Brief Report

Impact of building closures during the COVID-19 pandemic on *Legionella* infection risks

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ABSTRACT

Prolonged building closures are prevalent during the COVID-19 pandemic, resulting in extreme stagnation in building water systems. High-throughput sequencing analysis revealed significantly increased presence of *Legionella* due to extreme water stagnation, highlighting elevated exposure risks to *Legionella* from building water systems during re-opening of previously closed buildings.
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Building closures, extending from weeks to months, have been common measures to reduce the spread of COVID-19. Prolonged closures inevitably lead to extreme stagnation in building water systems (BWS), which occurs when water stops flowing for extended periods, particularly in multistory buildings with complex plumbing. Water stagnation has been implicated for increased colonization of BWS by *Legionella*,[1] which is the pathogen causing Legionnaires' disease and Pontiac fever. Surveillance data in the U.S. shows that the large majority of *Legionella* infections are single, sporadic cases,[2] suggesting the broad occurrence of *Legionella* in BWS. The primary route of exposure to *Legionella* has been identified as inhalation of aerosols generated from *Legionella*-contaminated BWS in built environments including homes, hotels, schools, and hospitals.[3]

With the re-opening of buildings previously closed due to COVID-19, the risk of *Legionella* infection from extreme water stagnation has become a major concern. However, no information is available on *Legionella* contamination in BWS with extreme stagnation, which was rarely encountered until the COVID-19 pandemic. Therefore, with the aim of this study to determine the impact of extreme water stagnation due to building closures on *Legionella* contamination, microbial contaminants in the BWS of university dormitory buildings

following a 2-month shutdown were investigated. This study represents the first report on the impact of extreme water stagnation on the occurrence of *Legionella* in BWS.

METHODS

Cold tap water samples with 2-month stagnation, overnight stagnation, or no stagnation were collected from ten BWS during the COVID-19 shutdown in 2020 (June-August) in on-campus multistory dormitory buildings with complex indoor plumbing. Two-month stagnant water samples were collected as the first 4 liters of water from the tap without flushing following a 2-month shutdown when the dormitories were uninhabited and water usage ceased. Water samples with overnight stagnation or no stagnation were collected 2 weeks after the dormitories were re-opened. Samples with overnight stagnation were collected as the first 4 liters of water from the tap before 6 AM in the morning. Subsequently, the taps were flushed at maximum flow for 10 min before samples with no stagnation were collected. Heterotrophic plate counts (HPC) were used as a measure of the overall level of microbial contamination in the water samples and quantified as colony forming units (CFUs).[4] Legionellae were identified by cultivation-independent high-throughput sequencing of bacterial 16S rRNA genes, which is more advantageous than techniques relying on cultivation and qPCR. Cultivation has long been recognized with the inability to enumerate all legionellae populations.[3] qPCR techniques, however, face the uncertainties in covering the growing diversity of *Legionella* reported in recent literature, particularly in environmental samples.[2] For high-throughput sequencing, bacterial cells were collected by membrane filtration followed by

DNA extraction, PCR amplification, and paired-end sequencing with the NovaSeq platform (Illumina, San Diego, CA, USA).[4] Sequencing reads were processed with QIIME2 version 2020.6 with the DADA2 pipeline.[5] Assignment of taxonomic ranks to sequences was performed with reference to the Silva database (version 132.8).[6] Raw sequencing reads were deposited at the NCBI Sequence Read Archive database with accession numbers SAMN19656238-SAMN19656281.

RESULTS

To determine the impact of extreme stagnation on overall microbial contamination of BWS, HPC results were compared between water samples with 2-month stagnation, overnight stagnation, and no stagnation. It is evident that bacterial contamination deteriorated significantly with water stagnation as HPC increased dramatically with the extension of the stagnation period (Fig 1). Fresh tap water with no stagnation exhibited the lowest level of microbial contamination, with the HPC averaging 6 CFU/L. With overnight stagnation, microbial contamination increased significantly to an average HPC of 512 CFU/L. With the stagnation period extended to 2 months,

microbial contamination measured by HPC increased further to an average of 2.6×10^5 CFU/L, demonstrating the significant impact of water stagnation on microbial contamination in BWS.

