

## Original Article



## Prevalence of Opportunistic Pathogens and Diversity of Microbial Communities in the Water System of a Pulmonary Hospital\*

TANG Wei<sup>1</sup>, MAO Yu<sup>1</sup>, LI Qiu Yan<sup>1</sup>, MENG Die<sup>2</sup>, CHEN Ling<sup>1,3</sup>, WANG Hong<sup>1,3</sup>,  
ZHU Ren<sup>2</sup>, and ZHANG Wei Xian<sup>1,3,#</sup>

1. State Key Laboratory for Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China; 2. Department of Infection Control, Shanghai Pulmonary Hospital, Tongji University, Shanghai 200433, China; 3. Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, China

### Abstract

**Objective** Our objective was to investigate the occurrence of opportunistic pathogens and characterize the bacterial community structures in the water system of a pulmonary hospital.

**Methods** The water samples were collected from automatic and manual faucets in the consulting room, treatment room, dressing room, respiratory ward, and other non-medical rooms in three buildings of the hospital. Quantitative polymerase chain reaction was used to quantify the load of several waterborne opportunistic pathogens and related microorganisms, including *Legionella* spp., *Mycobacterium* spp., and *M. avium*. Illumina sequencing targeting 16S rRNA genes was performed to profile bacterial communities.

**Results** The occurrence rates of *Legionella* spp., *Mycobacterium* spp., and *M. avium* were 100%, 100%, and 76%, respectively in all samples. Higher occurrence rates of *M. avium* were observed in the outpatient service building (building 1, 91.7%) and respiration department and wards (building 2, 80%) than in the office building (building 3), where no *M. avium* was found. *M. avium* were more abundant in automatic faucets (average  $2.21 \times 10^4$  gene copies/L) than in manual faucets (average  $1.03 \times 10^4$  gene copies/mL) ( $P < 0.01$ ). *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, and *Acidobacteria* were the dominant bacterial phyla. Disinfectant residuals, nitrate, and temperature were found to be the key environmental factors driving microbial community structure shifts in water systems.

**Conclusion** This study revealed a high level of colonization of water faucets by opportunistic pathogens and provided insight into the characteristics of microbial communities in a hospital water system and approaches to reduce risks of microbial contamination.

**Key words:** Opportunistic pathogen; Microbial community; Hospital water system; *Mycobacterium avium*

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#Correspondence should be addressed to ZHANG Wei Xian, PhD, E-mail: zhangwx@tongji.edu.cn

Biographical note of the first author: TANG Wei, male, born in 1987, PhD candidate, majoring in environmental microbiology, bioinformatics, environmental health and ecotoxicology.

## INTRODUCTION

Drinking-water distribution systems including premise plumbing are complex microbial ecosystems that may harbor a diversity of bacteria, protozoa, virus, and fungi. As the most frequently studied group of microorganisms in water systems, bacteria play a key role in water quality and drinking water safety. In general, *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Verrucomicrobia*, and *Actinobacteria* are the most frequently reported abundant bacterial phyla in the water systems<sup>[1]</sup>. Notably, the presence of pathogens or opportunistic pathogens in these phyla, such as *Legionella pneumophila* (*L. pneumophila*) and *Mycobacterium avium* (*M. avium*), can pose potential health threats to humans. *L. pneumophila* are Gram-negative obligate aerobic bacteria of the phylum *Proteobacteria*, which can cause Legionnaires' disease (LD) or Pontiac fever. Similar to some species within the *Actinobacteria* phylum, non-tuberculous mycobacteria (NTM) can cause pulmonary diseases resembling tuberculosis, lymphadenitis, soft tissue infection, or disseminated infection in immunocompromised individuals such as acquired immune deficiency syndrome (AIDS) and transplanted patients<sup>[2]</sup>. So far, although over 150 different species of NTM have been reported, NTM infections are most commonly associated with species such as *M. avium* complex, *M. abscessus*, *M. fortuitum*, and *M. kansasii*<sup>[3]</sup>.

