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Management of Legionella in Water Systems

Committee on Management of Legionella in Water Systems

Water Science and Technology Board

Board on Life Sciences

Board on Population Health and Public Practice

Division on Earth and Life Studies

Health and Medicine Division

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COMMITTEE ON MANAGEMENT OF LEGIONELLA IN WATER SYSTEMS

JOAN B. ROSE, NAE, Chair, Michigan State University, East Lansing
NICHOLAS J. ASHBOLT, University of Alberta, Edmonton, Canada
RUTH L. BERKELMAN, NAM, Emory University, Atlanta, Georgia
BRUCE J. GUTELIUS, New York City Department of Health and Mental Hygiene
CHARLES N. HAAS, Drexel University, Philadelphia, Pennsylvania
MARK W. LECHEVALLIER, Dr. Water Consulting, LLC, Morrison, Colorado
JOHN T. LETSON, Memorial Sloan-Kettering Cancer Center, New York City, New York
STEVEN A. PERGAM, Fred Hutchinson Cancer Research Center and the University of Washington, Seattle
MICHÈLE PRÉVOST, Polytechnique Montréal, Canada
AMY PRUDEN, Virginia Polytechnic and State University, Blacksburg
MICHELE S. SWANSON, University of Michigan, Ann Arbor
PAUL W. J. J. van der WIELEN, KWR Water Research Institute, Nieuwegein, The Netherlands
LAN CHI NGUYEN WEEKES, La Cité, Ottawa, Canada

National Academies Staff

LAURA J. EHLERS, Study Director, Water Science and Technology Board ANDREA HODGSON, Program Officer, Board on Life Sciences KATHLEEN STRATTON, Scholar, Board on Population Health and Public Practice ERIC EDKIN, Program Coordinator, Board on Earth Sciences and Resources RAYMOND M. CHAPPETTA, Senior Program Assistant, Board on Earth Sciences and Resources

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Stagnation. Stagnant areas in premise plumbing support more cultivable legionellae (including *L. pneumophila*) than other parts of premise plumbing (Fisher-Hoch et al., 1982; Tobin et al., 1981). Ciesielski et al. (1984) showed that hot-water tanks with stagnant water support higher *L. pneumophila* numbers (10⁵ to 10⁶ CFU/L) compared to hot-water tanks in which water was continuously replaced (less than 10⁴ CFU/L). Compared to non-stagnant water, stagnant water has lower or no disinfectant residual (Fisher-Hoch et al., 1982; Wang et al., 2012), lower water temperatures (Patterson et al., 1994), higher concentrations of organics (LeChevallier et al., 1996; Wang et al., 2012), lower dissolved oxygen concentrations (Wang et al., 2012), higher biomass concentrations (Lautenschlager et al., 2010), altered microbial community composition (Dai et al., 2018a; Lautenschlager et al., 2010), and higher numbers of protozoan hosts (Wang et al., 2015b)—factors that all influence *L. pneumophila* growth.

Corrosion. The impact of corrosion products on *Legionella* proliferation is multifaceted (Brazeau and Edwards, 2013). By consuming residual disinfectant, these compounds create a more favourable environment for *Legionella* growth. Increased bioavailability of various metal corrosion products, such as iron, may also upregulate virulence in legionellae, stimulate general biofilm growth (Buse et al., 2012), and contribute to *Legionella* growth in hot-water heaters (Dai et al., 2018b; Ji et al., 2017; Proctor et al., 2017). Iron released during the recent massive corrosion event in Flint, Michigan, contributed to loss of chlorine residual and, as a required nutrient (Reeves et al., 1981; States et al., 1985; Warren and Miller, 1979), this metal was also hypothesized to stimulate *Legionella* growth (Rhoads et al., 2017a). Van der Lugt et al. (2017) also recently reported that iron rust in stainless-steel shower heads resulted in increased *Legionella anisa* plate counts. Corrosion products can also promote heterotrophic biofilm growth by producing electron donors, such as hydrogen, and by stimulating autotrophic metabolism and fixation of organic carbon in the system (Rhoads et al., 2017b).