Alarmingly, increases in overall microbial contamination were accompanied by the proliferation of *Legionella*. Analysis of the microbial contaminants profiled by 16S rRNA gene-based sequencing revealed that *Legionella* spp. were present in all water samples, regardless of the occurrence of water stagnation or not (Fig 2). Nevertheless, fresh tap water with no stagnation exhibited lowest levels of *Legionella*, with an average relative abundance of 0.03% in all bacterial populations present in the water, which increased significantly to 0.15% after overnight stagnation (Fig 2). When the period of stagnation increased to 2 months, the relative abundance of *Legionella* underwent another significant increase to 0.34%. It should be noted that increases in the relative abundance of *Legionella* with water stagnation indicate more rapid growth of *Legionella* as compared with other bacterial populations in water. Given that the overall level of bacterial contamination in BWS measured as HPC rose significantly with water stagnation, it could be reasoned that increases in *Legionella* contamination were even greater during water stagnation.
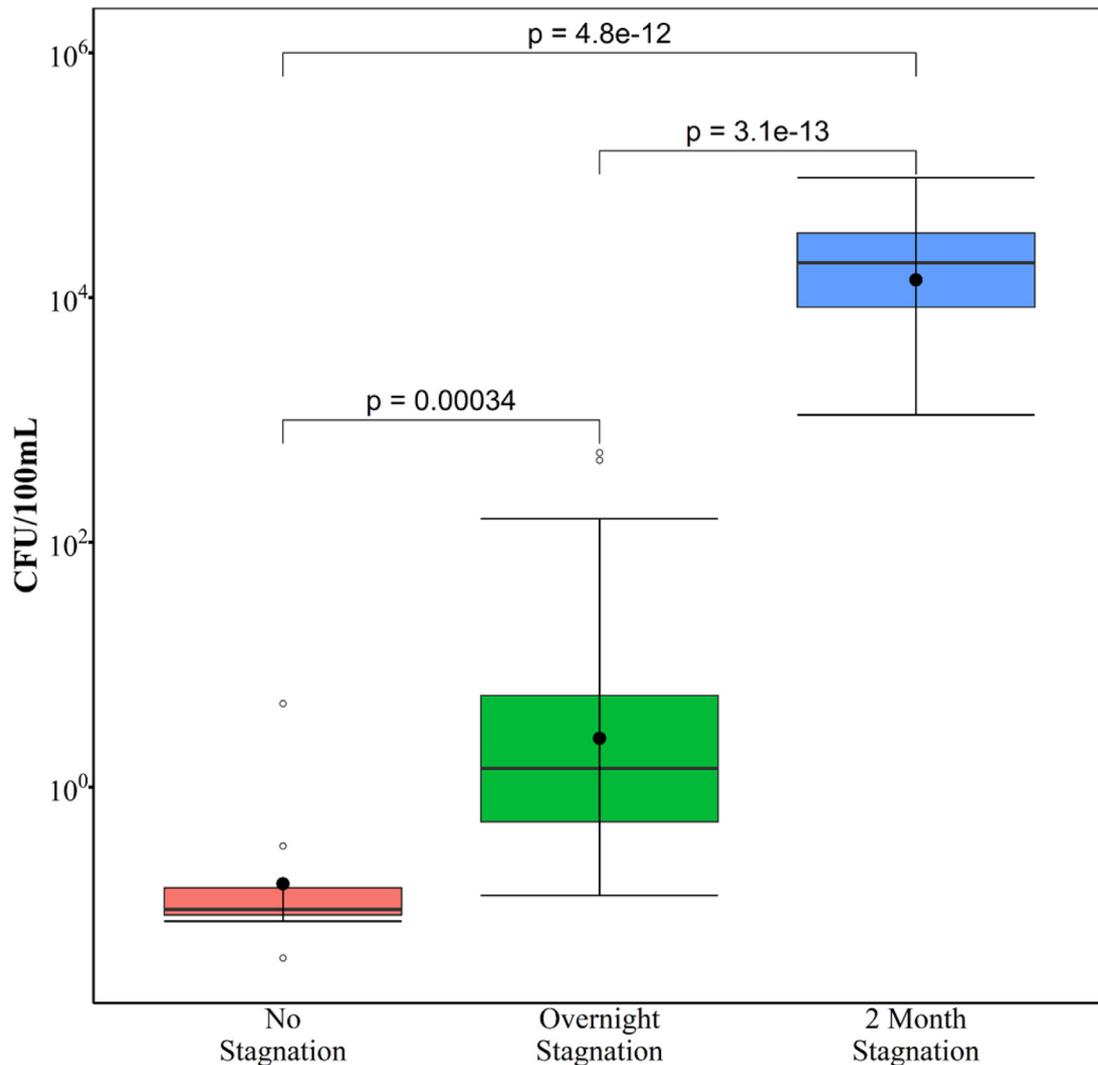