Owing to their ability to resist stressful environmental conditions (such as oxygen deprivation, nutrition deficiency, temperature shocks, pH changes, and chlorine resistance), *Legionella* and NTM are often found in a wide range of natural and artificial aquatic environments<sup>[4,5]</sup>. According to previous studies, these bacteria have been widely detected in municipal water treatment and distribution systems<sup>[6,7]</sup>. *Legionella* and NTM colonization in water distribution systems are influenced by many factors, including water temperature, organic matter, pipe material, corrosion, nutrient levels, hydraulic conditions, and biofilm formation in pipe networks<sup>[8-10]</sup>. The U.S. Environmental Protection Agency (EPA) has included *M. avium* and *L. pneumophila* in the contaminate candidate list 4 (CCL4), which is a list of contaminants that are currently not subject to any proposed or promulgated national primary drinking water regulations<sup>[11]</sup>.

Many modes have been identified for the transmission of these opportunistic pathogens to

humans. Inhalation or micro aspiration of aerosols generated by contaminated water, such as from nebulizers, showers, faucets, fountains, hot water systems, and cooling towers, is the most common route of exposure. The hospital environment is of importance in the cross-transmission of opportunistic pathogens. For example, pathogen contamination on the surfaces of medical devices or other healthcare facilities is a potential exposure route to opportunistic pathogens for susceptible people, which increases the potential risk of healthcare-associated infections. A review of 27 LD outbreaks investigated by the U.S. CDC shown that 33% LD cases are linked to healthcare<sup>[12,13]</sup>. Airborne/aerosols mode of infection are also important migration routes in hospital. *Legionella* spp. and NTM can survive in water systems and spread in the form of aerosols during showering, bathing, or through faucet water<sup>[14]</sup>. Like other bacteria, these opportunistic pathogens mostly grow in biofilms, which provide an ecological niche for microorganisms on the surfaces of plumbing materials. Once established within the water system, opportunistic pathogens are difficult to be eradicated.

There is an extensive concern that nosocomial infections are associated with waterborne opportunistic pathogens. Some studies indicated that the water system is one of the main sources for LD outbreaks in hospitals<sup>[15,16]</sup>. *Legionella* spp. in hospital drinking water samples have been associated with patient isolates in Italy<sup>[17]</sup>. The aerators/rectifiers connected to the faucets of the water supply in a Japanese hospital were also suspected to be related to the increased frequency of NTM-positive disease cases<sup>[18]</sup>. However, there are gaps in the knowledge about the existence of opportunistic pathogens in the water systems of respiratory disease hospitals that hospitalize many patients with pulmonary diseases susceptible to infection with opportunistic pathogens. In addition, little is known about the composition of microbial community, especially that of opportunistic pathogens, associated with bacterial populations in hospital water systems. Considering the complexity of hospital water systems and the vulnerability of hospitalized patients, it is important to conduct studies to survey the presence of opportunistic pathogens and investigate the composition of microbial community in water systems of respiratory disease hospitals.

In this study, representative opportunistic pathogens such as *Legionella* spp., *Mycobacterium*

spp., and *M. avium* were targeted in a pulmonary hospital water system. This hospital is one of the largest hospitals specializing in respiratory disease treatment and research in China. In addition, amplicon sequencing of 16S rRNA genes was performed. The aims of this study are as follows: (1) to reveal the occurrence of opportunistic pathogens in tap water; (2) to evaluate the correlations of water quality parameters like temperature, pH, total chlorine, nitrate, nitrite, and ammonia with opportunistic pathogens; (3) to characterize microbial communities and to explore the influence of drinking water systems on the drinking water microbiome. The information from this study might be useful for improving hospital water systems to prevent nosocomial infections caused by opportunistic pathogens.

## MATERIALS AND METHODS

### Sample Collection and Processing

The work was conducted in a pulmonary hospital in Shanghai, China, which is one of the largest hospitals specialized in respiratory disease treatment, research and education in China. This hospital was founded in 1933, covering an area of 103,000 square meters. There are 1,348 employees, 13 clinical departments (such as thoracic surgery, oncology department, respiration department, tuberculosis department, occupational disease department), and 11 medical technique departments. It reports 1,044,704 outpatient visits and discharges 88,768 patients on a yearly average.