Pipe Materials. Pipe material may influence growth of *L. pneumophila*. For example, rubber material in a model pipe system enhanced growth of *L. pneumophila*, except when a biocide (thiuram) was present in the rubber material (Niedeveld et al., 1986). Plastic pipe materials can also enhance growth of *L. pneumophila*, especially those used in premise plumbing, such as soft PVC (PVC-P), polyethylene, polypropylene, or polybutylene materials (Rogers et al., 1994a,b; van der Kooij et al., 2002). The biofilm concentration on each type of pipe material correlates with *L. pneumophila* load (Learbuch et al., 2019). Therefore, pipe materials most likely affect *L. pneumophila* growth indirectly: higher biofilm concentrations support more protozoan hosts, which generate higher counts of *L. pneumophila* (van der Kooij et al., 2017). European standardized laboratory tests have demonstrated that, compared to an inert material such as glass, rubber (natural and synthetic), soft PVC (i.e., PVC-P), polyethylene, polypropylene, and polybutylene significantly enhance microbial growth (Hambsch et al., 2014) because of the growth-promoting organic compounds that these materials release. In contrast, stainless steel, PVC-C, and PVC-U did not enhance growth of *L. pneumophila* in these laboratory tests. A field study of several buildings demonstrated that the highest cultivable legionellae numbers were present in the biofilm on rubber components of taps (van Hoof et al., 2014), consistent with various laboratory test results.

In premise plumbing, the impact of copper pipes on legionellae is not consistent among studies, possibly due to differences in biofilm microbiota and the physiological status of cells. Several laboratory studies report that copper inhibits growth of *L. pneumophila* (e.g., Learbuch et al., 2019; Rogers et al., 1994b; Schoenen et al., 1988). In addition, Danish buildings with copper premise plumbing showed lower cultivable legionellae counts than buildings with iron pipes (Pringler et al., 2002). In contrast, others observed enhanced growth of *L. pneumophila* on copper compared to PVC-U or PVC-C (Buse et al., 2014a,b; Gião et al., 2015; van der Kooij et al., 2002). Likewise, by comparing bacteria growing in

tubing downstream of biofilm reactors with copper versus PVC-U coupons, Lu et al. (2014) also noted that injected *L. pneumophila* actually survive better downstream of copper. A companion paper by the same group (Buse et al., 2014a) further indicated that the copper coupons were colonized by and released a greater number of *L. pneumophila* when co-inoculated with *Acanthamoebae polyphaga* and measured by qPCR, but *L. pneumophila* were only cultivable from PVC-U coupons.

There are several possible explanations for the apparent enigma of net effects of copper plumbing on *Legionella*. First, van der Kooij and colleagues (2005) observed that new unused copper material initially inhibited growth of *L. pneumophila* due to the release of copper ions, but when the copper material was corroded, release of copper ions was reduced and inhibition of *L. pneumophila* no longer occurred. Interactions of the copper pipe with the local water chemistry is also important to consider. Proctor et al. (2017) noted that benefits of copper pipe for depressing *L. pneumophila* levels were only apparent at or below 41°C. Above 53°C, *L. pneumophila* were no longer detectable, and thus pipe material did not matter. Buse et al. (2017) noted that a higher pH (greater than 8.2), which limits dissolution, can also limit antimicrobial activity of copper pipe. Build-up of corrosion byproducts over time also limits the antimicrobial activity of copper toward *Legionella* (van der Kooij et al., 2005). In addition to having stronger antimicrobial properties than solid Cu, free Cu²⁺ in solution can induce other reactions, such as corrosion and associated hydrogen production, which could indirectly impact *Legionella* (Proctor et al., 2017; Rhoads et al., 2017b).

