields and disciplines. "Holistic", in this context, can be defined as considering multiple parts as interconnected and part of a whole, rather than as disconnected components. The current approach can be segmented both by discipline, and by specific microorganism of interest. Moreover, decision processes must balance many interests, including other health outcomes, cost, and energy. Additional local considerations and boundaries of water jurisdictions further segment approaches to manage DWPI.

First, while experts trained in various disciplines are making advances to understanding DWPI behavior, there is a lack of means to efficiently and effectively share findings across disciplines, in part due to terminology unique to each field (Table 2). Often, literature reviews focus on either clinical disease (Johnson and Odell, 2014), microorganism fate and transport (Falkinham, 2018), or persistence in drinking water systems (Dowdell et al., 2019). DWPI research from these disciplines often occurs in parallel fields without sufficient connection and tends to neglect translational work that bridges research and practice. For example, due to limited resources, epidemiological outbreak investigations may not aim to link clinical and environmental samples (e.g., the majority or patients diagnosed with Legionellosis in 2016–2017 in the US did not have an exposure source (healthcare setting, travel history, or senior living facility) identified (CDC, 2020a) – noting that person-to-person spread is considered negligible (Correia et al., 2016).) Both research and field investigations will benefit from increased communication.

Second, and perhaps more importantly, disciplinary teams often focus on one microorganism due to logistical limitations and available methods, as reflected in literature reviews that focus on one microorganism (Bédard et al., 2016; Falkinham, 2018, 2009; NASEM, 2019). Coinfections or polymicrobial infections with DWPIs are possible (Naito et al., 2020; Dev and Ashbolt, 2020) but data regarding co-infection within the same species of DWPI or with multiple DWPIs, as well as regarding the ability for a DWPI infection to serve as a pre-disposing factor for other infectious agents is a data gap. Some reviews of microbial ecology (Berry et al., 2006; Proctor and Hammes, 2015a), or broad scoping reviews (Falkinham et al., 2015) can take multiple pathogens into consideration, but this does not necessarily translate to specific interventions designed for practitioners. With most guidance directed at individual DWPI, we have noted examples of incompatible recommendations. For instance, adding free chlorine at the building level can be effective in reducing concentrations of *L. pneumophila*, but might increase NTM occurrence in building water system (Moore et al., 2006; Rhoads et al., 2017b). Similarly, the use of monochloramines may offer enhanced diffusion into biofilms for pathogen inactivation, but as a weaker oxidant will prove less effective against NTM (Revetta et al., 2013). The authors have noted a lack of studies that simultaneously tackle the full suite of DWPI. Even clinical or plumbing management recommendations targeted at a single microorganism type may fail to take into account host micro-eukaryotes (Thomas et al., 2010) that may be critical for pathogen recovery (Dey et al., 2019a) and strain variability within specific groups, such as that exemplified by the wide variation in virulence and health outcomes as a result of infection with different species within the *Mycobacterium* genus (Tortoli, 2014). Future recommendations should consider the impact on the full suite of DWPI.

In this paper, we outline similarities and differences among DWPI that can lead to conflicting guidance and present research needs that could help with the development of a necessary consensus across disciplines. We consider these similarities and differences across three main categories: clinical and public health, microbial ecology, and engineering controls. We highlight several opportunities to encourage research to be more cross-disciplinary and to increase the translation of research findings into practice. We present this commentary to advocate for a more holistic approach to DWPI research, legislative action, building codes, monitoring efforts, and professional practice to reduce the burden of disease associated with DWPI. The authors represent a diverse background of primary disciplines and organisms of interest (Table S1) to emphasize the importance of cross-disciplinary cooperation.

2. CLINICAL MEDICINE & PUBLIC HEALTH

While clinical medicine focuses on the treatment and outcomes of individual patients, the field of public health addresses the prevention of disease and promotion of health at the population level. We have chosen to discuss them together here as both focus on human health. The overlap in research and practice. DWPI have long been the focus of both clinical and public health research; however, in some cases, disparate goals have resulted in a lack of alignment among disease surveillance and reporting, clinical diagnosis, and assessment of DWPI risks. Multiple DWPI hazards exist, and the ability to detect DWPI-associated diseases and to predict their impacts varies. Holistically assessing these factors and impacts is nontrivial, especially as exposures may relate to episodic and complex phenomena at play in plumbing systems.

2.1 Reporting

In the U.S., Legionellosis is currently the only nationally notifiable group of diseases caused by DWPIs to the Centers for Disease Control and Prevention (CDC) (CDC, 2019b). NTM infections and positive isolates are required to be reported in some states (Table 1). The wide variation among NTM reporting requirements has resulted in calls for a national reporting requirement for NTM (Daley and Salfinger, 2016; Donohue, 2018; Winthrop et al., 2017). Certain infections with DWPI may meet criteria for reporting in the CDC National Healthcare Safety Network for healthcare-associated infections, such as *Pseudomonas*-related blood stream infections. Drug-resistant *P. aeruginosa* infections are tracked by CDC through its Antibiotic Resistance (AR) Laboratory Network, however the AR lab network is “not a surveillance network and does not represent testing of every isolate in every state” (CDC, 2019c). Certain states such as Minnesota and Wisconsin require reporting of free-living amoebic infections, including *Acanthamoeba* spp., *N. fowleri*, *Balamuthia* spp., and *Sappinia* spp. (Minnesota Department of Health, 2019a; Wisconsin Department of Health Services, 2018), and primary amoebic encephalitis caused by *N. fowleri* is state-reportable in Florida, Texas, and Louisiana (CDC, 2017). This patchwork of surveillance and notification requirements, often motivated by high-profile outbreaks, highlights the need for unified DWPI associated disease reporting to fully assess disease burdens. Even so, mild infections often go undiagnosed or untreated, while healthcare-associated infections are also likely underestimated (Perkins et al., 2019; Spagnolo et al., 2016). Overall, reported cases are the “tip of the iceberg” (WHO-Europe, 2019; Yang et al., 2012).

2.2 Diagnosis and epidemiologic case definitions

An epidemiological case definition is “a set of standard criteria for classifying whether a person has a particular disease, syndrome, or other health conditions” (CDC, 2012a). Some of these definitions have been developed into national standards that are comparable across clinical settings and locations. A standardized case definition is an important step toward making large-scale comparisons of DWPI. Case definition criteria can be more limited during an outbreak investigation, and the strictness of the definition depends on the purposes for classifying the occurrence of a disease (CDC, 2012a). A case definition is not the same as a clinical diagnostic criterion, where the former is used to aid an epidemiologic investigation and the latter is used to make treatment decisions for individual patients

(LaMorte, 2016). Clinicians' ability to diagnose DWPI diseases vary, but most approaches are based on culture or molecular analysis of the pathogen in bodily fluids, or observation of antibodies to a particular infection.

With regard to standardized case definitions, Legionnaires' disease has a clear case definition (CDC, 2021b), but a standard case definition for Pontiac fever, which is generally considered to be a milder form of legionellosis without pneumonia, has only recently been established (CSTE, 2019, 2010, 2005; Tossa et al., 2006). Similarly, case definitions have only recently been standardized for free-living amoebae (CDC, 2012b) and NTM (CSTE, 2017; Oregon Health Authority, 2018) infections, while *P. aeruginosa* infections are diagnosed via bacterial culture as a primary or secondary bloodstream infection; secondary bloodstream infections are not reportable as Laboratory Confirmed Bloodstream Infections to the CDC National Healthcare Safety Network (CDC, 2020b). Other case definition criteria are used for antibiotic-resistant *P. aeruginosa* infections (Minnesota Department of Health, 2019b; Walters et al., 2019). For *M. avium* and other NTM, the American Thoracic Society/Infectious Disease Society of America and the British Thoracic Society define a confirmed case as one with NTM grown from two separate sputum samples, or a single positive sample from a broncoalveolar lavage or a lung biopsy (Griffith et al., 2007; Haworth et al., 2017). In addition, the Council of State and Territorial Epidemiologists (CSTE) has recently published a case definition for extrapulmonary NTM (CSTE, 2017; Oregon Health Authority, 2018). Granulomatous amoebic encephalitis (GAE, *i.e.* amoebic infection of the central nervous system) is often diagnosable, but rarely before it becomes fatal (Reed et al., 1997; Sell et al., 1997). Factors including (i) extended disease progression, especially for NTM which may have a progression over years (Field et al., 2004; Lewis et al., 1960), (ii) lack of identification needed to reach a diagnosis or treatment (*e.g.* otitis externa or "swimmer's ear" can be treated without identifying the causative organism as *P. aeruginosa*), and (iii) lack of clinical awareness (Alaga et al., 2018) can obscure connections between environmental exposure and disease (Johnson and Odell, 2014; Provoost et al., 2018). The use of medical decision trees, tools with visual descriptions of a series of diagnostic tools and subsequent outcomes and downstream decisions to aid in arriving at a diagnosis, have been long-employed in the medical literature (Detsky et al. 1997). Such tools would be beneficial in the DWPI context for codifying information that could distinguish between different DWPI disease outcomes, as well as to highlight gaps where additional information could be collected to clinicians in differentiating between DWPI infections and elucidating causal pathways(Detsky et al., 1997).

Testing is critical for diagnosis, but convenience, reproducibility, and accuracy (sensitivity, or the probability of correctly identifying a positive case of disease, and specificity, the probability of correctly testing negative in the absence of disease) of available testing technologies varies by DWPI, and the compatibility of environmental and clinical tests varies due to conflicting goals, as discussed below. Taken together, these challenges for diagnosis amplify the underreporting of DWPI disease. Accurate reporting will require (1) rapid, reproducible, and precise testing methodologies for DWPI diseases, (2) diagnostic protocols that include clear decision trees for routine inclusion of DWPI in clinical workups, and (3) national reporting requirements and databases for DWPI.

Epidemiological investigations offer a clear opportunity for cross-disciplinary work. Clinical medicine can identify disease (and report it), while public health specialists will look for environmental sources of exposure to link to the outcome. This requires timely cooperation and communication so that environments can be sampled close to the exposure window. Linkage typically requires work at the subspecies level and should look for co-infections with other DWPI. Engineers can also contribute to epidemiological studies by identifying key exposure points that can be added to epidemiological surveys of exposure history (e.g., do you live near a water feature?). Successful quantification of both exposure and outcomes can help validate risk models.

2.3 An Example of Holistic Thinking: Risk Assessment and Management

In assessing risks from DWPI, a traditional approach may focus on risks of a single pathogen, epidemiological observations and studies, or even costs associated with treating a single disease. However, a combination of metrics would be needed to holistically evaluate and prioritize the impacts, costs, and tradeoffs of DWPI in different contexts, and substantial value judgements would be necessary when communicating these types of analyses for legislative and preventative efforts. Further, complexity is added when competing objectives of many stakeholders (e.g., energy costs, liabilities, and management objectives unrelated to DWPI) must be considered. In this section, we describe how the current approach to risk assessment includes aspects of a holistic approach and can be further improved to incorporate metrics from other disciplines.