Drinking water samples were collected from three buildings during March to May, 2018 in this hospital, where chloramine was used as the secondary disinfectant. The first building is used for outpatient service, the second building are wards of respiration department, and the third building is an office building free of medical services, serving as a control site in this study. The detailed information for sampling is shown in [Supplementary Table S1](#) (available in [www.besjournal.com](http://www.besjournal.com)). Samples (1,000 mL) were collected after flushing for 3 min, and then the bulk water sample was filtered through a sterile 0.22  $\mu\text{m}$  nitrocellulose membrane (Millipore, MA) using a peristaltic pump. A filter membrane with collected biomass was transferred into a 2 mL tube (the lysing matrix A tube) from FastDNA SPIN Kit (MP Biomedicals, OH). The sample was further processed according to the manufacturer's protocol. The extracted DNA was

purified, precipitated, and dissolved in 50  $\mu\text{L}$  of sterile nuclease-free water. The concentration and purity of the total DNA were measured using NanoDrop 2000 (Thermo Fisher Scientific).

### Water Quality Analysis

Temperature, pH, total chlorine, nitrate, nitrite, and ammonia were measured at the time of collection. pH and temperature were monitored using a portable pH meter (Bante Instrument, Bante903P). Total chlorine, nitrate, nitrite, and ammonia were analyzed using HACH reagents, on a DR900 colorimeter (HACH, Loveland, CO).

### Quantitative PCR (qPCR)

*Legionella* spp., *Mycobacterium* spp., *M. avium*, and total bacterial 16S rRNA genes were quantified by qPCR assays using previously published primers and probes<sup>[19]</sup>. All qPCR reactions were performed in triplicate in a 10  $\mu\text{L}$  system containing TaqMan Probes or SybrGreen Premix (TaKaRa). For SYBR Green assays, the 10  $\mu\text{L}$  reaction mixture contained 5  $\mu\text{L}$  of SYBR Green Premix, 0.8  $\mu\text{L}$  each of 5  $\mu\text{mol/L}$  forward and reverse primers, 0.16  $\mu\text{L}$  ROX, 2.24  $\mu\text{L}$  DNase/RNase-free water, and 1  $\mu\text{L}$  template DNA. For TaqMan assays, the 10  $\mu\text{L}$  reaction mixture contained 5  $\mu\text{L}$  of Probes Premix, 0.4  $\mu\text{L}$  each of 5  $\mu\text{mol/L}$  primers, 0.35  $\mu\text{L}$  of 10  $\mu\text{mol/L}$  probe, 0.1  $\mu\text{L}$  ROX, 2.75  $\mu\text{L}$  DNase/RNase-free water, and 1  $\mu\text{L}$  template DNA. The qPCR conditions were as follows: 30 s at 95  $^{\circ}\text{C}$ , 40 cycles of 5 s at 95  $^{\circ}\text{C}$ , 34 s at 55  $^{\circ}\text{C}$ , and 40 s at 72  $^{\circ}\text{C}$  (for 16S rRNA genes); 1 min at 95  $^{\circ}\text{C}$ , 40 cycles of 5 s at 95  $^{\circ}\text{C}$ , 34 s at 64  $^{\circ}\text{C}$ , and 40 s at 72  $^{\circ}\text{C}$  (for *M. avium*); 30 s at 95  $^{\circ}\text{C}$ , 40 cycles of 5 s at 95  $^{\circ}\text{C}$ , 34 s at 58.5  $^{\circ}\text{C}$ , and 40 s at 72  $^{\circ}\text{C}$  (for *Legionella* spp.); 30 s at 95  $^{\circ}\text{C}$ , 45 cycles of 5 s at 95  $^{\circ}\text{C}$ , 34 s at 55  $^{\circ}\text{C}$ , and 40 s at 72  $^{\circ}\text{C}$  (for *Mycobacterium* spp.). Samples were diluted at 1:10 ratios to minimize PCR inhibition. Standards (positive controls) and sterile water (negative controls) were included in each qPCR run. qPCR standards were analyzed to determine the amplification efficiencies (range: between 90% and 110%) and the correlation coefficient ( $R^2 > 0.99$  for all the standard curves). Samples yielding a detectable threshold cycle in at least two experiments out of three were scored as positive.