Copper might also induce a VBNC-like state for L. pneumophila, as has been suggested for Pseudomonas aeruginosa (Flemming et al., 2014). Induction of a VBNC-like state through copper exposure decreased the number of L. pneumophila detected by cultivation (Learbuch et al., 2019; Rogers et al., 1994a; Schoenen et al., 1988; van der Kooij et al., 2002), but not the number quantified by DNA-based methods (Buse et al., 2014a,b; Gião et al., 2015). Consistent with this hypothesis, after batch incubations with copper ions, Proctor et al. (2017) reported sharper decreases in L. pneumophila numbers by plate counts versus qPCR. Also, copper (and other) materials influence the microbial composition of premise plumbing biofilms (Buse et al., 2014a; Proctor et al, 2018), with copper resulting in less biofilm growth than various hard and soft plastics (Proctor et al., 2018; van der Kooij et al., 2017). Interestingly, while less biofilm may accumulate on copper materials than on plastics, the types of bacteria and amoeba present could be more supportive of L. pneumophila growth than those on plastics (Buse et al., 2014a,b; Gião et al., 2015). In particular, V. vermiformis is the L. pneumophila host most often associated with warm- and hot-water (largely copper-pipe) systems (Buse et al., 2017; Ji et al., 2017). Hence, along with biofilm concentration, the species composition of the biofilm is important for growth of amoebae that favor L. pneumophila replication. Overall, L. pneumophila growth appears enhanced in biofilms dominated with α-Proteobacteria, key prey for protozoan hosts (van der Kooij et al., 2018).

Once within the complex plumbing of a large building, *L. pneumophila* may persist given the right combinations of temperature, stagnation, and subsequent loss of residual disinfectant, often exacerbated by the presence of iron oxides/hydroxioxides/humics (Butterfield et al., 2002) and other pipe corrosion products (Rhoads et al., 2017b). *L. pneumophila* strains have remained detectable in simulated building water systems for a long time (i.e., up to 2.4 years) (Paszko-Kolva et al., 1992; Skaliy and McEachern, 1979; Wadowsky and Yee, 1985), with the one apparent clone in buildings causing outbreaks over decades (Garcia-Nunez et al., 2008). This prolonged survival in water has been attributed to the organism's ability to produce and store poly-3-hydrobutyrate, a carbon/energy source when nutrients are scarce (James et al., 1999; Mauchline et al., 1992). Recently, Shaheen and Ashbolt (2019) showed that viable cells of a *L. pneumophila* serogroup 1 strain remained in a dormant-like state associated with amoebae for over two years in drinking water. Such persistence may be associated with the expression of a Type II

Iron Corrosion and Inorganic Nutrients

Much of U.S. water distribution systems consist of century-old unlined iron mains, which are beyond their designed lifespan and subject to substantial corrosion as well as intrusion during water main breaks. Corrosion of pipe surfaces provides not only a habitat for bacterial proliferation and protection from chlorine disinfectant residuals but also a source of nutrients. Aerobic microbial respiration consumes oxygen, resulting in a reduced redox environment that can accelerate corrosion and produce a disinfectant demand. Corrosion of pipe surfaces and deposition of corrosion products can also create tubercles and surface roughness that protect biofilm organisms from hydraulic shear (Characklis and Marshall, 1990). The resulting turbulent flow can help transport nutrients and detritus, further enhancing the biofilm environment.

Growth of certain microbes is also promoted by other inorganic substances can also serve as electron donors or acceptors including methane, ferrous iron, reduced sulfur compounds, hydrogen gas, manganese, ammonia, and nitrite. These substances can stimulate autotrophs to fix organic carbon into the system, leading to more bacterial cells and associated organic matter. The accumulation of organic carbon and reduced inorganic compounds (e.g., iron, nitrite, sulfides) in biofilms can create a disinfectant demand that protects the attached microbes from being inactivated. In particular, iron-oxidizing bacteria oxidize ferrous iron to produce ferric iron oxides. Not only is iron a known nutrient for *Legionella*, it also reacts with chlorine, thereby increasing microbial risk by removing the disinfectant residual.

Plumbing Materials

Plumbing materials are an important factor to consider in *Legionella* control. Common plumbing materials in buildings include copper, iron, and numerous plastics, with cross-linked polyethylene (PEX) and cross-linked polyvinyl chloride (PVC) being particularly suitable for hot-water plumbing because of their tolerance of higher temperatures. Each pipe material will influence the building-level water chemistry and shape the biofilms that colonize premise plumbing in a unique manner (Ji et al., 2015). Being able to identify a pipe material that most effectively limits proliferation of *Legionella* for a given water chemistry and building type would be valuable as a passive barrier. It is important to recognize that water chemistry varies regionally, seasonally, and as dictated by various upstream water treatment processes (Dai et al., 2018), making it difficult to predict how incoming water will react with different pipe materials.

