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Water Stagnation and Flow Obstruction Reduces the Quality of Potable Water and Increases the Risk of Legionelloses

*Muhammad Atif Nisar, Kirstin E. Ross, Melissa H. Brown, Richard Bentham and Harriet Whiley**

College of Science and Engineering, Flinders University, Bedford Park, SA, Australia

Legionella is an opportunistic waterborne pathogen associated with Legionnaires' disease and Pontiac fever. Despite improved public awareness, the incidence of *Legionella* associated infections has been increasing. Aerosols generated from engineered potable water systems are a demonstrated cause of both nosocomial and community-acquired legionellosis. The ecology of *Legionella* in these systems is complex with multiple factors impacting their colonization and persistence. Flow dynamics has been identified as an important factor and stagnation in cooling towers is an accepted risk for increased *Legionella* growth; however, less is known about the impact of flow dynamic on *Legionella* in potable water systems. This is especially complex due to the inherent intermittent and variable usage observed within outlets of a potable water system. This systematic literature review examines the role of fluid dynamics and stagnation on the colonization and growth of *Legionella* in potable water systems. Twenty two of 24 identified studies show a positive association between stagnation zones and increased colonization of *Legionella*. These zones included dead legs, dead ends, storage tanks, and obstructed water flow (such as intermittent usage or flow restriction). Prolonged stagnation in building plumbing systems also deteriorates the quality of thermally or chemically treated potable water. This stimulates the colonization of *Legionella* established biofilms. Such biofilms are intrinsically resistant to disinfection procedures and accelerate the rate of decay of chemical disinfectants. Sub-lethal doses of disinfectants and the presence of protozoan hosts in stationary water promote generation of viable but non-culturable *Legionella* cells. This results in false negatives in surveillance methods that use culture methodology. In conclusion, elimination of temporal and permanent stagnation points can improve the quality of potable water, efficacy of disinfectants, and reduce the risk of legionellosis. Current guidelines and water safety plans recognize the risks associated with permanent stagnation point (dead ends and dead legs); however, there is a need for greater emphasis on controlling temporal stagnation arising from intermittent usage.

Keywords: Legionnaires' disease, legionellosis, building, plumbing, water stagnation, flow dynamics, dead legs, dead ends

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****Correspondence:***
Harriet Whiley
harriet.whiley@flinders.edu.au

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INTRODUCTION

The importance of *Legionella* as an opportunistic waterborne intracellular human pathogen is well-documented (Berjeaud et al., 2016). It is frequently associated with nosocomial and community-acquired legionellosis (Pontiac fever and Legionnaires' disease [LD]) in immunocompromised and immunosuppressed individuals (Fields et al., 2002). *Legionella* is ubiquitously present in constructed water systems. It is frequently detected in domestic and hospital water systems, cooling towers, humidifiers, fountains, and pools, (Bartram et al., 2007; Fitzhenry et al., 2017).

Despite recent advancements in disease surveillance and management systems, the legionellosis incidence and outbreak rate remains high. In the USA, there were 290 reported outbreaks of legionellosis from 2009 to 2017 (Centers for Disease Control Prevention, 2020) and in 2017, 28 outbreaks were reported in nine European Union countries. In Europe, the legionellosis notification rate also increased from 12 cases/million in 2013 to 18 cases/million in 2017 (European Centre for Disease Prevention and Control, 2019).

According to the USA Centers for Disease Control and Prevention, from 2013 to 2015 ∼67% potable waterborne outbreaks were caused by *Legionella pneumophila* (Shah et al., 2018). Plumbing networks and potable water distribution systems of hotels, healthcare facilities, and residential buildings were identified as *Legionella* hotspots (Benedict et al., 2017). Clearly, understanding the environmental factors that affect the survival and growth of *Legionella* in potable water is essential to inform improved management and control strategies.

The growth of *Legionella* in potable water systems is primarily associated with biofilms and the free-living amoeba hosts feeding on biofilms (Ashbolt, 2015). This provides *Legionella* protection from severe physiochemical stresses (Thomas et al., 2004; Dupuy et al., 2011). The environmental conditions that influence the formation of biofilms are essential for the control of *Legionella*. These include stagnation and the presence of plumbing dead legs and dead ends that provide favorable conditions for microbial growth (Bartram et al., 2007). Dead legs (isolated pipes with limited or no water flow) and dead ends (redundant capped pipe which completely obstruct water flow) are well-established as factors contributing to stagnation (National Academies of Sciences Engineering Medicine, 2019). Stagnation or areas with inappropriate hydraulic dynamics are likely to result in the failure of disinfection procedures (Ling et al., 2018). Due to the complex relationship between different environmental variables it can be challenging to study role of stagnation in *Legionella* growth. This systematic literature review examines the specific role that water stagnation plays in *Legionella* colonization and growth within potable water systems. Studies on potable water systems including surveillance and laboratory-based studies that examine the relationship between stagnation and fluid dynamics on water quality and *Legionella* colonization are explored.

MATERIALS AND METHODS

The protocol of this systematic literature review was designed according to the instructions of the PRISMA statement (**Figure 1**). The databases Scopus and Web of Science were searched for all articles written in English and published prior to April 2020. In the databases the search terms ("*Legionella pneumophila*" OR "*L. pneumophila*" OR *Legionella* OR legionellosis OR "Legionnaires' disease" OR "Pontiac fever") AND (flow* OR "dead end*" OR "dead leg*" OR "water circulation" OR "water recirculation" OR "Reynolds number" OR stagnation OR stagnant OR "stationary water" OR turbid OR turbidity OR usage*) AND (plumbing OR pipework OR pipe OR artificial OR piping OR building OR manufactured OR engineered OR potable OR manmade* OR biofilm OR water) were applied. Initially, all identified records were combined and duplicates removed. Subsequently, articles were manually screened by reading the titles and abstracts and excluding those that discussed engineering works, bacterial colonization or biofilms without specifically referring to *Legionella*. Papers were also excluded that referred to stagnation in municipal water supplies as this is a different issue compared with building water supplies. Finally, the remaining articles were analyzed in full and excluded if they did not examine or describe the relationship between *Legionella* colonization, growth, survival, and water stagnation, or recirculation in building water systems.