### Illumina MiSeq Amplicon Sequencing

The V3–V4 region of 16S rRNA genes were amplified for all DNA extracts with primers 338F (forward primer sequence: 5'-ACTCCTACGGG AGGCAGCA-3') and 806R (reverse primer sequence:

5'-GGACTACHVGGGTWTCTAAT-3'). PCRs were performed with a 20  $\mu$ L system using TransStart Fastpfu DNA Polymerase (Beijing TransGen Biotech). The PCR products from triplicate amplifications were examined using a 2% agarose gel, and then purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) according to the manufacturer's instructions. Purified amplicons were pooled in equimolar amounts prior to amplicons sequencing on an Illumina MiSeq platform using paired-end 250 bp kits according to the standard protocols at the Majorbio Bio-Pharm Technology Corporation in Shanghai, China.

Paired-end reads were stitched and screened using Trimmomatic to remove short sequences, singletons, and noisy reads, prior to alignment using FLASH<sup>[20,21]</sup>. Chimeras were identified and removed from further analysis using UCHIME<sup>[22]</sup>. Sequences were clustered into operational taxonomic units (OTUs) using the average neighbor algorithm with a similarity cut-off of 97%<sup>[23]</sup>. The taxonomic information of sequences was aligned against the Greengenes database, with a threshold confidence level of 70%<sup>[24]</sup>.

The software Mothur was used to analyze the alpha diversity indices, including the coverage, observed OTUs richness (Sobs), Shannon index, abundance-based coverage estimator (ACE) and Chao1<sup>[25]</sup>. The intersection of OTUs lists for different samples was analyzed using the vegan packages in R to reveal overlapped and unique OTUs in different buildings. Non-metric multidimensional scaling (NMDS) analysis and analysis of similarity (ANOSIM) were performed based on generated Bray-Curtis distance matrices to evaluate the variation of the microbial community structure among different sample groups. Redundancy analysis (RDA) was used to evaluate the relationship between the abundance of microbial species and environmental variables, using the vegan and ggplot2 packages in R v3.5.0 software.

### Statistical Analysis

qPCR and physiochemical data were analyzed

with a significance level  $< 0.05$ . The Shapiro-Wilk test was used to test the normality of data. One-way analysis of variance (ANOVA) or Kruskal-Wallis  $H$  test was performed to compare target microbes in different buildings depending on the normality and variance of the data. Wilcoxon rank-sum test was used to compare physiochemical and/or biological data between the two different types of faucets. Spearman correlation analysis was used to identify associations between qPCR data and physiochemical parameters using the IBM SPSS statistics v24.0 software. Maximal Information-based Nonparametric Exploration (MINE) analysis, a tool for finding the pairwise associations (linear or non-linear association) in a dataset, with a false detection rate correction at 0.05 was used to characterize the associations between *Legionella* spp., *Mycobacterium* spp., and other bacterial genera<sup>[26,27]</sup>.

## RESULTS

### Water Quality Characteristics in the Hospital Water System

The water quality parameters in the hospital water system are shown in [Table 1](#) and [Supplementary Table S2](#), available in [www.besjournal.com](http://www.besjournal.com). The temperature, pH, total chlorine, NO<sub>3</sub>-N, NO<sub>2</sub>-N, and NH<sub>4</sub>-N varied in the range of 12.7–27.5 °C, 7.17–7.91, 0.06–0.93 mg/L, 0.3–2.9 mg/L,  $< 0.001$ –0.046 mg/L, and 0.07–0.20 mg/L among all water samples, respectively. The levels of total chlorine in building 2 were significantly higher than in building 1 and building 3 ( $P < 0.05$ ). NO<sub>3</sub>-N shared a similar trend: building 3  $<$  building 1  $<$  building 2 ( $P < 0.05$ ). However, the variation of temperature was opposite, with the average temperature in building 3 about 7.0 °C higher than that of building 2 ( $P < 0.05$ ). The concentrations of NH<sub>4</sub>-N in building 1 and building 2 were approximately two times higher than building 3 ( $P < 0.05$ ). No significant difference was observed for NO<sub>2</sub>-N among the three buildings.