The prevalence of infections by DWPI varies (Table 1), although some rank-order differences have been estimated: NTM (up to 41.3 per 100,000) > Legionnaires' disease (up to 1.36 per 100,000) > *P. aeruginosa* > *Acanthamoeba* > *N. fowleri* (Falkinham et al., 2015; Strollo et al., 2015). Furthermore, disease prognoses vary greatly; for example, *N. fowleri* associated meningoencephalitis has a >95% case fatality rate (Wang et al., 2018), while the case fatality rate for healthcare-associated Legionnaires' disease is up to 25% in some susceptible populations (CDC, 2021c). Quantitative microbial risk assessment (QMRA) may be useful for prioritization, as it aims to understand underlying mechanisms, relative contributions of processes and modeling rare outcomes, among other attributes bridging environmental concentrations to outcomes of interest via dose-response models (Haas et al., 2014; World Health Organization, 2016). QMRA is especially useful as a companion tool to traditional epidemiological methods which can necessitate large studies in order to observe potentially small effect size. Dose-response models used in QMRA are available for quantifying relationships between exposure and the probability of an outcome for DWPI (Table S2). A growing body of QMRA literature addresses *Legionella* spp. (Hamilton and Haas, 2016), NTM (Hamilton et al., 2017; Rice et al., 2005), *P. aeruginosa* (Dean and Mitchell, 2020a; Rasheduzzaman et al., 2019), and *N. fowleri* (Dean et al., 2019; Rasheduzzaman et al., 2019), but important limitations remain as many variables must be considered. For example, the likelihood of infection for a DWPI is not only dependent on the species/strain of microorganism, the virulence of the microorganism at a given time, and host characteristics (*e.g.*, immune status/age/demographics), but also on the fate and transport of the microorganism in the environment, and exposure route and dose. For example, men over 55 years of age are at higher risk for *L. pneumophila* while

NTM tends to disproportionately infect slender, taller, post-menopausal women (Chalmers et al., 2018; Kartalija et al., 2013). New dose-response models will be useful for microbial ecologists, water quality engineers, and ultimately legislators seeking to identify risk-based monitoring targets for environmental control of these organisms. Such efforts to derive risk-based concentration monitoring targets have already begun for *Acanthamoeba* spp. (Dean and Mitchell, 2020b), *P. aeruginosa* and *N. fowleri* (Rasheduzzaman et al., 2019) and *L. pneumophila* (Hamilton et al., 2019).

Risk prioritization could also be assigned based on cost, but tracking disease and quantifying costs of illness are challenging (Adam et al., 2017). Multiple approaches are available in the economic, risk, decision analysis, and policy analysis literature to inform decisions that take into account multiple pathogen considerations (*e.g.* (Mitchell-Blackwood et al., 2011)). These approaches not only consider the direct losses (*e.g.,* hospitalization costs, Table 1, (Collier et al., 2021)), but also secondary considerations such as non-hospitalized infections (i.e., missed work, lowered productivity) and mortality when allocating scarce resources (Institute of Medicine, 2009; National Research Council, 1990). For example, Corso et al. (2003) identified healthcare costs and productivity losses from the 1993 cryptosporidiosis outbreak in Milwaukee, Wisconsin using costs from categories of people with mild, moderate, and severe illness using a retrospective analysis, arriving at an estimate of $96.2 million in losses (2003 US dollars) (Corso et al., 2003). In addition to direct costs, disease has many indirect costs, including loss of trust in the safety of municipal drinking water. In considering costs, the expense of trade-offs, including alternative risks and costs of preventative measures should be considered. For example, raising the temperature of a water heater can decrease risk of DWPI infection, but will increase the risk of scalding and energy costs. Such analyses would improve if all DWPI were reportable, noting that cryptosporidiosis did not become a nationally notifiable disease until 1995 after the catastrophic Milwaukee outbreak (CDC, 1995).

The level of "acceptable" risk is typically dependent upon the hazard, consequences, and ability to control exposure (NASEM, 2019). Drinking water efforts for policy standardization have typically relied on health-risk based target benchmarks such as one in 10,000 infections per person per year (Lim and Jiang, 2013; Regli et al., 1991). Per-exposure versus annual risks are often chosen as a decision basis depending on whether exposures are continuous or short term, leading to different monitoring approaches, with annual-based approaches focusing less on short-term (hazardous event) variations (NASEM, 2019; Signor and Ashbolt, 2009). The choice of an acceptable risk value, like one in 10,000, is a policy decision and may be too stringent in certain situations (Haas, 1996; Lim and Jiang, 2013). Other endpoint metrics are available such as the disability adjusted life year (DALY) to measure the health impacts of a disease incorporating both fatal and non-fatal outcomes (a 10^{-6} DALY per person per year is often used as a reference point) (Sinclair et al., 2015; WHO, 2015). A DALY value is available for Legionellosis (van Lier et al., 2016), however, this value is location- and context-specific. Regardless of the quantification method used, there is no such thing as "zero risk" (De Keuckelaere et al., 2015).

Research needs for DWPI risk assessment and prioritization can be summarized as follows: (1) an increased understanding of infectivity, including new dose response models for emerging DWPI for various exposure routes (*e.g.* aspiration), endpoints, susceptibilities, (2) the impact of underlying human characteristics and behavioral factors (*e.g.* smoking, use of tap water for non-recommended uses such as neti-pots, contact lenses, and medical uses, occupational (co)exposures of host populations and impact on risk), (3) knowledge of virulent types amongst each of the DWPI species to aid monitoring, (4) improved exposure information for aerosol exposures as well as non-potable uses such as cooling towers, healthcare equipment, dental equipment, and other known sources of infection, (5) consideration of multiple costs and risk tradeoffs (*e.g.* increasing water heater temperatures to inactivate *Legionella* spp. results in higher energy costs), and (6) careful consideration of risk benchmark metrics in various decision- making processes. The insurance industry (Section 4.5), may provide an opportunity to address risks and consider costs across multiple sectors.

2.4 Proposed Approach

Clinicians, public health practitioners, and other health researchers who wish to participate in a holistic approach to DWPI can embrace several strategies. Recognizing that these proposed approaches are not always easy to implement, they are ordered from most to least readily achievable.

- Educate at-risk individuals about their personal risk factors, and provide them with the resources to protect their water systems and modify behavior to minimize personal risk.
- Timely studies across public health and medicine that link exposures and disease outcomes/outbreaks, improving linkages among professionals in the medical and environmental space for this purpose. Following a case investigation, communicate with water providers and building managers about potential risks and strategies for future disease prevention, regardless of whether positive environmental samples were collected, in order to increase awareness of potential problems.
- Report every instance of DWPI to a centralized database (Box 1).
- Make reporting of all DWPI mandatory, rather than just some pathogens, and to the extent possible, match clinical cases with environmental samples within linked reporting databases.
- Using risk assessment to systematically identify and prioritize research gaps, understand tradeoffs, and improve modeling efforts toward predicting and preventing rather than reacting to DWPI illness
- Rapid, more granular public health data collection for DWPI-associated illnesses and expansion beyond *Legionella* spp., which also considers people presenting with symptoms associated with DWPI for further testing and diagnosis

3. MICROBIOLOGY AND MICROBIAL ECOLOGY

Each DWPI has a unique set of ideal environmental conditions for proliferation. These conditions generally overlap to allow for growth within the environment, drinking water systems, and the human body, but clear differences in niches have emerged. In studying microorganisms in isolated laboratory conditions, important discoveries are made, but studies can also be designed to emulate realistic conditions to inform complex cross-species interactions, as well as actionable engineering strategies and policies.

3.1 Key Microbial Physiological Considerations

Fundamental microbiology studies have revealed several innate physiological and structural traits that might predict where DWPI are likely to be found in the environment. Hydrophobicity and surface charge are important factors in determining the partitioning of cells/aggregates in a drinking water system (e.g., planktonic vs. surface microlayer vs. biofilm) and the likelihood of aerosolization (Falkinham, 2018; Parker et al., 1983). Many DWPI are hydrophobic and prefer to grow on surfaces in biofilms (Falkinham, 2009). NTM are especially notable given their waxy, "acid-fast" cell walls, which make them particularly hydrophobic and readily aerosolized (Falkinham, 2018; Parker et al., 1983). Surface charge may also dictate ecological distribution and infectivity (Steed and Falkinham, 2006; Stormer and Falkinham, 1989; van Oss et al., 1975). For example, differences in the electrophoretic mobility among *L. pneumophila* serogroups likely contributed to differences in serogroup behaviors (Buse et al., 2018). In contrast, several *M. avium* complex species exhibited less variability in electrophoretic mobility (Lytle et al., 2004). It is also important to recognize the way DWPI may be presented in exposure scenarios, such as packaged within amoebal vesicles, trophozoites, or cysts (Shaheen and Ashbolt, 2018). Further understanding of these differences is critical to identifying likely hotspots and explaining disease patterns.

DWPI growth rates vary considerably, impacting incubation periods between exposure and symptom onset, preferred growth location, and ideal culture detection methods. While doubling times and growth conditions can vary within a species, generally the growth rates for *M. avium*. and *Pseudomonas* spp. Are generally low (Sharaby et al., 2017; van der Kooij et al., 1982). These growth rates are lower in the drinking water environment than in clinical or laboratory settings due to oligotrophic conditions, this can mean that culture-based recovery of environmental DWPI isolates can require >10 days (Lalancette et al., 2017). In this field, growth is considered rapid if organisms are detectable in less than 7 days. Specific strains and species also exhibit wide variation. For example, *Mycobacterium malmoense* requires 8–12 weeks for culture detection, while *M. abscessus* and other "rapidly growing" NTM can be detected in <7 days) (Bartlett et al., 2000). Caution should be taken in extrapolating conclusions regarding growth rates as a slow growth rate does not necessarily imply slow metabolism; mycobacterial increase in numbers is slow because a great deal of energy is expended in synthesizing the long-chain (C_{60}-C_{80}) lipids that comprise the outer membrane (Brennan and Nikaido, 1995). It is critical to understand these basic pure culture behaviors in order to (1) understand microbial incubation periods and colonization patterns across ecosystems, (2) develop robust cost-effective methods (Box 2), and (3) design engineering solutions (e.g., intermittent flush, heat shock) for multiple DWPI simultaneously.

Many DWPI survive stress due to harsh conditions in potable water (*e.g.*, low nutrients, temperature, and exposure to chlorine residuals) by resorting to a dormant or protective state. This includes the poorly understood viable but nonculturable (VBNC) state (Alleron et al., 2008; Dopp et al., 2017; Li et al., 2014) and survival within protozoa, which has been observed for many bacterial DWPI (Thomas et al., 2010). These survival mechanisms can result in false non-detects for culture-based methods and sometimes molecular methods (*e.g.*, when amoebae are not lysed effectively). Importantly, these seemingly non-active microorganisms are still capable of infection (Dey et al., 2019b), with several studies showing the reactivation of DWPI after they are exposed to a less stressful environment (Dietersdorfer et al., 2018), such as within the human body. The importance of detecting viability varies across stakeholders (Section 3.5), but improved methods to quickly detect and differentiate between different physiological states would be widely beneficial across disciplines.