Fig 1. Comparison of overall microbial contamination between water samples with no stagnation, overnight stagnation, or 2-month stagnation. Data shown are heterotrophic plate counts (HPC) measured in colony forming units (CFUs). The horizontal line in each box represents the median; the black dot represents the mean; the upper and lower boundaries of each box indicate the 75th and 25th percentiles, respectively; the whiskers designate the statistical maximum and minimum; and the open circles represent outliers. Statistical significance is indicated by *P* values based on Student's t test.
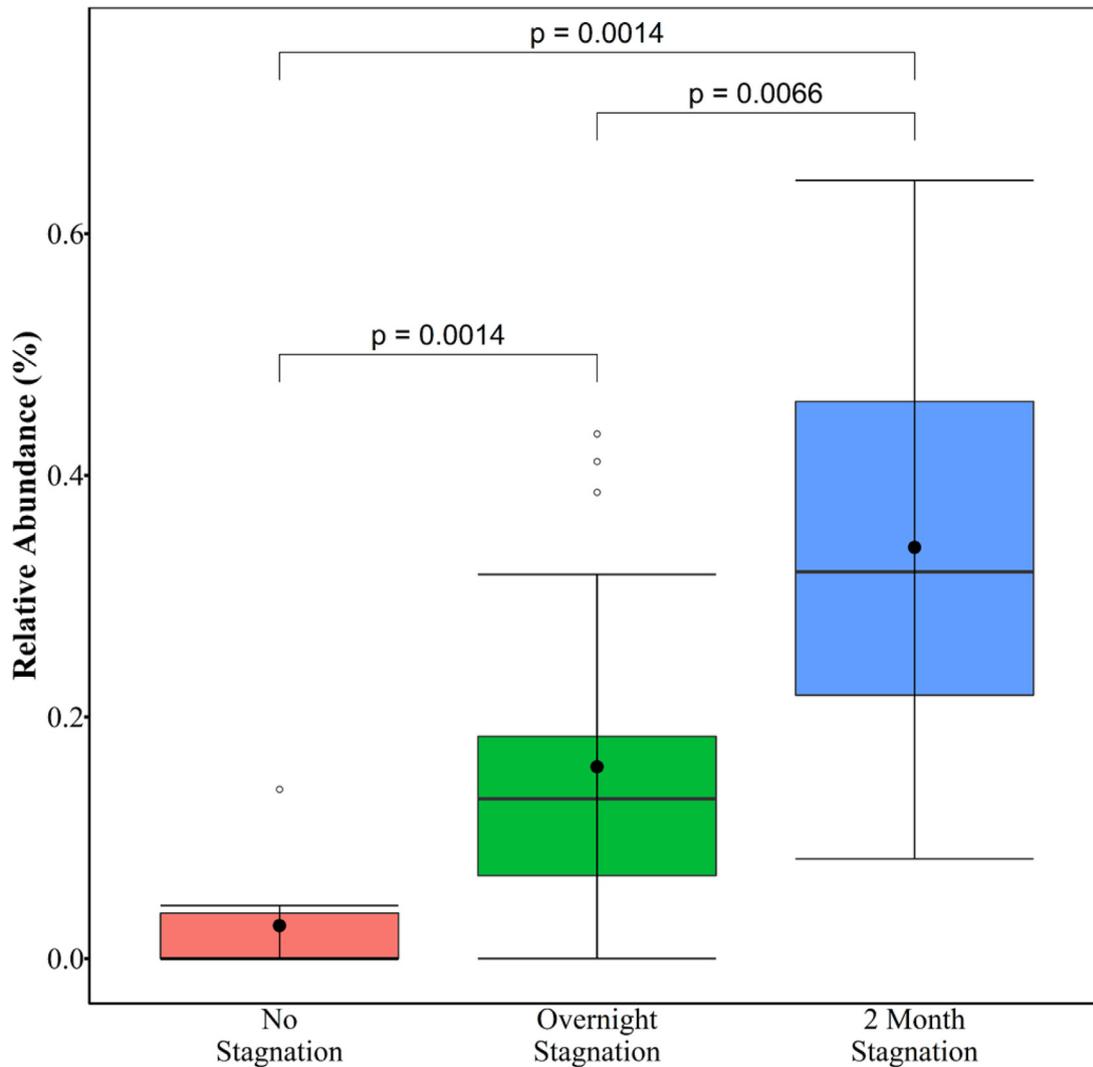
Fig 2. Comparison of *Legionella* contamination between water samples with no stagnation, overnight stagnation, or 2-month stagnation. Data shown are the relative abundance% of *Legionella* sequences in all bacterial sequences identified by 16S rRNA gene-based amplicon library sequencing. The horizontal line in each box represents the median; the black dot represents the mean; the upper and lower boundaries of each box indicate the 75th and 25th percentiles, respectively; the whiskers designate the statistical maximum and minimum; and the open circles represent outliers. Statistical significance is indicated by *P* values according to the Wilcoxon ranked sum test.

DISCUSSION

Responses to the COVID-19 pandemic have included prolonged closures of various types of buildings, leading to extreme water stagnation rarely encountered in BWS. While water stagnation has been linked to deterioration of water quality,[7,8] there is an alarming lack of knowledge on the linkages between extreme water stagnation and exposure risks to *Legionella*, the most common cause of waterborne disease outbreaks reported in the U.S.[9] In this study, sequencing-based analysis of microbial contaminants in BWS detected substantial increases in *Legionella* abundance with the extension of water stagnation (Fig 2). Representing the first report on the impact of extreme water stagnation on *Legionella* contamination, this study highlights the urgent need to mitigate *Legionella* infection risks in BWS during re-opening of buildings previously closed in response to COVID-19.

CONCLUSIONS

This study revealed significantly elevated microbial contamination, particularly by *Legionella*, in building water systems linked to extreme water stagnation due to building closures implemented to control the spread of COVID-19. These findings underline elevated *Legionella* infection risks in buildings experiencing prolonged closure.

References

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