RESULTS

A total of 395 abstracts were obtained from the Web of Science and Scopus. After applying the described inclusion and exclusion criteria (as presented in **Figure 1**), 24 research articles describing a relationship between water stagnation and *Legionella* or *L. pneumophila* survival and colonization were identified as suitable for inclusion in this review (**Table 1**).

Study Sites

Seventeen of 24 studies (**Table 1**) investigated hot or cold water storage tanks and building water distribution systems; 7/24 studies were *in vitro* modeling experiments. The majority of the real-world studies were from investigations of hospital water systems (12/17) and almost half of these were conducted in response to a legionellosis outbreak (5/12). Half of the studies (14/24) discussed the relationship between *Legionella* contamination or colonization and permanent stagnation points (dead ends 6/24, dead legs 8/24). In the majority of studies, there was limited information provided describing fluid or water dynamics.

Building and Plumbing System Studies

According to **Table 1**, *L. pneumophila* serogroup (sg) 1 frequently contaminates water and plumbing system of hospitals and municipal buildings. *L. pneumophila* sg1 is the most common serogroup associated with legionellosis. Few studies reported contamination of other *L. pneumophila* serotypes (sg2–4, sg2–14, sg2–16, sg3, sg4, sg6, sg10, and sg10–14) and *Legionella* species

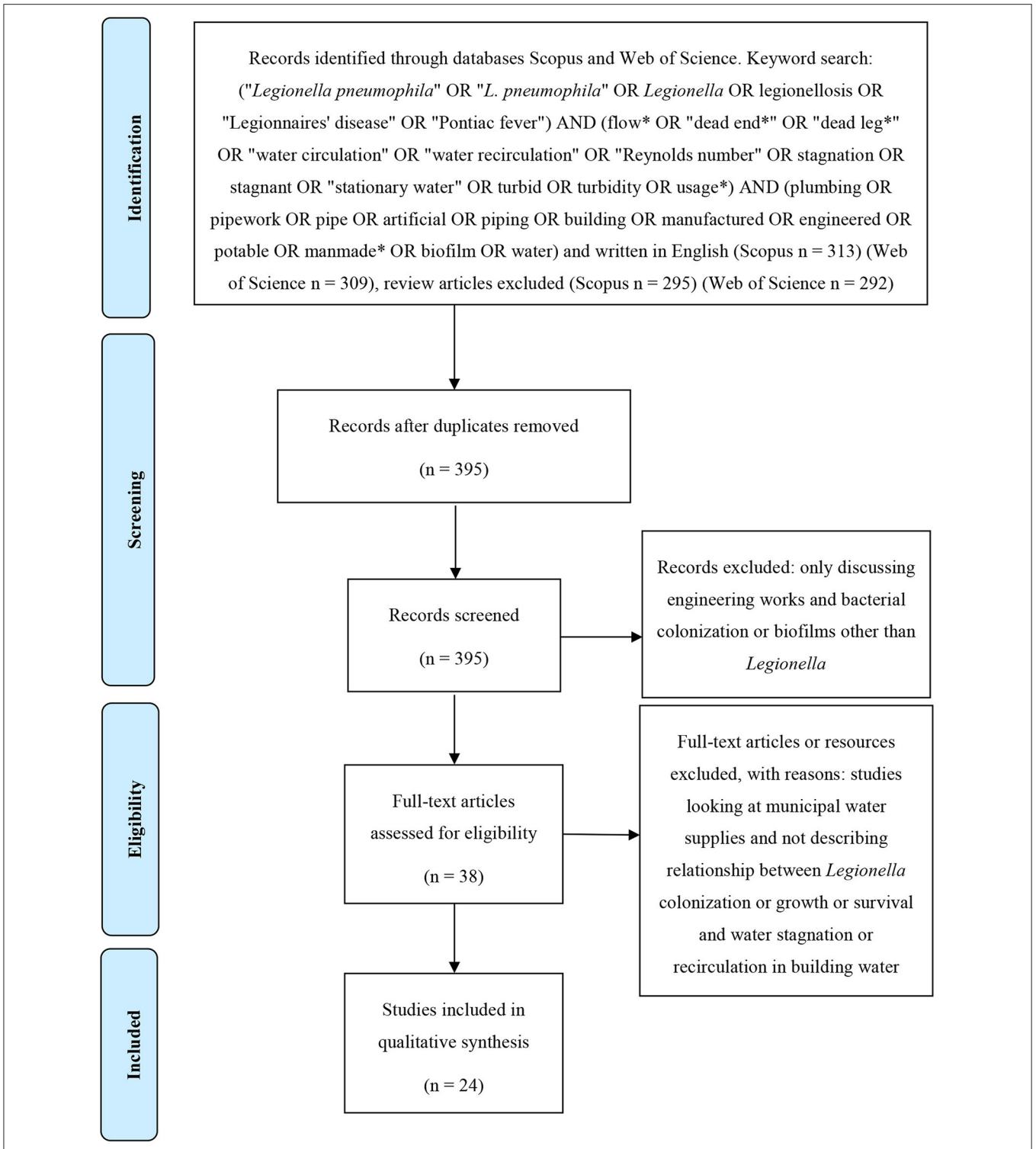

FIGURE 1 | Overview of the PRISMA strategy used for selection of articles.

(*L. anisa*, *L. steigerwaltii*, *L. feelii*, *L. longbeachae*, *L. micdadei*, and *L. rubrilucens*).

Sixteen out of 17 studies examining building plumbing systems showed a positive association between stagnation and increased *Legionella* colonization/persistence. Ten out of 17 studies of the building plumbing systems demonstrated that permanent stagnation points (dead ends 5/17, dead legs 5/17) were positively associated

TABLE 1 | Relationship between water stagnation and *Legionella* colonization.