3.2 Environmental Conditions

DWPI respond to a variety of physio-chemical factors, including temperature, disinfectant residual type and concentration, oxygen levels, and other water chemistry characteristics. Many of these factors have been studied with direct implications for engineering solutions, indicating cooperation between these disciplines. Care must be taken in interpreting results from different scales of experiments, that can range from pure culture to studies of full-scale buildings. As studies often focus on only one DWPI or limited environmental factors, it is difficult to determine the applicability of findings (1) across DWPI, (2) in different systems, or (3) at different scales of study. Still, some differences in niche seem to emerge amongst DWPI.

Ideal temperatures for most DWPI are similar (28–35 °C), but growth can occur outside this range. For example, *P. aeruginosa* can grow between 10 and 42 °C (Brown, 1957). While most DWPI are considered inactive at temperatures greater than 55 to 60 °C, their recovery after exposure to higher temperatures (*e.g.*, 63 °C for *L. pneumophila* (Borella et al., 2004) and 65 °C for NTM (Merkal and Crawford, 1979)) may indicate they are present in the VBNC state (*i.e.,* VBNC *L. pneumophila* after exposure to 70 °C (Allegra et al., 2008)) or exhibit other survival mechanisms under such conditions. Conclusions about responses to temperature extremes are especially difficult to scale-up to engineered water systems because *in situ* temperatures are difficult to measure at various scales (e.g. within a biofilm or at a pipe wall versus at the center of the water column), and survival may further be aided by biofilms, protozoa, and areas not exposed to maximum or minimum system temperatures. As temperature may allow for niche differentiation, this presents an opportunity for further multi-DWPI studies

The chemical characteristics of drinking water vary based on source (*e.g.*, groundwater vs. surface water), residual disinfectant type (*i.e.*, chloramine vs. free chlorine), and specific building parameters (*i.e.*, pipe material), all of which individually and collectively influence the growth and survival of DWPI and their hosts. As all of these water chemistry factors can differ across studies, it is difficult to (1) isolate factors of concern, and (2) compare results for different DWPI. Comparison of disinfectant efficacy is especially difficult. Even for a single organism, Ct (disinfection concentration (C) × time (t) [mg*min/L]) inactivation values can vary based on strain, material type, presence of biofilm, pH, and/or temperature

(Buse et al., 2019; Dowdell et al., 2019). Still, each DWPI also appears to demonstrate slightly different tolerances towards chloramine and free chlorine; chloramine has a strong effect on *L. pneumophila* and *P. aeruginosa*, but is less effective towards inactivating NTM (Dowdell et al., 2019; Lytle et al., 2021; Moore et al., 2006; Rhoads et al., 2017b). *M. avium* complex and *P. aeruginosa* typically exhibit high resistance to both disinfectants (Grobe et al., 2001; Taylor et al., 2000). To allow more accurate conclusions regarding disinfectants' effects on DWPI, it is critical to (1) fully report all water chemistry variables, (2) study multiple DWPIs within the same experimental framework.

The concentrations of metal ions can also impact growth and survival of DWPI, but are not always considered in studies at multiple scales. Iron, typically originating from iron pipes, may be a critical nutrient for *Legionella* spp., NTM, and *P. aeruginosa* growth, although in different quantities. Copper, originating from copper-silver ionization systems or from copper pipes, is generally considered to have antimicrobial properties (Cachafeiro et al., 2007; Landeen et al., 1989; Lin et al., 1996; Liu et al., 1994). Furthermore, copper has also been linked to VBNC induction in *P. aeruginosa* (Bédard et al., 2014; Dopp et al., 2017; Dwidjosiswojo et al., 2011). Still, these results are difficult to scale to engineering solutions, especially considering the variability of metal concentrations both spatially and temporally within building water systems and changes in metal chemistry throughout the lifetime of a building (Brazeau and Edwards, 2013; Rhoads et al., 2017b; Salehi et al., 2020). For example, while the use of copper pipes has been suggested as a control mechanism for *L. pneumophila* (Learbuch et al., 2019; Rogers et al., 1994), the effects on *L. pneumophila* and other DWPI might be short-lived, pH dependent, and modified in the presence of biofilm (Buse et al., 2017; Elguindi et al., 2009; Proctor et al., 2017; Rhoads et al., 2017b). Under certain conditions, the presence of copper may even selectively encourage the growth of *L. pneumophila* (Proctor et al., 2017; Rhoads et al., 2017b), by changing the pipe microbiome (Buse et al., 2014). Elucidating the impact of metals on the broader microbiome under varied water chemistry conditions will allow better understanding of conflicting findings in full-scale systems.

Each DWPI also has a unique set of nutrient and oxygen requirements, with some DWPI surviving under extreme conditions that may provide competitive advantages. The *M. avium* complex is considered microaerobic, growing in as little as 6% oxygen, and surviving in anaerobic conditions (Lewis and Falkinham, 2015). *P. aeruginosa* is generally considered aerobic, but can grow anaerobically utilizing nitrate as a terminal electron acceptor (Palmer et al., 2007). *L. pneumophila*, on the other hand, is an obligate aerobe, requires amino acids produced by other microorganisms, and can grow necrotrophically (Temmerman et al., 2006). Amoebae similarly rely on other microorganisms, digesting them via phagocytosis (Samba-Louaka et al., 2019). Better understanding of this niche differentiation will assist in finding mitigation strategies that are effective for mitigating the full suite of DWPI.

3.3 DWPI Interactions

When DWPI are studied together, relationships (parasitic, symbiotic and competitive) are uncovered that would not be seen in monoculture studies. Many DWPI, including *Legionella* spp. and NTM can be phagocytized by free-living protozoa, and amoebae (Delafont et al., 2018), which facilitates replication, offers protection, and enhances virulence (Buse and Ashbolt, 2011; Cateau et al., 2011; Cirillo et al., 1997). Amoebic relationships may account for the preference of *L. pneumophila* in biofilms with high cell densities (van der Kooij et al., 2017) versus the preference of *Pseudomonas* spp. and *Mycobacterium* spp. in biofilms with low cell densities (Proctor et al., 2018, 2016). Some of these protozoan hosts are also DWPI themselves (e.g., *Acanthamoeba*). *P. aeruginosa* can suppress the growth of *L. pneumophila* (Kimura et al., 2009) and may prevent their attachment to simple biofilms (Stewart et al., 2012), likely through the production of bacteriocin-like substances that suppress competitors (Guerrieri et al., 2008). Also, there appears to be preference by amoeba for prey that are not amoeba-resisting (Shaheen and Ashbolt, 2021). In continuously chlorinated cooling towers, *Legionella* spp. and *Pseudomonas* spp. had negative associations due to direct or indirect relationships with hosts (Paranjape et al., 2020). In addition, DWPI can compete with one another and other drinking water bacteria for uptake by amoebic hosts, potentially affecting inactivation and virulence (Berry et al., 2010; Cirillo et al., 1997; Declerck et al., 2005; Thomas and Ashbolt, 2011). It may be possible to leverage these findings to eliminate DWPI from water systems.

3.4 An Example of Holistic Thinking: Antibiotic Resistance

The differentiation of niches amongst DWPI is especially well exemplified with respect to antibiotic resistance (AR) (Box 1). The resistance of microorganisms to antibiotics has been widely studied across multiple disciplines and multiple DWPI, embodying key aspects of the proposed holistic approach. Epidemiologists and clinicians traditionally study AR with respect to identifying sources, limiting their spread, and determining the best treatment approaches. Ecologists study environmental niches conducive to growth of AR organisms, conditions that select for resistant strains, and microenvironments favorable to the transfer of AR genes via cell-to-cell via horizontal gene transfer. Concerns regarding AR are emerging for engineers, who have studies how engineered systems can serve as barriers to the spread of AR or, conversely, can inadvertently promote dissemination of AR. Identifying solutions to these pressing and unconventional challenges necessitates collaboration among researchers across disciplines, but holistic solutions are needed given that each organism presents unique challenges. Holistic consideration of these challenges by engineers, ecologists, and epidemiologists working in collaboration is necessary for parallel control of these disparate AR-DWPIs in water systems.

3.5 An Example of Holistic Thinking: Method Development

The methods used to measure DWPI by different disciplines often create a barrier because each field has different goals (Table 3). A public health specialist or epidemiologist requires in-depth identification (*e.g.*, serotyping, sequence typing) to trace outbreaks, while a physician focuses on information pertinent to diagnosis and treatment (*e.g.*, symptoms may be enough for some diseases/organisms, while complete typing is needed for others). Thus, positive diagnostic tests (*e.g.*, urinary antigen tests) may not be paired with more in-depth analysis (*e.g.*, sputum culturing, water testing) on a routine basis. Environmental monitoring may also use culture-based techniques, but clinical culture media are often not suitable to recover nutrient-starved DWPI cells typically found in drinking water. A microbial ecologist may only be interested in the presence of a microorganism, and thus

DNA-based molecular methods may be adequate (*i.e.*, composite capture of viable, VBNC, and dead cells). The meaning of the unit gene copy per volume is not immediately relatable to quantities reported from traditional culture methods (i.e., colony forming units (CFU) per unit volume), and relative abundance (*e.g.*, percent abundance from amplicon sequencing data) does not directly translate into the absolute concentrations needed for risk assessment. A monitoring program requires relatively quick and easy methods for live- or revivable (VBNC)-pathogen detection and emerging methods for achieving this report often rely on non-traditional units of measurement (*e.g.*, most probable number (MPN) per unit volume). Other engineers need immediate indicators for a change in physicochemical, nutrient, or total bacterial conditions and may be particularly interested in methods designed to denote hydraulic changes rather than direct correlations with human pathogens. Even if these do not relate directly to DWPI growth or survival, they could indicate a failure in treatment or hydraulic issues and need for corrective action. Building owners may not be interested in finding definitive evidence of DWPI because of legal liability and may prefer more general water quality metrics that could be indicative of conditions conducive to DWPI growth (*e.g.*, temperature, chlorine concentration).

All available methods have benefits and drawbacks, and a single method cannot address all questions. At the same time, financial, time, and expertise limitations will not typically allow for application of all methods in all circumstances. Better understanding of the differences and relative benefits of information provided by these methods can allow for (i) communication between disciplines, and (ii) better design of studies that will translate more effectively across disciplines. Similarly, convenient environmental sampling methods could be designed with compatibility for downstream processing like sequence-based typing (Mercante and Winchell, 2015; Scaturro et al., 2005) or whole genome sequencing (Haworth et al., 2017) to allow for links with healthcare cases. Using select methods in concert can substantially increase the value of studies (*e.g.*, allowing interpretation of various units of measurement), but requires pooling of resources.