Although copper pipe has well-known antimicrobial properties, it does not universally control *Legionella*. Indeed, copper has been associated with decreased, increased, and comparable numbers of *Legionella* relative to other pipe materials (Rhoads et al., 2017b). As described in Chapter 2, the age of copper pipe, temperature, pH, and general water chemistry influence the dissolution chemistry and overall antimicrobial action of copper towards *Legionella*. The composition of the biofilm community also matters, e.g., interactive effects of amoebae and copper appear to favor survival of *Legionella* (Buse et al., 2017; Ji et al., 2017). Thus, it is clear that copper pipe cannot be the sole agent to control *Legionella*; other microbiological, chemical, and site-specific factors needs to be considered.

PEX and other heat-tolerant flexible polymeric plastic materials have gained popularity for their ease of use for hot-water plumbing. These materials, however, are well known to leach organic carbon and can stimulate bacterial growth (Proctor et al., 2018). In particular, flexible pipe materials commonly employed to plumb showerheads are especially vulnerable to biofilm formation and microbial growth, producing total bacterial cell counts ranging from 10⁶ (PE-Xc—applied as a rigid control plastic) to 10⁸

systems, such as wastewater treatment plants, because of the nature and scale of these systems. Other competing goals, such as commitment to water and energy savings for green building certification, must also be taken into consideration. Water management plans (discussed in detail in Chapter 5) are essential to *Legionella* control for any water system, as they provide the opportunity to adapt and tailor the strategy to the specific system of concern and employ and integrate all applicable barriers (see Table 4-6).

Two rows in Table 4-6 do not correspond precisely to controls discussed in this chapter. First, source water quality is listed (rather than the narrower nutrient limitation), as there are important water quality considerations at each stage of a building's life cycle and multiple control strategies will affect water quality. Second, there is a final row on water management plans for protecting a building from a *Legionella* outbreak because having a plan itself is a critical control. (Such plans are discussed in detail in Chapter 5.)

The conclusions and recommendations below highlight key lessons regarding *Legionella* control strategies for the building and device types discussed in this chapter.

For all types of buildings, hot-water heater temperature should be maintained above 60°C (140°F) and the hot-water temperature to distal points should exceed 55°C (131°F). Maintaining temperature outside *Legionella's* preferred growth range is the paramount *Legionella* control strategy for all buildings that provide hot water and has been proven successful by numerous longitudinal field studies. Temperature control is most effective in large, complex hot-water systems that are hydraulically balanced, with dead-end pipes removed and faulty devices that compromise the distribution of hot water identified and replaced.

There is growing evidence that, compared to free chlorine, a monochloramine residual better controls *Legionella* risk from building water systems, although the reasons for the improved performance are not yet clear. It is possible that amoebae trophozoites are more sensitive to monochloramine, causing the amoebae to encyst and thus preventing the proliferation of *Legionella* within their host. Additional research is needed to examine the precise action of monochloramine on *Legionella* persistence and growth within pipeline biofilms. Better understanding of the potential for nitrification in building plumbing is also required, as this reaction could negatively impact the effectiveness of a chloramine residual for *Legionella* management.

Additional research is needed to evaluate the potential for nutrient limitation (concentration and composition) to control *Legionella* growth in distribution and building water systems. These studies should examine, in full-scale drinking water systems, the impact of nutrient reduction on the concentration and composition of the microbiome in biofilms and water including amoebae growth and life stages and the subsequent effect on occurrence and decrease of pathogenic *Legionella* species.

New NSF/ANSI standards regarding microbial growth potential of materials are needed so that water utilities, plumbers, and building contractors can include *Legionella* control when making decisions about pipe material usage. Certain plastic components (e.g., PEX) tend to lead to bacterial proliferation. Iron components in distribution systems and premise plumbing should be replaced or otherwise managed with appropriate corrosion control to avoid disinfectant decay and release of iron as a nutrient for *Legionella*. Because of conflicting accounts in the literature about their role in *Legionella* growth, copper pipes cannot be relied on as a barrier to *Legionella* colonization and growth. More research is needed to identify circumstances when copper's antimicrobial properties are enhanced.