Settings	Species/Serogroup/ Sequence type/ Type culture	Comments	Geographic location	References
Building and plumbing system studies Hospital hot water storage tanks (capacity 7,600 L each)	*L. pneumophila* sg1	Post nosocomial LD outbreak prevention measures adopted. Prevention of stagnation in hot water (45.5–47.5°C) tanks reduced *L. pneumophila* population.	Colorado, USA	Ciesielski et al., 1984
Residential and institutional cold and hot water distribution system	*L. pneumophila* sg1	Stagnation, low hydraulic flow and low chlorine concentration (0–0.7 mg/L) resulted in *L. pneumophila* contamination.	Ohio, USA	Voss et al., 1985
Hospital hot water storage tanks (capacity 10,000 L) and distribution system	*L. pneumophila* sg1 (0.7%), sg6 (87%), and sg10 (7%) *L. longbeachae* (4.3%) *L. micdadei* (1.3%)	Post nosocomial legionellosis infections in immunocompromised patients control measures adopted. Increase in flow rate, maintaining temperature at 60°C and elimination of dead ends reduced bacterial population in hot water.	Brussels, Belgium	Ezzeddine et al., 1989
Hospital hot and cold water storage distribution system	*L. pneumophila* sg1 *L. anisa* *L. steigerwaltii* *L. feelii*	Post fatal nosocomial LD outbreak prevention measures adopted. Removal of fire hydrant spurs (dead legs) connected to storage tanks reduced the bacterial contamination.	Glasgow, Scotland	Patterson et al., 1994
Hospital hot water storage (15,000 L) and distribution system	*L. pneumophila* sg1	10 year surveillance program designed to control nosocomial LD. Circulation of hot water (>55°C) identified as the best way to reduce risk of nosocomial LD.	Jönköping, Sweden	Darelid et al., 2002
Hospital water storage (capacity ≈ 19,68,000 L) and water (hot and cold) distribution system	*Legionella*	Removal of dead legs and plumbing system repair unable to produce any profound effect on *Legionella* control. Disinfection of water with ClO_2 (0.3–0.5 mg/L) best way to control *Legionella*.	Pennsylvania, USA	Sidari et al., 2004
Hospital water distribution system	*L. pneumophila* sg1	Probing of nosocomial legionellosis infections demonstrated that dead end pipe source of *Legionella*.	Jesenice, Slovenia	Tercelj-Zorman et al., 2004
Hotel, office, school, store, and assembly hall hot water distribution system	*L. pneumophila* sg1 and sg4 *L. anisa* Non-culturable *Legionella*	Hot water system of 40% buildings found contaminated with *Legionella*. Buildings with central hot water storage system (66.7%) showed higher prevalence of *Legionella* than buildings with central hot water circulation system (38.5%) or local instantaneous hot water production system (20%).	Osaka, Japan	Edagawa et al., 2008
Hospital hot water distribution system	*L. pneumophila* sg1 (18.8%), sg2–4 (68.3%), and other sg (12.9%)	2 year hyperchlorination, regular maintenance of boiler and storage tanks, replacement of showerheads and increase in boiler outlet temperature (60°C) were unable to eliminate *Legionella* contamination for longer period of time. Dead legs suspected as reason of recolonization of *Legionella* in hot water system. High temperature (50–60°C), ClO_2 (0.2–0.7 mg/L) disinfection and removal of dead legs reduced *Legionella* contamination.	Milan, Italy	Tesauro et al., 2010
Nursing home hot and cold water distribution system	*L. pneumophila* sg1 (ST23)	Probing of nosocomial legionellosis outbreak demonstrated that closed pipes and disturbance in water flow sites promoted bacterial contamination.	Ljubljana, Slovenia	Trop Skaza et al., 2012
Hospital hot water distribution system	*L. pneumophila*	By increasing flow rate (> 0.2 m/s) and maintaining 55°C temperature in tap resulted in 93.1–46.1% reduction of *L. pneumophila* population within 4 weeks in tap water.	Québec, Canada	Boppe et al., 2016

(Continued)