The investment in test method development and availability varies considerably by DWPI. Culture-based and culture-independent methods have been developed for the detection and quantification of most DWPI, but their convenience and standardization vary. Easy-to-use environmental detection tests, like Pseudalert® and Legiolert®, target single pathogen types. For *L. pneumophila*, a simple urinary antigen test is available for clinical testing (albeit only for the detection of serogroup 1). While convenient, the urinary antigen test results in the underestimation of other disease-causing serogroups or species (e.g., *L. longbeachae*) (Mercante and Winchell, 2015) and does not produce isolates for disease tracking. More rapid serologic tests based on enzyme immunoassays are under development for NTM, but are not commonly employed (Aksamit et al., 2014; Haworth et al., 2017). While culture-based methods generally require specialized expertise, the CDC administers a control program for *L. pneumophila*: the ELITE program (Environmental *Legionella* Isolation Techniques Evaluation). Development of easier, faster methods is needed across to address all DWPI, as is the development of quality control programs. As it is believed that amoeba and protozoa may play important roles in DWPI persistence in drinking water systems, a method to identify DWPIs that have been resident in those protists (e.g., DNA modification) would be valuable. Moreover, faster standardized methods would allow for the testing of multiple organisms at once, especially in environmental samples. Currently, measuring all DWPI in a sample can be burdensome, even for research projects. A possible workflow for an "ideal universal method", which considers multiple DWPIs, multiple field-specific interests, as well as the real-world constraints of time and cost, is proposed in Box 2.

3.6 Proposed Approach:

Microbial ecologists, who primarily participate in research on the bench scale, can embrace several strategies to make their research more useful from a holistic perspective.

- Rapid, and well documented, well quality-controlled, clearly reporting sampling strategies that are deliberately designed to answer research questions relevant to cross-cutting objectives in health, microbiology, and engineering.
- Identify methods to distinguish virulent (from avirulent) DWPI species to allow monitoring of DWPIs that are of greatest public health significance.
- Given the need for CT values for DWPI for informing engineering interventions and complexities of comparing values derived from piecemeal experimental studies, a systematic review and meta-analysis of kinetic parameters (e.g., inactivation and growth under different temperature, disinfectant residual, water chemistry, and temporal conditions) for DWPIs is needed for identifying research gaps and data needs.
- Include and measure multiple DWPIs in research projects, so that the potential effect of altered environmental conditions (*e.g.*, temperature, disinfectant residual levels) can be understood for all DWPI within comparable conditions. Comparison of conditions across existing published studies is difficult.
- Conduct mixed community experiments under conditions representative of real systems (alongside studying full-scale drinking water systems, which are more difficult to control). Complementary pure culture, mixed culture and full scale experiments will allows the field to build on basic understanding and move towards applicable engineering solutions.
- Work with other scientific fields to elucidate transmission dynamics of multiple DWPI between media. It would be useful to understand the fluxes from biofilm to water, water to aerosol, aerosol to inhalation, and inhalation to infection under multiple water physio-chemical scenarios. This would require accurate and comparable quantification of organisms in multiple media (see methods below).
- Develop methods that can (i) easily be used for multiple DWPI detection and quantification, and (ii) be used on multiple media (bodily fluids, water, biofilm) with a single understandable metric (Box 2) and (iii) move toward near real-time DWPI detection.
- Harmonize environmental measurements with clinical approaches for understanding source-to-receptor exposures along the full exposure continuum.
- Track the development of DWPI-AR within studies by routinely incorporating AR-tracking methods (e.g., qPCR) (Box 1). This is done in some studies (e.g.,

Cocuzza et al., 2021; Sikora et al., 2017; Vaz-Moreira et al., 2012) already, but efforts can be expanded.

4. ENGINEERING & OTHER BUILDING PRACTITIONERS

Since DWPI were first identified, water quality engineers have made efforts to reduce their proliferation and eliminate them from water systems, with many interventions focused on *Legionella pneumophila* alone. Initial interventions adapted methods used at the treatment plant (*i.e.*, adding traditional disinfectants), and could only be applied by trained operators and building managers. The field has since expanded to an examination of building and plumbing system design itself, with maintenance-free strategies introduced at the design-stage by architects and engineers. Borrowing from the field of industrial hygiene, hazard removal or employment of maintenance-free engineering controls is preferable to relying upon building operation habits or having individuals responsible for reducing their own exposure (CDC and NIOSH, 2015). Nonetheless, implementation of many engineering strategies for pathogen control will involve multiple professions (e.g., architects, plumbers, building code officials and building managers), requiring effective communication.

4.1 Applying Disinfectant

Since loss of disinfectant residual is associated with microbial growth (Berry et al., 2006; Proctor and Hammes, 2015b), boosting the concentrations of disinfectants either in the distribution system or in the plumbing of large buildings is a promising DWPI engineering control. While there are many reported successes of on-site disinfectant systems, there are trade-offs between disinfectant types (e.g., efficacy discussed above, disinfection byproducts, and corrosion of materials (Giovanardi et al., 2020) and differential selection of one DWPI over another. Chloramine residuals persist longer and have greater effect on biofilms than chlorine in distribution systems, but their relative efficacies can be reversed in building water system under conditions that accelerate chloramine decay (Zhang et al., 2009). In comparing samples from buildings where either chlorine or chloramine were applied in the supply's distribution system, *L. pneumophila w*as detected with similar frequency with both disinfectants, while certain NTM were detected more often in chloraminated systems (Donohue et al., 2019). In the distribution system, the choice of disinfectant type is often driven by concerns other than DWPI, including disinfectant byproduct formation.

Applying disinfectant at the building level can provide additional control, but an important nuance is how disinfectant is applied. Chloramine addition at the building level is able to control *Legionella* spp., *P. aeruginosa* and NTM when applied correctly (Duda et al., 2014; Lytle et al., 2021). However, in cooling towers, continuous chlorine treatment appeared to suppress *Legionella* spp. while promoting *Pseudomonas* spp. (Paranjape et al., 2020). The choice of oxidant (e.g., chloramine, chlorine, or chlorine dioxide) can also influence the specific species or strains in a system (Marchesi et al., 2016), therefore, the choice of residual disinfectant and delivery mechanism may select for certain DWPI species or strains. The choice of disinfectant type at this level is often driven by cost, experience, and maintenance concerns. Additionally, adding on-site disinfectant still may not protect all distal ends, can be expensive, and may result in a regulatory burden (additional testing and permits (SDWA, 1996)), deterring some building owners from preventative action. Ultimately, an effective disinfectant-based solution, if achievable, will rely heavily on building design features, upstream water quality, and importantly, competent operators, which may not be feasible in all buildings.

Non-traditional disinfection approaches like ultraviolet (UV)-irradiation, ozonation, and copper-silver ionization have been applied in buildings (Lin et al., 2011). Neither UV nor ozone provide a strong residual, and *Mycobacterium* spp. and *Legionella* spp. have been shown to survive in full-scale ozone contactors (Kotlarz et al., 2018). Copper-silver ionization generally is effective for reducing culturable *L. pneumophila* (Lin et al., 2011); however, its effectiveness is highly dependent on chemical water quality characteristics (Lin et al., 2002), and resistance can develop over time (June and Dziewulski, 2018), thus, vigilant maintenance is required (Office of Inspector General, 2013). Copper-silver ionization has not been tested extensively for other DWPI, and may be less effective against NTM (Huang et al., 2008; June and Dziewulski, 2018; Kusnetsov et al., 2001). Overall, the effectiveness of non-traditional disinfection approaches against all DWPI, as well as the skill and capability of system operators must be taken into consideration for long-term successful treatment. Different types of filters and devices (UV, UV-LED) installed at the point of use are available commercially and can be installed at faucets and showerheads to prevent exposure to bacteria and DWPI (NASEM, 2019). Water quality (e.g., turbidity) will affect their efficacy and the duration of their use before breakthrough. Manufacturers test products using ISO 22196:2011, which involves incubation of pure cultures of bacteria on the antimicrobial surface found inside the showerhead (ISO, 2011) and may not represent realistic conditions. Nonetheless, successful implementation of such devices has been observed. Since DWPI can also grow in point-of-use filters (Chaidez and Gerba, 2004; Rodgers et al., 1999), frequent replacement is often recommended, requiring vigilant building operators. Recent product improvements incorporate biocides and include inactivation technologies that extend the life of these devices. Other filters, like granular activated carbon filters may create an ideal niche for *L. pneumophila* and NTM (Dai et al., 2018; Rodgers et al., 1999; Wu et al., 2017). To ensure public safety, validation of these devices under varied realistic scenarios with multiple DWPI is needed.

4.2 Temperature Control

In applying findings about temperature (discussed above) to full-scale systems, there are several strategies to keep water out of ideal growth ranges. In large hot water systems, recirculation helps maintain elevated temperatures capable of inactivating DWPI throughout the system. Maintaining temperatures > 60 °C in the water heater and > 55 °C at distal points of the system is recommended for prevention of *L. pneumophila* growth (Bédard et al., 2015a; Darelid et al., 2002; NASEM, 2019). However, some studies indicate that recirculation at improper (*i.e.* too low) temperatures can increase *L. pneumophila* densities, presumably due to heat losses prolonging the time within the optimal growth conditions (Rhoads et al. 2015). Similarly, increasing pipe insulation seems beneficial, in that hot water can stay hotter, but sometimes adding insulation can increase the time of ideal *Legionella* spp. growth temperatures during stagnation in hot water lines (Bédard et al., 2015a; Van

Kenhove et al., 2019a). While cold water systems are not typically the focus for *Legionella* spp. research, toilets can also transmit *L. pneumophila* (Couturier et al., 2020), and ideal growth temperature can be achieved in cold water systems with co-location of hot/cold pipes or where warm ambient temperatures are consistently present. Other DWPI including *P. aeruginosa* (Bédard et al., 2015b) are more often studied in cold water systems. Studies are needed regarding efficacy of optimizing temperature designs against non-*Legionella* DWPIs and the prevalence of all DWPIs in cold water systems.

One-time or periodic treatment can also be applied to remediate potential problems (*e.g.*, recommissioning of buildings after long stagnation or disuse (Proctor et al., 2020)). Heat-shock methods have found some success for *L. pneumophila* control, but recovery, recolonization, and development of resistance or acclimation are common (Allegra et al., 2011; Farhat et al., 2010; Ji et al., 2018; Temmerman et al., 2006), likely attributable to dissipation of temperature in distal ends, VBNC protective states, or even the ability of *L. pneumophila* to subsist necrotrophically on other cells killed by the intervention. Biofilm "cleaning methods" (e.g., ice slurry) are also available commercially, but without independent verification. Hyperchlorination or shock chlorination, *i.e.*, temporarily increasing disinfectant concentrations, has also been employed, but mitigation has been short lived and damage to pipe materials is a concern (Lin et al., 2011; Proctor et al., 2020). The recommended strength (*i.e.*, dose of chlorine, temperature), duration, and frequency of these treatments must consider the varied DWPI growth rates, physiology and resistance to treatment, as well as specific characteristics of treated buildings.