TABLE 1 | Continued

Settings	Species/Serogroup/ Sequence type/ Type culture	Comments	Geographic location	References
Residential building, nursing home, sports facilities, hotels, and kitchen water distribution system	*Legionella*	Comprehensive surveillance study demonstrated that temperature, pipe length measures, and stagnation are three parameters to predict *Legionella* contamination in drinking waters system. Stagnant hot water (45°C) can easily get contaminate with *Legionella* (predicted risk 77.2%).	Cologne, Germany	Volker et al., 2016
Hospital hot water distribution system	*L. pneumophila* sg3, sg2–4, sg6, and sg10–14	Installation of time flow taps (flow rate 64–192 L/day) in proximity of dead legs reduced the bacterial contamination in hot water system (temperature: 38.2 ± 2.1–46.8 ± 2.1°C and free chlorine: 0.05 ± 0.007–0.30 ± 0.01 mg/L).	North western Tuscany, Italy	Totaro et al., 2018
Hospital hot water distribution system	*L. pneumophila* sg2– 4 (predominant) and sg1	Dead legs and low-use taps identified as sites of *Legionella* colonization in hot water system. Maintaining temperature at 55°C and water recirculation managed bacterial colonization.	Catalonia, Spain	Gavalda et al., 2019
Hospital hot water distribution system	*L. pneumophila* sg1 (ST1 and ST104) *L. rubrilucens* *L. anisa*	WTP828 (water team process 828: 34% wt/wt H_2O_2 and 0.003% wt/wt Ag^+ salt) disinfectant efficiently reduced bacterial load. Efficacy of disinfectant increased by plumbing repairs i.e., removal of dead ends and management of water stagnation.	Cotignola, Italy	Girolamini et al., 2019
Hotel water distribution system	*L. pneumophila* sg1 (ST763)	Probing of LD infections demonstrated that potable water (temperature: 60.4–122.9°F, chlorine: 0.4–2 mg/L, bromine: 2–4 ppm) system was contaminated due to dead end and stagnation.	Missouri, USA	Ahmed et al., 2019
Hospital hot water distribution system	*L. pneumophila* sg1, sg2–14, and sg2–16	Installation of time flow taps (flow rate: 192 L/day) in correspondence to dead ends, proper hot water recirculation (44.8–53.2°C) and chlorination (0.23–0.36 mg/L) effectively reduced the bacterial population within 24 h−15 days.	Pisa, Italy	Totaro et al., 2020
***In vitro* model plumbing studies**				
Pilot scale domestic water system of loops and dead legs	*L. pneumophila* sg1	Continuous flow of ClO_2 (0.5 mg/L) and chlorine (2.5 mg/L) treated water reduces the bacterial population in loops, whereas continuous flow (7 days) of ClO_2 treated water is only effective way to reduce bacterial population in dead legs. Copper dead legs possess intrinsic antibacterial property and inhibits proper colonization and growth of *Legionella*.	France	Thomas et al., 2004
Pilot scale domestic water distribution model	*Legionella*	Regular 20% renewal of dead legs water unable to produce any impact on *Legionella* population. However, regular complete replacement of dead legs water eliminates culturable *Legionella* within 7 days.	France	Loret et al., 2005
Stagnant water model	*L. pneumophila* sg1 (ATCC 33152)	Heat treated (60°C) stagnant tap water in dead ends promotes growth of *L. pneumophila* in biofilms. Moreover, it also increases the diversity of eukaryotic microbes in the biofilm.	Belgium	Vervaeren et al., 2006
Plumbing model of three parallel pipe	*L. pneumophila* sg1 and sg6	Bacteria survived and proliferated in turbulent flowing (Re: 10,000–40,000), laminar flowing (Re: 355–2000) and stagnant water. Stagnation did not promote *Legionella* colonization.	USA	Liu et al., 2006
Pilot scale 1 stainless steel hot water system (volume: 316 L)	*L. pneumophila* *Legionella*	Dead legs (stagnant point) water harbored 1–2 log higher concentration of bacteria than loops (flow rate 15 L/min)	France	Farhat et al., 2010

(Continued)

TABLE 1 | Continued

Settings	Species/Serogroup/ Sequence type/ Type culture	Comments	Geographic location	References
Dual pipe loop system (50 L volume)	*Legionella*	Biofilm of *Legionella* and *Naegleria fowleri* ATCC 30894 developed in stagnant water (dead-end, reservoirs, and hydrant source).	USA	Biyela et al., 2012
Pilot scale household hot water system (volume: 71.9 L)	*L. pneumophila*	*L. pneumophila* population persisted at a high density in stagnant/low frequency usage taps than dynamic water. Hot stagnant water (temperature 51°C) supported selection of *L. pneumophila* (125 times more) than dynamic hot water.	USA	Rhoads et al., 2015

LD, Legionnaires' disease; ClO_2, Chlorine dioxide gas; wt/wt, weight/weight; Re, Reynolds number.

with increased *Legionella* contamination. Three studies presented in **Table 1** specifically demonstrated that restricted water circulation resulted in persistence of *Legionella* in hospital hot water storage tanks with capacities of ≈7,600–15,000 L (Ciesielski et al., 1984; Ezzeddine et al., 1989; Darelid et al., 2002).

Ten building water system studies reported a reduction in *Legionella* numbers after increasing water flow rates and removal of dead legs or dead ends. Some studies specifically used hot water recirculation (Darelid et al., 2002; Gavalda et al., 2019) or an increase in the flow rate of hot water (Ezzeddine et al., 1989; Boppe et al., 2016) to reduce *Legionella* contamination. In Italy (2018–2020), installation of time flow taps with 64–192 L/day flow rate in the vicinity of dead legs and dead ends effectively reduced *L. pneumophila* contamination from hot water supplies of hospitals (Totaro et al., 2018, 2020).

The one study that did not find any positive association between stagnation and *Legionella* growth was by Sidari et al. (2004). This study examined water storage and distribution in a 437 bed hospital in Pennsylvania after numerous cases of nosocomial LD over a 5 year period (1994–1999). Flushing of hot water temporarily reduced the concentration of *Legionella*; however, this study found that removal of dead legs and routine chlorination was not sufficient to reduce *Legionella* contamination. Finally, ClO_2 (0.3–0.5 mg/L) treatment for 21 months resulted in complete removal of *Legionella* (culture negative).

In vitro Model Plumbing Studies

As with the real-world studies, in 6/7 *in vitro* model plumbing studies a positive relationship between water stagnation and *Legionella* colonization was identified (**Table 1**). Several model systems demonstrated that stagnation promoted genesis of stable *Legionella*–eukaryotic microbe biofilms (Vervaeren et al., 2006; Biyela et al., 2012). Farhat et al. (2010) constructed a pilot scale hot water system consisting of both dynamic water loops (flow rate: 15 L/min) and dead leg stagnation areas. They estimated the *Legionella* concentration (using culture, qPCR, and epifluorescent microscopic counts) was 1–2 log greater in stagnant points. Two studies also investigated the role of ClO_2 treated water in elimination of *Legionella* contamination from dead legs (Thomas et al., 2004; Loret et al., 2005). It was found that 20% replacement of dead leg water with ClO_2 treated water was not sufficient to completely eliminate culturable *Legionella*. However, regular complete replacement of ClO_2 treated water in dead legs was able to eliminate culturable *Legionella* within 7 days (Loret et al., 2005). Another pilot scale domestic water model consisting of both dynamic and stagnant water channels demonstrated that continuous flow of ClO_2 treated water for 7 days significantly reduced *L. pneumophila* from copper dead legs (Thomas et al., 2004).