4.3 Building Design Features

Many aspects of plumbing design are dictated in building code, but most codes were not developed with microbial quality or DWPI growth in mind. Especially with changing trends in water conservation, an update to these building codes is warranted. For example, despite lower water flows, pipe diameter requirements remain unchanged since 1940 Hunter design codes (Hunter, 1940), resulting in over-sized pipes, long stagnation times, and low linear flowrates, which are hypothesized to influence biofilm and water quality (Julien et al., 2020b). On the other hand, smaller pipes increase surface-area-to-volume ratios, increasing the influence of biofilm on bulk water microbial quality. Competing objectives will make it challenging to achieve consensus for updated codes.

Decisions made during the design and construction of a building can have consequences for water flow patterns, temperature, and residence time of water in that building for its entire lifetime. In particular, buildings are not designed with "microbially-informed" parameters in mind. In Germany, the length of distal ends is restricted to 3 m to limit *Legionella* spp. growth (NASEM, 2019). Furthermore, by placing the toilet at the end of a trunk-and-branch line, water flow can be maximized through the pipes. Even the orientation of pipes (up or down from the pipe to the fixture) has implications on temperature and hence DWPI growth due to induction of convective mixing (Rhoads et al., 2015). Minimizing the water system footprint by reducing the length of distal pipes, reducing the number of fixtures, and installing water fixtures close together (i.e., minimizing wet walls) would help simplify investigations of DWPI colonization, remove *de facto* dead-ends, lower construction costs, and decrease energy needs for hot water, but achieving these collective goals requires communication between many silos of expertise.

In most plumbing designs, distal ends still present a challenge since water treatment will only affect the distal fixture by opening that particular fixture during treatment. To address this, some new plumbing designs and management protocols either automatically drain pipes or direct manual draining of pipes to dryness between uses. As NTM, *P. aeruginosa*, and fungi thrive in such semi- moist environments (*e.g.,* such as showerheads (Gebert et al., 2018; Hageskal et al., 2009; Novak Babič et al., 2017), tap aerators, and sink drains (Walker and Moore, 2015)), these changes have the potential to alter microbial ecology within plumbing. NTM are particularly resistant to desiccation (Archuleta et al., 2002). Electronic non-touch faucets were designed, in part, to reduce microbial contamination problems from faucets functioning as fomites, yet are associated with colonization and growth of many DWPI (Bédard et al., 2016; Kotsanas et al., 2008; Livni et al., 2008; Sydnor et al., 2012). Due to these unintended consequences, new plumbing designs should be thoroughly tested for risk of colonization internally by multiple DWPI as well as for their ability to serve as fomites on external-facing surfaces.

The propensity of pipe material to support DWPI survival, persistence and growth are often studied. Iron or copper can have positive (iron), negative (copper-silver), or mixed (copper) effects on DWPI growth (Buse et al., 2014; Haig et al., 2020; Lin et al., 2002, 1996). Corrosion can also accelerate decay of disinfectant residual (Nguyen et al., 2012; Rhoads et al., 2017a), reducing stress on all DWPI. Many plastic pipes leach assimilable organic carbon (Bucheli-Witschel et al., 2012; van der Kooij, 1992), a nutrient for growth of many DWPIs as they are heterotrophs. The type and amount of carbon leaching varies considerably amongst plastics, and can affect microbial communities and DWPI (Proctor et al., 2016). One limitation in pipe material research is the duration of studies, as most are limited to a few days, weeks, or months of pipe use. The hydrophobicity of pipe materials may have an influence on initial attachment (Simões et al., 2007), and copper may have anti-microbial effects (Learbuch et al., 2019; Rogers et al., 1994), but the long-term effects are questionable (Buse et al., 2014; Gião et al., 2015; Proctor et al., 2017; Rhoads et al., 2017b). Case studies can offer insights into long-term effects of plumbing design, but interpretation must consider multiple confounding variables. Consensus on which pipe material to use when or where will require rigorous long-term controlled and field-validation studies.

Commissioning, the final stage of building construction, can also impact water quality for the lifetime of the building. Careful hydraulic balancing (*i.e.*, calibrating pressures) helps maintain proper temperatures in both hot and cold water systems, if done properly (NASEM, 2019). While some standards for the commissioning process exist, they do not necessarily consider water quality. Often, water is allowed to stagnate in pipes for weeks before buildings are occupied (Inkinen et al., 2014), allowing time for biofilm formation. ASHRAE suggests considering shock-chlorination after four weeks of stagnation (ASHRAE, 2018). According to a survey of the many professional groups involved, professional responsibility for the commissioning phase is unclear, often resulting in missed steps and minimal review

(Potts and Wall, 2002). While the building owner is responsible for water quality, many people influence it before they take possession of the property.

4.4 Building Maintenance

Day-to-day, decisions regarding building water system can drastically affect DWPI growth. Some of these, such as local stagnation at a fixture, are difficult to control, but others, like water heater settings, are being incorporated into building water management plans aimed at curbing DWPI.

Long stagnation times are generally thought to permit DWPI growth, e.g., due to reduced chlorine residual. Studies of green buildings with water-saving devices that elevate water age often find DWPI (Rhoads et al., 2016) and samples taken after longer stagnation have higher microbial densities (Haig et al., 2020, 2018; Lautenschlager et al., 2010). Increased water age is also associated with a change in microbial community composition (Ji et al., 2017; Ling et al., 2018; Proctor et al., 2018), and even the type of NTM found (Haig et al., 2018). The community shift may indicate the importance of biofilm interactions with the water phase. The speed of biofilm-water interactions may account for the need for high frequency flushes to protect against *L. pneumophila* in some buildings (e.g., every 2 hours (Totaro et al., 2018)). However, flushing will not remove biofilms, and evidence suggests that increasing the flushing frequency may even cause greater net biofilm growth due to the delivery of fresh nutrients (Rhoads et al., 2015). It should be stressed that flushing after abnormally long stagnation times (e.g., upon return to a second home), may remove the immediate exposure threat from DWPI (Proctor et al., 2020). Even so, it is difficult to reach consensus amongst experts regarding best flushing practices (Proctor et al., 2020), and the direct relationship between stagnation and *Legionella* spp. growth is questioned (Rhoads and Hammes, 2021).

Depending on the system size and responsiveness changing water heater settings is a relatively simple way to control DWPI, however, scalding concerns and energy conservation efforts have resulted in low recommended temperatures (*e.g.*, 48 °C/120 °F). Such settings put hot water delivery pipes within the ideal temperature range of DWPI. Thermostatic mixing valves can be installed close to the point of use to allow higher temperatures and are recommended (NASEM, 2019), but they also have potential issues and require maintenance (Proctor et al., 2020). Instantaneous tankless water heaters may also allow temperature control while alleviating energy concerns (Brazeau and Edwards, 2013). Most research on water heater settings have focused on *L. pneumophila* (NASEM, 2019). Research considering the use of temperature to control other DWPIs is needed. Moreover, cost-benefit analysis to weigh health, scalding, and energy concerns are needed from multidisciplinary teams.

Water management plans vary widely but are all aimed at understanding and improving water quality. Plans should identify potential hazards (*e.g.*, aerosol-producing water features, low-use fixtures) and characterize the water system (*e.g.*, water heater operation, connections to other systems), which may be beyond the scope of information routinely available to building operators or feasible to collect on a routine basis. Control measures might be suggested, including physical controls, temperature management, disinfectant control, and pathogen testing. To date, plans sporadically include best-practices like cleaning showerheads and aerators, flushing hot water tanks, and minimizing aerosol exposure through increased ventilation in bathrooms (Masters et al., 2018). While success of water management plans is challenging to measure, simply having a water management plan may not be sufficient to improve water quality (van der Lugt et al., 2019). Moving forward, creators of water management plans likely require more guidance, training, site-specific information and access to specific expertise to develop and execute an effective document. Conflicting advice and interests (e.g., liability with testing) also influence the effectiveness of water management plans. Most water management plans are targeted at *L. pneumophila* prevention, including those required by the Centers for Medicare and Medicaid Services (CMS, 2018). Such a focus may be promoting practices that unintentionally create ideal conditions for other DWPI as was shown following the substitution of monochloramine for chlorine and the disappearance of *Legionella* spp., but an increase in *Mycobacterium* spp. numbers (Pryor et al., 2004). Moreover, maintenance of other water systems (*e.g.*, cooling towers) might be overlooked in these plans, but likely have a strong effect on disease.

4.5 An Example of Holistic Thinking: Creating Proactive Incentives

Implementation of engineering solutions and legislation after the discovery of a problem in the field is typically reactive, rather than proactive, and may not consider multiple hazards or unintended consequences of action. For example, legislation was enacted nationwide for drinking water in response to the 1993 Cryptosporidium outbreak (Gostin et al., 2000), and for cooling towers in New York City response to a 2015 Legionnaire's outbreak (Bassett and Balter, 2017). Even in communication of risk, there is a clear lack of multi-hazard consideration. Communication materials are available to inform the public on steps they can take to make decisions (*e.g.*, individual risk factors and behaviors) in order to manage DWPI, emphasizing that this is a shared responsibility between utilities, facilities managers, and individuals (Masters et al., 2018); however, most of the guidance available is for preventing risks of *Legionella* spp. at the exclusion of other water quality issues (Singh et al., 2020). The benefits of awareness and having a water management plan are not entirely conclusive (Clopper et al., 2021; van der Lugt et al., 2019), perhaps due to a variety in plan content and follow-through. However, a recent study suggested that 81% of *L. pneumophila* fatalities occurred in facilities without a water management plan (Clopper et al., 2021). In a survey of Minnesota hospitals, the majority of building managers expressed interest in getting assistance to set up a water management plan (Danila et al., 2018). More research to target the effective elements of water management plans will assist in creating effective plans.

A potential solution to raise awareness, and move towards more effective proactive solutions, like building water management plans, could be to incorporate building water maintenance into health and building insurance programs. This approach would take into consideration potential future savings on health care costs (due to lowered infection rates), and building owners' legal fees (due to lowered liability in case of infection). These plans can incentivize preventative actions, and even offset the energy, institutional, or testing costs associated with those preventative actions.

4.6 Proposed Approach

DWPI can be considered over the entire lifetime of the building, from design through everyday maintenance. The implementation of engineering solutions requires many non-engineering strategies.

- Encourage "microbially-informed design" of buildings through mechanisms such as LEED or national organizations
- Incorporate education about all DWPI into implementation of engineering solutions, such that architects, construction professionals, building owners, managers, and maintenance personnel have greater understanding of the significance of the work. This can include educating building tenants about personal risk factors for DWPI.
- Improving communication/education across disciplines e.g., regarding plumbing operation /codes /fixture designs among non-engineering communities and consideration of public health concerns by engineering community at the design phase
- Encourage building owners' and renters' insurance to include partial coverage of building water quality to incentivize preventative actions.
- Include previously developed water management plans in the sale of buildings, and require water testing as an inspection element, so that new tenants are aware of potential issues and address them. If testing isn't required, disclosure of previous water issues and testing, if conducted, should be required.
- Create a database of building testing data for all DWPI to collect existing data and encourage greater investigations into large data trends. This can include a more standardized reporting procedure for building size, sample type, and engineering solutions currently in place.
- Incorporate AR tracing into studies of real buildings, including epidemiological investigations of outbreaks to better understand how widespread AR is within engineered drinking water systems (Box 1). Investigations of engineering controls for AR can also be scaled up and tested in the field.