The one *in situ* model study that did not find any positive association with stagnation and *Legionella* colonization was conducted by Liu et al. (2006). This model consisted of three parallel pipes within a partially open system (5% of water continuously flowed through the system while 95% of the water was recirculated). The water temperature was maintained at 24°C, one pipe had laminar flow (Re: 355–2,000), one turbulent flow (Re: 10,000–40,000), and one was stagnant. Significantly higher concentrations of culturable *Legionella* were recovered from the biofilm in the pipe with turbulent flow followed by laminar flow with the lowest *Legionella* concentrations observed in the biofilm in the pipe with stagnant flow. However, a significant difference in *Legionella* concentrations in biofilm was observed after 1 week. These concentrations remained static for the remaining 5 weeks of sampling. The authors attributed this result to the turbulent flow increasing the mass transfer of the nutrients and oxygen from the water in the seeding tank. They also noted that the turbulence created may have been insufficient to detach the biofilm. There was no significant difference in planktonic *Legionella* between any of the treatments. This study concluded that intermittently used pipe work (dead legs) sustained aerobic microbial populations than dead ends (where nutrient depletion is more likely).

DISCUSSION

Legionella is a typical premise plumbing pathogen that can tolerate disinfectants, develop biofilms, survive in waterborne protozoa, and thrive in low levels of nutrients (Fields et al., 2002). Prolonged water stagnation can result in accumulation of nutrients and compromises disinfection, promoting colonization of premise plumbing pathogens in potable water systems

(Gauthier et al., 1999; Rhoads et al., 2016; Ling et al., 2018; Xu et al., 2018).

Influence of Water Stagnation on Bacterial Colonization

Microbial ecology of potable water is very complex. From water source to point of use, the varying environmental conditions modulate growth and composition of microbial communities. In building plumbing systems, water can stagnate permanently or temporarily (Prévost et al., 1997). Dead ends permanently stagnate water (Tercelj-Zorman et al., 2004); whereas, water storage tanks and temporal water usage can result in intermittent stagnation (Peter and Routledge, 2018). Prior to consumption, water can stagnate from a few hours to weeks in a building piping system (Manuel et al., 2009). This may result in the deterioration of water quality, and promote accumulation of biomass thereby increasing the concentration of intact and cultivable cells.

Temporary Stagnation

Water in municipal distribution pipelines rarely gets obstructed. As it enters domestic building plumbing systems it stagnates and changes the diversity of microbial communities (Pepper et al., 2004). It has been observed that overnight stagnation resulted in a 4 to 580-fold increase in microbial load (using heterotrophic plate counts), a 2 to 3-fold increase in total cell concentration (using flow cytometry), and a 2 to 8-fold increase in microbial activity (ATP analysis) (Lautenschlager et al., 2010). This was supported by a study which showed that a 2 week stagnation of a domestic buildings plumbing system resulted in a 2 log increase in microbial load (using flow cytometry) (Lipphaus et al., 2014).

Building design also impacts on stagnation and microbial water quality. A recent study demonstrated that water from the plumbing system of a net-zero energy residential building contained 5 log higher amount of bacterial 16S rDNA (6.46×10^7 gene unit/mL) and *Legionella* 16S rDNA (8.91×10^4 gene copies/mL) compared with a typical residential building plumbing system (400 gene unit/mL and 100–2.3×10^3 gene copies/mL, respectively). This variation, due to building structure and design, could be attributed to increased stagnation prior to consumption as the average stagnation time for a net-zero energy residential building is 2.7 days compared with 1 day in a typical residential building (Rhoads et al., 2016). *In vitro* green building hot water plumbing model studies also suggested limited water flushing and inadequate temperature (51°C) support colonization of *Legionella* and host protozoa (Rhoads et al., 2015). In conclusion, stagnation and aging of water in plumbing system of green energy buildings resulted in persistence of microbial contamination.

Permanent Stagnation

Dead legs and dead ends have been implicated in several nosocomial outbreaks of LD (Patterson et al., 1994; Tercelj-Zorman et al., 2004; Bartley et al., 2016). These permanent stagnation points should be avoided or managed during building construction or modifications (Bartram et al., 2007). However, it is impractical to remove them all. Recently, two studies of Italian hospitals demonstrated that installation of time flow taps (flow rate 64–192 L/day) in the vicinity of dead legs was successful in reducing *L. pneumophila* contamination in the hot water system (Totaro et al., 2018, 2020). In the first study, *L. pneumophila* from various serogroups including sg3, sg2–4, sg6, and sg10–14, with concentrations ranging from 1×10^2 to 1.3×10^5 CFU/L, was present in hospital hot water (temperature: 38.2 ± 2.1–46.8 ± 2.1°C and free chlorine: 0.05 ± 0.007–0.30 ± 0.01 mg/L). Instead of removing dead legs, time flow taps were installed in the outlet closest to each identified dead leg. This strategy successfully eliminated all culturable *L. pneumophila* (Totaro et al., 2018). In the second study, it was found that within 15 days of installation of time flow taps (flow rates 192 L/day) in the proximity of dead ends, *L. pneumophila* sg1, sg2–14, and sg2–16 contamination in hospital hot water (temperature: 44.8–53.2°C and free chlorine: 0.23–0.36 mg/L) was reduced from 2×10^2 to 1.4×10^5 CFU/L to no culturability (Totaro et al., 2020). These studies demonstrate that reducing temporary stagnation and increasing flow, even in the presence of permanent stagnation points, reduces the risk of *Legionella* contamination.

Several model studies have explored the impact of permanent stagnation on *Legionella* colonization. In a pilot scale model, bacterial biofilms in dead legs (pre-treatment population density 10^7 CFU/L and 10^8 genomic unit/L) survived thermal shock treatment and promoted rapid recolonization within 48 h (Farhat et al., 2010). Thus, permanent stagnation sites act as a reservoir of *Legionella* biofilms that play an important role in re-contamination of water.