5. CONCLUSIONS

Clearly, key similarities exist among DWPIs: exposure routes are similar and various niches in plumbing systems provide ideal growth conditions, often with similar temperature ranges for growth, slow growth, and preference for the biofilm phase. However, differences in DWPI physiology and response to the culmination of various conditions in building water system (*e.g.*, resistance factors, competition) can result in different susceptibilities to each DWPI among individuals and populations, while mitigation efforts are also not universally effective against each DWPI.

The most well-studied DWPI across many fields is undoubtedly *L. pneumophila*. While awareness and treatment of this microorganism is a positive step towards improving plumbing design and operation and better protecting public health and safer plumbing, ignoring other DWPIs could result in unintended consequences and increase the burden on the healthcare system. Strategies to reduce *L. pneumophila* tend to reduce overall biofilm concentration and increase disinfectant residual, and thus may create conditions ideal for NTM (Pryor et al., 2004) proliferation. The attention placed on *L. pneumophila* has supported development of rapid and easy diagnostic and environmental measuring techniques, which in turn has led to substantial advancement of understanding of *L. pneumophila* in both clinical and environmental realms. Our understanding of other microorganisms would benefit from similar development of rapid tests, which can be motivated with financial and legislative incentives.

Ultimately, strategies to holistically protect the public from pathogens capable of growth in drinking water will involve the consideration of all DWPIs together and across all disciplines. For example, more DWPI associated diseases should be reportable and microorganisms should be studied concurrently in buildings to better inform risk models. While a single solution that creates a “zero-risk” plumbing system is not possible, increased cooperation and multiple DWPI consideration will allow for informed balancing of risk and consequences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ACRONYMS USED

AR	antibiotic resistance
CDC	Centers for Disease Control and Prevention
CFU	colony forming units
CSTE	Council of State and Territorial Epidemiologists
DALY	disability adjusted life year
DWPI	drinking water-associated pathogens that can cause infections in immunocompromised individuals
GAE	granulomatous amoebic encephalitis
MAC	Mycobacterium avium complex
MCLG	maximum contaminant level goal

MPN	most probable number
NTM	nontuberculous mycobacteria
QMRA	quantitative microbial risk assessment
UV	ultraviolet
VBNC	viable but nonculturable

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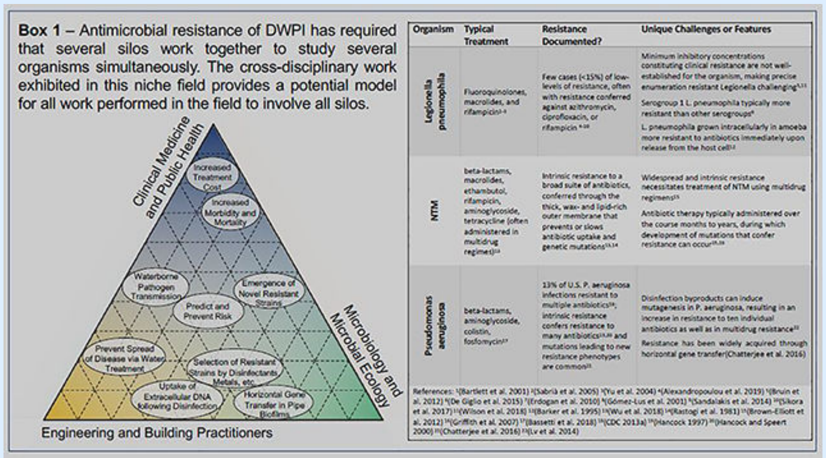
Box 1.

Various groups are responsible for water safety regarding drinking water-associated pathogens that can cause infections in immunocompromised individuals. Acronyms used: CDC = centers for disease control; HVAC = heating, ventilation and air conditioning *Citations in* Box 1 *[for the purpose of citation managers only..]:* [1](Bartlett et al., 2000) [2] (Sabrià et al., 2005) [3] (Yu et al., 2004) [4] (Alexandropoulou et al., 2019) [5] (Bruin et al., 2012) [6] (De Giglio et al., 2015) [7] (Erdogan et al., 2010) [8] (Gómez-Lus et al., 2001) [9](Sandalakis et al., 2014) [10] (Sikora et al., 2017) [11](Wilson et al., 2018) [12](Barker et al., 1995) [13](Wu et al., 2018) [14](Rastogi et al., 1981) [15](Brown-Elliott et al., 2012) [16](Griffith et al., 2007) [17](Bassetti et al., 2018) [18](CDC, 2013) [19](Hancock, 1997) [20](Hancock and Speert, 2000) [21](Chatterjee et al., 2016) [22](Lv et al., 2014)

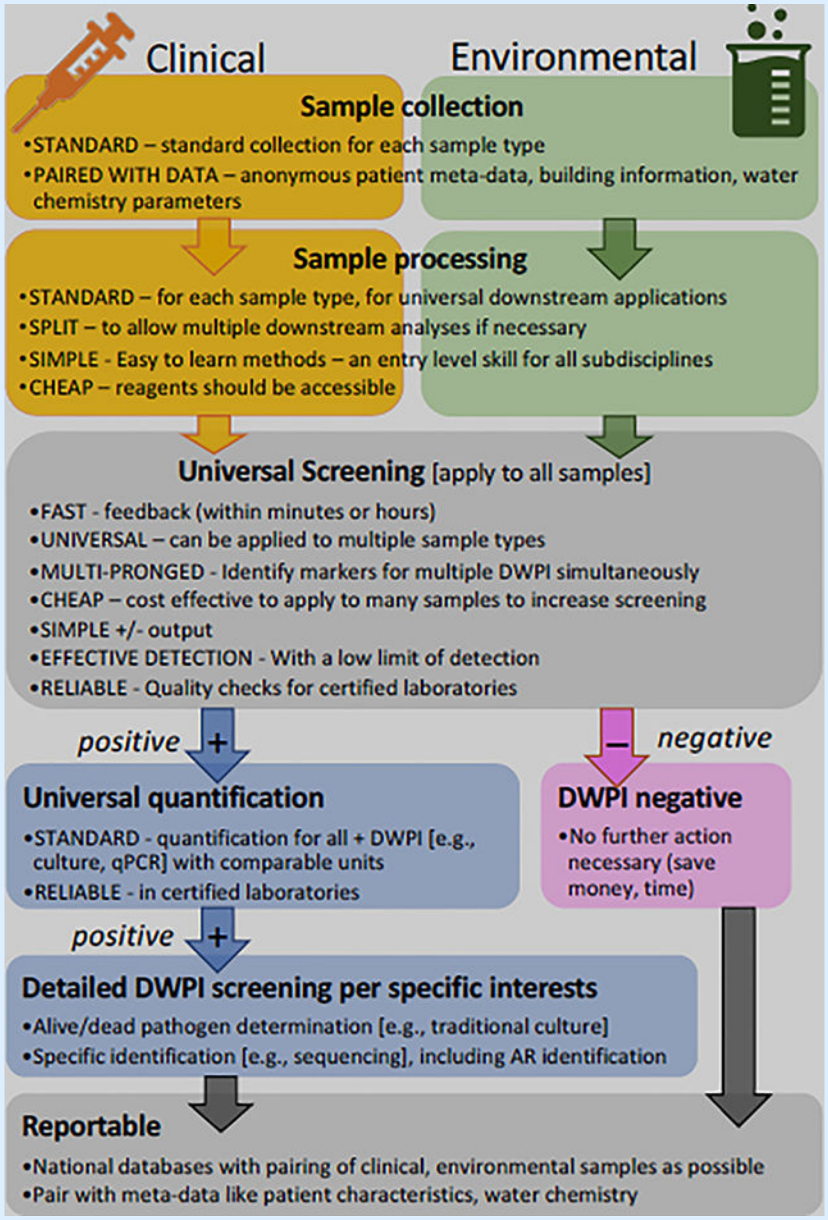


Organism	Typical Treatment	Resistance Documented?	Unique Challenges or Features
Legionella pneumophila	Fluoroquinolones, macrolides, and rifampicin	Few cases (<15%) of low-levels of resistance, often with resistance conferred against azithromycin, ciprofloxacin, or rifampicin	Minimum inhibitory concentrations constituting clinical resistance are not well-established for the organism, making precise enumeration resistant Legionella challenging Serogroup 1 L. pneumophila typically more resistant than other serogroups L. pneumophila grown intracellularly in amoeba more resistant to antibiotics immediately upon release from the host cell
NTM	beta-lactams, macrolides, ethambutol, rifampicin, aminoglycoside, tetracycline (often administered in multidrug regimes)	Intrinsic resistance to a broad suite of antibiotics, conferred through the thick, wax- and lipid-rich outer membrane that prevents or slows antibiotic uptake and genetic mutations	Widespread and intrinsic resistance necessitates treatment of NTM using multidrug regimens Antibiotic therapy typically administered over the course months to years, during which development of mutations that confer resistance can occur
Pseudomonas aeruginosa	beta-lactams, aminoglycoside, colistin, fosfomycin	13% of U.S. P. aeruginosa infections resistant to multiple antibiotics; intrinsic resistance confers resistance to many antibiotics and mutations leading to new resistance phenotypes are common	Disinfection byproducts can induce mutagenesis in P. aeruginosa, resulting in an increase in resistance to ten individual antibiotics as well as in multidrug resistance Resistance has been widely acquired through horizontal gene transfer(Chatterjee et al. 2016)

References: [1](Bartlett et al. 2001) [2](Sabrià et al. 2005) [3](Yu et al. 2004) [4](Alexandropoulou et al. 2019) [5](Bruin et al. 2012) [6](De Giglio et al. 2015) [7](Erdogan et al. 2010) [8](Gómez-Lus et al. 2001) [9](Sandalakis et al. 2014) [10](Sikora et al. 2017) [11](Wilson et al. 2018) [12](Barker et al. 1995) [13](Wu et al. 2018) [14](Rastogi et al. 1981) [15](Brown-Elliott et al. 2012) [16](Griffith et al. 2007) [17](Bassetti et al. 2018) [18](CDC 2013a) [19](Hancock 1997) [20](Hancock and Speert 2000) [21](Chatterjee et al. 2016) [22](Lv et al. 2014)

Box 2.

Proposed ideal method workflow that incorporates a universal screening step and reporting of all results to centralized databases.



Highlights

- Drinking water pathogen research often lacks cross-disciplinary communication
- Differences in pathogen behavior and management should be considered holistically
- Discipline-specific methods, vocabulary, and goals hinder cross-disciplinary work
- Comprehensive messaging and research regarding these pathogens is recommended

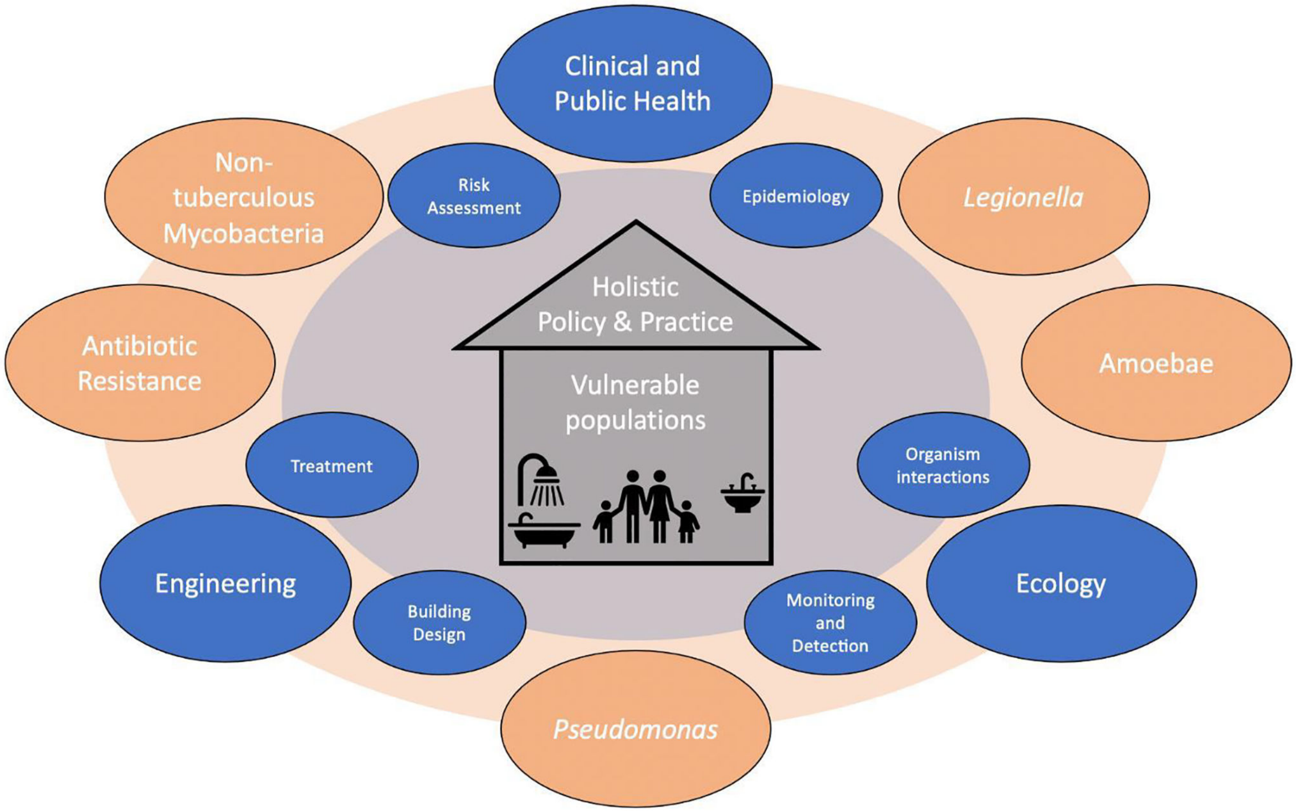


Figure 1.

Various groups are responsible for water safety regarding drinking water-associated pathogens that can cause infections in immunocompromised individuals. The professions and backgrounds of individuals within these groups may cross discipline silo boundaries or exist entirely outside of the disciplines – necessitating outreach on multiple fronts. Acronyms used: CDC = centers for disease control; HVAC = heating, ventilation, and air conditioning

Table 1.

Drinking water-associated pathogens (DWPIs) that can cause infections in immunocompromised individuals and associated diseases. DWPIs are defined as bacteria and amoebae that are (1) suited to grow in drinking water systems, particularly within building water systems and water fixtures, and (2) frequently cause disease in susceptible populations.

Organism	Disease	Infection incidence	Direct Healthcare cost[4]	Deaths/ year[4]	Reportable
Legionella pneumophila ß†	Legionellosis: Legionnaires' Disease and Pontiac Fever	8,000–50,000/ year[1]	402 million	995	CDC (national)[5]
Nontuberculous Mycobacterium **(NTM)** ß†§; **Mycobacterium avium complex (MAC)** ß† **& abscessus complex (MAB)** ß†	NTM lung disease, nodular bronchiectasis; skin and soft tissue infection	86,244/year[2]	1.53 billion	3,800	NTM-certain states w/ disease and/or positive isolate *
Pseudomonas aeruginosa ß®§	Otis externa ®	51,000/year (healthcare associated only)[3]	564 million	219	Certain states with antibiotic resistance **
Pseudomonas aeruginosa ß®§	*Pseudomonas* pneumonia †	51,000/year (healthcare associated only)[3]	453 million	730	Certain states with antibiotic resistance **
Pseudomonas aeruginosa ß®§	*Pseudomonas* septicemia §	51,000/year (healthcare associated only)[3]	214 million	695	Certain states with antibiotic resistance **

Other DWPI: Other Legionella species (e.g., L. longbeachae, micdadei, bozemanii, L. dumoffi) – ß†; Burkholderia cepacian complex – ß; Achromobacter – ß; Stenotrophomonas maltophila – ß, Acinetobacter baumannii – ß, Sphingomonas paucimobili – ß, Aeromonas hydrophila – ß, Hartmanella (Vermamoeba) – Æ, Acanthamoeba – Æ Ø, Naeglaria fowleri – Æ Ø€, – Balamuthia mandrillaris Æ Ø

References [1](Prussin et al., 2017) [2] (Strollo et al., 2015) [3](CDC, 2019d) [4](Collier et al., 2021) [5](CDC, 2019b)

Reportable states *Oregon, Nevada, New Mexico, Nebraska, Missouri, Mississippi, Wisconsin, Ohio, Virginia, New Jersey, and Maryland **Minnesota, Maryland, Wisconsin, Utah, New York, Tennessee, Washington, and Michigan (CDC, 2021d, 2021e; Minnesota Department of Health, 2019b); Florida as part of electronic laboratory reporting surveillance (Florida Health, 2016)

Symbol Key

Organism type: ß = Bacterium; Æ =Amoeba

Disease type/exposure: † = Pulmonary through inhalation or aspiration of aerosols; § = skin or soft tissue infection through dermal exposure ® = Ear infection; Ø = Granulomatous Amebic Encephalitis (GAE) through intranasal exposure like irrigation; € = Eye infection

Table 2.

Terms often used in the field with potential confusion between fields

	Term	Definition and usage in different contexts
Types of water in plumbing	Potable	Water used for drinking and/or food preparation, most often cold water. In some fields, potable is inclusive of hot water
	Non-potable water	Water unsuitable for drinking purposes but used for other activities, including irrigation, industrial uses, or other non-drinking purposes. (WateReuse, 2016)
	Drinking water	A term sometimes used to distinguish water identified strictly for drinking purposes (e.g., providing water to water fountains/drinking fountains/bubblers) and/or food preparation. In the US, piped drinking water may be used for non-potable purposes, as it is also routed to toilets and water heaters in most cases. This is sometimes a different system than the cold water piped to other water outlets in commercial buildings.
Components of plumbing	(Hot) Water heater	A closed vessel in which water is heated by the combustion of fuels, electricity, or any other source and is withdrawn for use external to the system at pressures not exceeding 160 psig [1100 kPa (gage)], including the apparatus by which heat is generated, and all controls and devices necessary to prevent water temperatures from exceeding 210°F (99°C). A water heating (and often storage) device that provides piped hot water for consumptive uses like showering, hand-washing, dishes, and laundry. Some models are tankless. Traditional models are powered by gas or electricity. (ASHRAE, 2020b)
	Boiler	A closed, pressure vessel that uses fuel or electricity for heating water or other fluids to supply steam or hot water for heating, humidification, or other applications. This is a more versatile water heating device that may provide water for consumptive uses, or for heating the building. (ASHRAE, 2020b)
	Water line/pipe	Colloquially used to refer to any water or wastewater pipe. Hot water line and hot water pipe system are interchangeable.
Bacterial life cycles	Inactivation	The interruption of growth metabolism and loss of a microorganism's ability to reproduce under suitable conditions; VBNC microorganisms represent one gray area for measurement of inactivation and microorganisms may recover from this state. The indicators we use to measure inactivation of organisms are often limited with issues related to quantification of living microbes, ability of microbes to resuscitate. Due to imperfect quantification on both ends of this spectrum (culture-based methods will underquantify viable organisms and molecular-based methods will quantify both dead and live organisms), all methods have variable detection limits that may also miss a fraction of viable organisms.
	Persistence	The precise definition of this term varies depending on the subfield. Environmental persistence refers to how well a microorganism or chemical substance is transported (a catch-all term that includes processes of removal, stress, biodegradation, and growth and death processes for biologics) in response to environmental conditions (Boethling et al. 2009). It is typically evaluated using a plot of occurrence (e.g. $\ln(C/C_0)$ for first-order inactivation or growth) over time. Medically, persistent infections refer to infections that remain relevant in the host for long periods of time despite treatment (Fisher et al., 2017).
	Antimicrobial resistance	The microorganism possesses physiological traits, intrinsic or horizontally acquired, that allow them to survive exposure to antibiotics, antimicrobials, and disinfecting agents. Resistance is operationally defined as an increase in the minimum inhibitory concentration (MIC) in response to exposure to an antibiotic.
	Decay	In biology, refers to the process of decomposition by which dead organic substances are broken down into simpler organic or inorganic matter. In chemistry, derived from radioactive decay, i.e., a reference to a chemical "half life" or time for the substance to reduce by a factor of 50% assuming a first-order practice. Informally, the term is often applied to refer to a decrease in a chemical species or bacterial population, however this term should not be used to refer to microorganism inactivation or death (Balaban et al., 2019).
	Disinfection	The process of inactivating pathogenic microorganisms. Primary disinfection is a common component of primary treatment of drinking water designed to inactivate microbial pathogens; secondary disinfection is used to maintain water quality achieved at the treatment plant throughout the distribution system up to the tap (WHO, 2017).
	Kill/dead	A state of an organism where resuscitation of metabolic activity is no longer possible; the end stage of the bacterial life cycle that occurs after all physiological processes have run their course (Colwell, 2000; Rice and Bayles, 2008)
	VBNC (viable but nonculturable)	Bacteria that are in a state of very low metabolic activity and do not divide due to environmental stress, and cannot be grown on standard culture media. However, the bacteria are alive and have the ability to become culturable once resuscitated (Li et al., 2014).