Water Stagnation and Failure of Disinfection Procedures

The accelerated decay of residual disinfectant significantly increases the risk of LD (Voss et al., 1985). Prolonged water stagnation, microbial communities, and organic nutrients accelerate the decay of disinfectants (Rhoads et al., 2016; Ling et al., 2018; Xu et al., 2018). According to the US CDC, from 2000 to 2014 70% of LD outbreaks were due to inadequate disinfectant concentrations in water supplies (Garrison et al., 2016). Currently in the USA, six disinfection procedures: Cu–Ag ionization, chlorine, chlorine dioxide, monochloramine, ozonisation, and ultraviolet disinfection, are used to control *Legionella* (Environmental Protection Agency, 2016b). In the USA, the concentration of chemical disinfectant is maintained in 95% potable water delivered to consumers (Environmental Protection Agency, 2016a). In studies from Austria, Germany, Netherlands, and Switzerland, the concentration of residual disinfectant in potable water (delivered to consumers) is not maintained (Rosario-Ortiz et al., 2016).

Decay of Disinfectant

Different chemical disinfectants are widely used to disinfectant potable water supplies (Pontius, 1990; Kim et al., 2002; World Health Organization, 2004). The residual disinfectant concentration in treated water does not remain constant and it gradually decreases within building plumbing systems. This continuous process of decay may result in complete degradation of chemical disinfectants, thereby increasing the chances of persistence of microbial contamination in building plumbing

systems (Vieira et al., 2004). Rates of decay have been associated with temporary or permanent stagnation, aging of plumbing systems, nature of plumbing material, and microbial biomass (Vieira et al., 2004; Patrick et al., 2012; Ling et al., 2018). Goyal and Patel (2015) reported continuous temporal stagnation in storage tanks for 22 h decreased residual chlorine concentration (from 0.2 to ∼0.12 mg/L) in domestic buildings. Similarly, Barbeau et al. (2005) reported that 24 h of temporary stagnation decreased chlorine content of water (from 0.6 to ∼0.3 mg/L in cement line ductile and 0.4–0.05 mg/L in gray cast iron pipe dead end) and increased the microbial count. Galvin (2011) demonstrated that the lower flow velocity observed in dead ends can result in a decrease in concentration of both chlorine and chloramine within 200 h. Laboratory model based experiments have also demonstrated that an increase in residence time of water results in decreased concentrations of chlorine, chloramine, and ozone and increased microbial contamination (Clark et al., 1994). Another model system showed that complete renewal of ClO_2 (0.5 mg/L) disinfected water in dead legs eliminated culturable *Legionella* (Loret et al., 2005).

Persistence of Intrinsically Disinfectant Resistant *Legionella*

Intrinsic resistance is a natural tolerance and resistance attributed to *Legionella* against physical and chemical water treatments (Steinert et al., 1998; Cooper and Hanlon, 2010). Biofilm in areas of stagnation within plumbing systems may harbor resistant populations and act as a continuous source of microbial contamination (Bartley et al., 2016). Studies have shown that currently available water disinfection methods (i.e., chlorine, chlorine dioxide, chloramines, hydrogen peroxide, ozone, copper, and silver ions) are only successful in reducing or eliminating *Legionella* populations transiently. Sidari et al. (2004) and Totaro et al. (2018) (see **Table 1**) demonstrated the inability of chlorine to eliminate all culturable *Legionella*. According to Totaro et al. (2018), circulation of hot chlorinated water in dead legs eliminated all chlorine sensitive culturable *L. pneumophila* sg2–4 and sg10–14 serotypes, though chlorine resistant and thermostable *L. pneumophila* sg3 and sg6 serotypes persisted in low concentrations.

A study conducted in a century old hospital in Italy found that continuous hyperchlorination (0.5–1 mg/L) for 5 years was not sufficient to completely eliminate *Legionella* contamination. Multiple factors including outdated piping, dead legs, improper water circulation and corrosion of plumbing materials were proposed as causes of *Legionella* persistence (Orsi et al., 2014). Similarly, a study conducted in another Italian hospital plumbing system demonstrated persistence of the same strain of *L. pneumophila* for a period of 15 years (1990–2004) despite thermal treatment, chlorination, and chlorine dioxide treatment (Scaturro et al., 2007). This demonstrates the role permanent stagnation (dead ends and dead legs) has in harboring and potentially selecting for more resistant strains through constant exposure to sub-lethal concentration of disinfectants (Cooper and Hanlon, 2010; Dupuy et al., 2011).

Stagnation and Microbial Biofilms

In water storage and distribution systems, 95% of the microbial population exists as biofilms attached to the inner surfaces and only 5% in the water (Flemming et al., 2002). According to an *in vitro* simulation experiment, plumbing coated with *Legionella* biofilms decayed free chlorine in stagnant water (48 h stagnation period) and increased the risk of legionellosis up to six times (Huang et al., 2020). Available literature shows that in potable water and building plumbing systems complex biofilms and amoebae hosts protect *Legionella* (Kilvington and Price, 1990; Thomas et al., 2004). Cargill et al. (1992) reported that in contrast to free living cells, *L. pneumophila* existing in complex biofilms can tolerate high doses of disinfectant. Specifically, the amoeba *Acanthamoeba* and *Vermamoeba* provide additional protection from prolonged and persistent water treatment processes (Kilvington and Price, 1990; Dupuy et al., 2011; Cervero-Arago et al., 2014; Dobrowsky et al., 2016). Donlan et al. (2005) observed that for *L. pneumophila*–amoebae complex biofilms, monochloramine (0.5 mg/L) was a more effective disinfectant compared to free chlorine (0.5 mg/L). Stagnant potable water allows formation of thick, dense, complex, and adherent biofilms, which accelerate decay of disinfectants (Tsagkari and Sloan, 2018). More importantly, chemical disinfectants are unable to penetrate such multispecies biofilms (Bridier et al., 2011).