	Term	Definition and usage in different contexts
Organism type	Indicator	Traditionally, indicator microorganisms are not necessarily pathogenic but are members of the intestinal flora of warm-blooded animals used to suggest the presence of pathogens or fecal contamination (see Bonde's criteria (Bonde, 1966)) (National Research Council, 2004). However, an indicator can be any proxy or sentinel for a particular outcome or process (Berg, 1978).
	Pathogen	Any microorganism capable of causing damage in a host. It is noted that there are concerns raised with this term due to the existence of pathogenic and non-pathogenic states (Pirofski and Casadevall, 2012).
	Opportunistic pathogen (OP)	A microorganism that can become pathogenic following a perturbation to their host (e.g. disease, wound, medication, prior infection, immunodeficiency, and ageing) (Brown et al., 2012), or in some cases that potentially can infect healthy individuals at higher doses. In drinking water contexts, they are often contrasted with fecal pathogens, by being capable of growth in the distribution system and by non-ingestion routes of exposure. See notes on "pathogen" above.
	Drinking water-associated pathogens that can cause infections in immunocompro mised individuals (DWPI)	Term proposed by this paper to be used to refer to a specific group of bacteria and amoebae that are (1) suited to grow in drinking water systems, particularly within building water system and (2) frequently cause disease in susceptible populations (e.g., people with immune compromise and/or lung disease), though the characteristics of susceptible populations can vary, and infections can still occur in healthy individuals. While present naturally in the environment (surface water, soil), DWPI typically (i) are not present in high quantities in finished water leaving the drinking water treatment plant, and (ii) multiply within building water system and/or the distribution network, often within biofilms. These organisms may cause exposure outside of "drinking water", but the term is used to distinguish problems oligotrophic "drinking" water, from specific issues encountered in other water supply systems like heating/cooling systems and gray water systems.
Pipe system	Plumbing, Building Plumbing, Premise Plumbing, Premises Plumbing Building water system	Plumbing is the pipes, tanks, fittings, and other devices that convey water within a building. Plumbing refers to both the supply side and waste side (drinking and wastewater). Premise plumbing is a term adopted to include plumbing and the service line leading to the building. A more grammatically correct term would be "premises" plumbing. Plumbing on the supply side may refer to both drinking water systems, that carry potable water to places where it can be consumed, as well as pipe systems that convey water for other purposes (e.g., heating and cooling systems, fire protection systems).
	Service line	The pipe that conveys water from the source of water for the building (i.e., municipal water system or well) into the building. The service line often has split ownership at the water meter between water provider and building owner.
	Distribution system, distribution network	The pipes used to carry water between a water or wastewater source and the user or end-stage purpose (e.g. resulting in intentional reuse or disposal to a water body and de-facto exposure). Often this refers to the buried pipes that convey water from a water treatment plant throughout the service area to individual buildings.
	Engineered water system	General term for any infrastructure created by humans used to store and/or convey water. For example, the term is used to refer to both the distribution system and plumbing.
Water Treatment	Treatment	Any biological, chemical, and/or physical process applied to water or wastewater that is designed to remove or otherwise reduce the risk from unwanted substances such as contaminants to improve quality for its desired purpose.
	Finished water	Water that has passed through a water treatment plant, such that all the treatment processes are completed, or finished. This water is ready to be delivered to consumers.
	Polishing	A treatment stage placed at the end of other treatment to bring the water to a more highly conditioned and more perfect state, e.g., a mixed bed of ion-exchange media installed as the final treatment step in the deionization process to remove last traces of undesirable ions. (McTigue and Symons, 2010)
Risk	Hazard	A substance or situation to which exposure would result in the ability to cause harm to humans or other organisms.
	Risk	The probability of an adverse outcome from exposure to a hazard; a function of both hazard and exposure. This definition can be broader across domains and applications and typically includes some acknowledgement of uncertainty and associated consequences of a particular scenario. The risk for pathogens can be quantified using quantitative microbial risk assessment (QMRA).
Disease terms	Median infectious dose (ID_{50}) or median affected dose (N_{50})	Descriptive parameters of a dose response relationship that describes the dose (number of microorganisms which a test subject is exposed to via a given route) at which a particular response or health endpoint (infection, illness, or death) is observed in 50% of the test subjects (animals, humans, cells, etc.). The term "infectious dose" or "minimal infective dose" is an imprecise descriptor colloquially used by the medical community to refer to the dose of microorganism required to establish an infection in the host and should be avoided when discussing risk.
	Dose response relationship	A mathematical function which describes the quantitative relationship between the dose of a microorganism (number of microorganisms which a test subject is exposed to via a given route)

	Term	Definition and usage in different contexts
		associated with the probability of a particular response or health endpoint (infection, illness, or death) in the test subjects.
	Health endpoint, outcome, or other sequelae	An outcome of a particular exposure event; this might be infection, illness, or death for microbes; for chemical exposures these can be carcinogenic or non-carcinogenic outcomes. The endpoint is typically specified in a dose-response experiment (e.g., shedding in feces, positive colonization sites, lesions in the lung, cancer, asthma, etc.) and can be continuous (on a numeric scale), quantal (number of positive or negative responses), or categorical (mild, moderate, etc.), but quantal data is typically used for microbial risk assessment dose response modeling. Valuation of a risk outcome is typically related to what endpoint is specified and therefore “meaningful” endpoints are typically chosen based on data availability and relevance to the risk scenario and/or disease process of interest. Definition of endpoint depends on the application (e.g., human health vs. ecological). Sequela represent longer-term impacts or outcomes following a microbial colonization and/or infection and resulting processes.
	Colonization	Colonization can occur in the context of the environmental system (e.g., colonizing a plumbing system biofilm in an environmental science and engineering context) or within the human body (at a target organ such as the lungs in a health risk context). Colonization indicates that a microorganism is able to establish itself and grow at a particular location or portal of entry (e.g., a target organ or pipe). In the medical context, colonization does not necessitate interaction between the host and organism, therefore it may be asymptomatic and does not necessarily indicate clinical expression or immune response (Dani, 2014).
	Infection	Survival and initiation of infectious processes and/or immune response in a host resulting from interplay between host and pathogen, whether or not it develops into a disease (Dani, 2014).
	(Infectious) disease	The development of an illness (presenting signs and symptoms) caused by a specific infectious agent or its toxic product that results from transmission of that agent or its product from its source to a susceptible host.
	Contaminated	The presence of a microorganism or substance that is hazardous to human health if exposure were to occur, or hazardous to other organisms depending on the context. This term is more often used in the engineering field to describe engineered or environmental systems (e.g., rivers, water).

Table 3.

Features of methods used to detect and quantify drinking water-associated pathogens that can cause infections in immunocompromised individuals (DWPI). Molecular methods are abbreviated as follows: polymerase chain reaction (PCR), quantitative-PCR (qPCR) and digital PCR (dPCR).

Method		What reaction/ substance does it measure?	Live/ dead?	Sample type (typical)	Description of method	Specificity of target	Enable type matching? §	Primary question it answers	Questions it cannot answer	Sector used by	ISO approved?	T rec
Urinary antigen test		Antigen	Y	Urine, bodily fluids	Confirms presence/ absence of antigen in sample	Serogroup (only for *L. pneumophila* SG1)	N	Is or was the human body fighting this organism recently?	Was the body fighting it before, or a different strain of it?	Health	N *	Mi
Culture		Growth	Y	Sputum, Water (sometimes filtered), tissue	Quantifies growth in specific nutrient rich conditions.	Specificity varies, typically species.	Y – must do prompt-ly	Can the organism grow right now under specific conditions?	Could the organism grow before, later, or under more realistic conditions? Is the organisms in a VBNC state?	Health, Ecology, Engineering	Y (N for NTM)	7+
PCR, qPCR, ddPCR	DNA-based	DNA (in all cells)	N	Water (filtered), swabs	PCR – confirms presence/ absence of a sequence. qPCR and dPCR – quantify a sequence.	Specificity varies typically genus, species or indicator genes.	Y ***	Is or was a gene present in the sample, and how much, absolutely?	Is it capable of infection/the thing that caused infection? (for DNA – is it alive?)	Ecology, Engineering, [Health as secondary method]	Y‡	8–
	RNA-based	RNA (only in live cells)	Y								N	
	PMA/ EMA based	DNA [live/dead stained]	Y **								N	8– hrs
'omics methods (e.g., amplicon sequencing, metagenomics, shotgun sequencing)		DNA (in all cells)	N	Water (filtered), swabs	Identifies relative abundance of sequences in communities. For taxonomic profiling or genome assembly.	Varies: Amplicon sequencing – resolution to genus level, metagenomics to species and in some cases strain level.	Y***	Is or was a gene present in the sample, and how much, relatively? How does occurrence of several DWPI compare?	Is it capable of infection/the thing that caused infection? (for DNA – is it alive?)	Ecology	N	1–
		RNA (only in live cells)	Y									
		DNA [live/dead stained]	Y **									
Pseudalert		Growth	Y	Water	Most probable number based on growth in chambered trays	Species – *P. aeruginosa* only	Y – must do prompt-ly	Can the organism grow right now under specific conditions?	Could the organism grow before, later, or under more realistic conditions? Is the organisms VBNC?	Engineering	Y	1 d
Legiolert		Growth	Y			Species – *L. pneumophila* only					N	7 d
Adenosine tri-phosphate (ATP)		Chemical cycled by living cells	Y	Drinking water	Quantifies 'total activity' of microbes	Non-specific – total bacterial measures ****	N	Are cells generally active? More so than usual?	Is there a specific organism of concern present?	Engineering, Ecology (primarily in research now)	N	Sec

Method	What reaction/ substance does it measure?	Live/ dead?	Sample type (typical)	Description of method	Specificity of target	Enable type matching? §	Primary question it answers	Questions it cannot answer	Sector used by	ISO approved?	T rec
Flow cytometry	Nucleic acids within cells	Y	Drinking water	Quantifies number of cells (total or intact)		N	How many cells are there? (How many are intact?)	*Total growth not consistently linked with any DWPI, but can indicate general temporal/ spatial conditions conducive to microbial growth.*		N	Mi
Heterotrophic Plate Counts	Growth	Y	Drinking water (sometimes filtered)	Quantifies growth of heterotrophs in relatively nutrient rich conditions.		N	Can heterotrophs grow right now under specific conditions?		Engineering (regulated drinking water method)	Y	2–

§As detection methods – none of these can alone be used for definitively determining the exact source of a disease. Typically, a more in-depth method must be applied to determine sequence, type, or serogroup matching.

*ISO does not typically approve diagnostic tests. This is CDC approved, however.

**Success of PMA and EMA based methods for differentiating between live and dead cells is debated.

***Extracted nucleic acids (RNA, DNA, or EMA/PMA differentiated DNA) used w/metagenome sequencing can identify more in depth characteristics. This is typically expensive.

****Flow cytometry measures have been developed specifically for *Legionella*, involving a immunomagnetic separation pre-processing step – but have not been widely validated.

‡In 2019, ISO approved a molecular method for *Legionella* and *Legionella pneumophila*.