Stagnation in Building Hot Water System

The studies presented in **Table 1** demonstrate that due to stagnation points and low water consumption, building hot water systems will develop *Legionella* contamination. Unlike cold water, hot water systems contained diverse species and serotypes of *Legionella* (Voss et al., 1985; Trop Skaza et al., 2012; Totaro et al., 2018; Girolamini et al., 2019). An *in vitro* study demonstrated colonization of diverse species of *Legionella* i.e., *L. pneumophila*, *L. anisa*, *L. taurinensis,* and *L. drancourtii*, and protozoan hosts i.e., alveolata members, *Bodonidae*, *Euglenozoa*, *Neobodo curvifilu*, *Thecamoebae*, *Vannella*, and *Vermamoeba vermiformis* in a hot water plumbing model. Moreover, it was also noticed that pathogenic *L. pneumophila* and *L. anisa* developed stable biofilms with protozoa (*Thecamoebae*, *Vannella*, and *V. vermiformis*) in storage tanks and survived during thermal (70°C/30 min) and chemical (biodispersant: tensio-active Ferrofos® 5260 and biocide: H_2O_2-peracetic acid) treatments. After such treatments, these stable biofilms re-contaminated hot water within the entire plumbing model (Farhat et al., 2012). Saby et al. (2005) analyzed hot water plumbing system models (materials: steel, galvanized steel, and chlorinated polyvinyl chloride) and concluded that chemical disinfection procedures (H_2O_2, continuous chlorination, hyperchlorination, and peracetic acid treatment) temporarily eradicated established *Legionella* biofilm. The only solution to eradicate *Legionella* biofilms was to maintain water temperature at >55°C at all points, which required continuous circulation of hot water. In another pilot study (material: stainless steel), it was noticed that thermal treatment (70°C/30 min) of the plumbing system containing a well-established *Legionella* (10^3 CFU/cm^2) biofilm decreased culturable *Legionella*. It was also observed that existence of dead legs in plumbing system promoted rapid recontamination of

remaining water (Farhat et al., 2010). All studies discussing hot water supplies to buildings showed that stagnation of hot water in storage tanks and dead legs or dead ends led to a reduction in water temperature (<45°C), and rapid decay of disinfectants promoting colonization of *Legionella*. This was exacerbated in warm water systems as dissipation of the residual chemical disinfectant was further accelerated at high temperature (Vasconcelos et al., 1997; Ndiongue et al., 2005).

Nutrient and Oxygen Supply Hypothesis

One argument presented by Liu et al. (2006) that counters the positive association of stagnation with *Legionella* contamination of biofilms is the reduced oxygen and nutrient content present in areas of stagnation compared with areas of turbulent flow. However, *L. pneumophila* sg1 has been shown to survive in drinking water under low nutrient availability for more than 2 years (Paszko-Kolva et al., 1992). *L. pneumophila* can also proliferate efficiently at 6–6.7 mg/L concentration of dissolved oxygen. However, at oxygen concentration <2.2 mg/L it can survive but stops multiplying (Wadowsky et al., 1985). *L. pneumophila* (sg1, sg2, sg3, sg4, and sg6) has also been isolated from different water bodies (i.e., lakes and rivers) with dissolved oxygen concentrations of 0.3–9.6 mg/L (Fliermans et al., 1981). In water at temperatures of 20–45°C, the concentration of dissolved oxygen ranges from 9.06 to 5.94 mg/L (Environmental Protection Agency, 1989), which is sufficient for *Legionella* survival and growth. Furthermore, upon environmental stresses (nutrient starvation, low oxygen, osmolarity alterations, pH, and temperature fluctuations) bacteria in biofilms activate stress tolerance mechanisms, which can lead to genesis of viable but non-culturable (VBNC) cells (Yamamoto et al., 1996; Fux et al., 2005). Previous research has also shown replication within protozoan hosts (Buse et al., 2013) and presence of disinfectants (Allegra et al., 2008; Turetgen, 2008; Mustapha et al., 2015; Whiley et al., 2017; Casini et al., 2018) promotes *Legionella* transformation into VBNC cells. As such, studies that rely solely on culture methods of detection [such as the study by Liu et al. (2006)] will not detect VBNC *Legionella* and underestimate the numbers present (Whiley, 2016).

Current Methods of *Legionella* Screening

In stagnation areas, limited nutrient availability and sub-lethal doses of disinfectant promote generation of VBNC *Legionella* (Li et al., 2014). According to Farhat et al. (2010)

TABLE 2 | Methods available for screening and detection of *Legionella* contamination from potable water and building plumbing system.

Techniques	Description	Limitations	References
Microbiological culturing and isolation	Gold standard to detect and identify *Legionella* contamination. Processed water sample/biomass is cultured on buffered charcoal yeast extract (BCYE) agar. Isolated bacterial colonies are further identified by serological/molecular assays. Its detection limit is 35 CFU/L.	Two weeks required to grow *Legionella* from potable water samples. Only detects culturable *Legionella* and is unable to screen any VBNC *Legionella*.	Volker et al., 2016; International Organization for Standardization, 2017; Standards Australia, 2017
Legionella–amoebae co–culture assay	Most appropriate method to identify VBNC *Legionella*. Processed samples are inoculated on amoebae (i.e., *Acanthamoeba*) culture plate. Then plates are regularly examined microscopically to identify any cytological and morphological modifications in amoebae cells.	Longer periods of incubation required to resuscitate VBNC *Legionella*. Difficult to quantify density of VBNC *Legionella*.	Garcia et al., 2007; Conza et al., 2013; Epalle et al., 2015
Fluorescent *in situ* hybridization	It is a whole cell-based screening method. Nucleic acid (16S rRNA) or antibody-based probes are used for visual detection of cells. It can be modified to detect and estimate VBNC *Legionella*.	Probes can interact with 16S rRNA of dead cells. Probes can cross react with background environmental bacteria.	Declerck et al., 2003; Delgado-Viscogliosi et al., 2005; Kirschner et al., 2012
Flow cytometry	It is a membrane integrity-based assay to identify VBNC *Legionella*. In this method differential live/dead stains (SYBR green/propidium iodide, Syto9/propidium iodide and thiazole orange/propidium iodide) and/or labeled probes are used to characterize VBNC and dead cells of *Legionella*. Its detection limit is 45–150 cells/L.	Only detects specific *Legionella* species/strains/serogroups/serotypes, so effective for controlled *in vitro* studies. Universal probes which cover entire *Legionella* complex are not available.	Allegra et al., 2008; Füchslin et al., 2010; Keserue et al., 2012
PCR detection and enumeration	Rapid and efficient method to detect and quantify *Legionella* contamination. Processed water sample/biomass is subject to 16S rDNA (*Legionella*) and *mip* (*L. pneumophila*) genes qPCR assay. Its detection limit is 500 GU/L.	Only detects DNA of *Legionella* and unable to differentiate culturable, VBNC and dead cells of *Legionella*.	Wellinghausen et al., 2001; International Organization for Standardization, 2019
Viability–qPCR	In this method, prior to nucleic acid extraction and qPCR the sample is processed with cell membrane impermeable nucleic acid intercalating dyes (ethidium monoazide or propidium monoazide). It is a good method to detect and quantify VBNC *Legionella*.	Presence of background bacteria in high density (common in environmental samples) challenges validity. Not suitable for *Legionella* quantification from biofilm samples.	Delgado-Viscogliosi et al., 2009; Ditommaso et al., 2014, 2015; Taylor et al., 2014

CFU/L, colony–forming unit/liter; GU/L, genomic unit/liter.

thermal treatment of water decreased the numbers of culturable *Legionella* temporarily, but increased concentration of VBNC cells. A 1 year pilot study demonstrated that chemical disinfection (Ferrocid® 8591, Ferrofos® 5260, H_2O_2 and peracetic acid) decreased culturable *Legionella* and *L. pneumophila* from 0.5 to 2 log, however application of a PCR analysis showed existence of VBNC in high density (Farhat et al., 2011). As mentioned earlier, at stagnation points *Legionella* also exists in complex biofilms, and amoebae hosts dwell in such microbial communities as well. It is well-known that amoebae hosts (*V. vermiformis*) are capable to transform culturable *Legionella* into VBNC (Buse et al., 2013). VBNC are pathogenic in nature and infect amoebae and human cell lines (Cervero-Arago et al., 2019). Proteomic profiling suggests VBNC are able to synthesize some virulence factors and proteins involved in different metabolic pathways (Alleron et al., 2013). *Acanthamoeba castellanii* and *A. polyphaga* resuscitate VBNC synthesized by starvation (Steinert et al., 1997) and disinfectant treatment (Garcia et al., 2007), respectively. *In vitro* studies showed *A. polyphaga* resuscitated *Legionella* are infectious for alveolar epithelial and macrophage like cells (Epalle et al., 2015). So far the underlying mechanisms of VBNC biogenesis and resuscitation are not yet well-understood.

Multiple methods are available to screen and detect *Legionella* contamination in potable water and building plumbing systems (**Table 2**). Any method that can identify and estimate both culturable and VBNC *Legionella* is most appropriate to monitor building plumbing system and potable water. Microbiological culturing (International Organization for Standardization, 2017) and qPCR (International Organization for Standardization, 2019) are approaches recommended by ISO (international organization for standardization), however both methods are unable to provide information about VBNC *Legionella*. Fluorescence *in situ* hybridization (Delgado-Viscogliosi et al., 2005) and viability qPCR (Ditommaso et al., 2014) are two techniques that can be used to estimate populations of VBNC *Legionella*, however validity of both assays is challenged by high density of sample background bacteria other than *Legionella*. Differential live/dead stain flow cytometry is widely used for *in vitro* disinfectant efficacy and plumbing model experiments to estimate the population of VBNC *Legionella* (Allegra et al., 2008, 2011; Wang et al., 2010; Mustapha et al., 2015). Some researchers have tried to develop and use dye labeled *Legionella* specific antibodies for detection of VBNC, however these antibodies are highly specific and can only detect specific strain/serogroup/serotypes (Füchslin et al., 2010; Keserue et al., 2012). A universal probe, which can cover all members of genus *Legionella*, is required to be effective to detect and estimate VBNC contamination from building plumbing systems. *Legionella*–amoebae co–culturing is one of the best techniques to identify VBNC contamination. In this assay, suspected samples are cultivated on amoebae hosts (preferably *Acanthamoeba*) and VBNC resuscitation is monitored microscopically (Conza et al., 2013). Garcia et al. (2007) artificially generated VBNC and then resuscitated them back into culturable *Legionella* using an *Acanthamoeba* host. Using an *Acanthamoeba* co–culture assay, Conza et al. (2013) estimated the quantity of VBNC *Legionella* (10^2-10^6 cells/g) from a composting facility. A major drawback of *Legionella*–amoebae co–culturing is the requirement of incubation for prolonged time. However, to our knowledge available literature has not discussed the application of co–culture assays to estimate VBNC contamination from building plumbing system.

CONCLUSION

Restricted water circulation and temporary or permanent water stagnation allows *Legionella* to colonize in building plumbing systems and water storage facilities. Aging of water, stagnation, and microbial biofilms accelerate decay of residual disinfectants and deteriorates water quality in buildings. Further research is required to better understand role of complex *Legionella*–protozoa biofilms in degradation of disinfectant in stationary water. To achieve long term disinfection of potable water continuous circulation of thermally or chemically treated water in buildings is the only solution to prevent outbreaks of legionellosis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MN and HW drafted and edited the manuscript, HW, KR, MB, and RB corrected and contributed to the manuscript. All authors approved of the final manuscript.

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