**Table 1.** Physiochemical parameters of water samples collected from the hospital water system

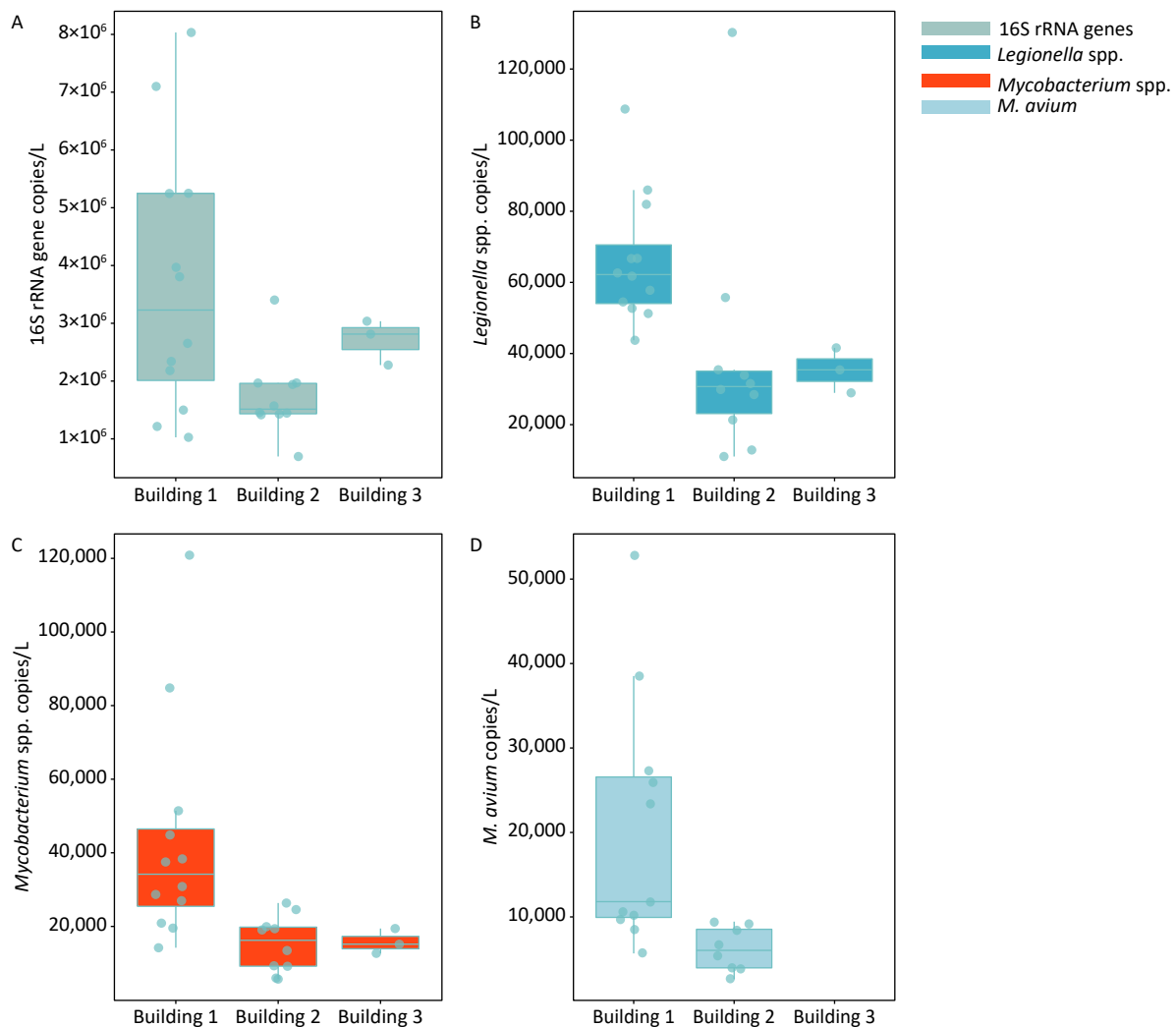
Item	Temperature (°C)	pH	Total chlorine (mg/L)	NO <sub>3</sub> -N (mg/L)	NO <sub>2</sub> -N (mg/L)	NH <sub>4</sub> -N (mg/L)
Building 1	21.6 $\pm$ 3.1	7.58 $\pm$ 0.12	0.60 $\pm$ 0.24	1.4 $\pm$ 0.6	0.006 $\pm$ 0.004	0.16 $\pm$ 0.03
Building 2	18.4 $\pm$ 5.0	7.39 $\pm$ 0.16	0.83 $\pm$ 0.10	2.1 $\pm$ 0.4	0.008 $\pm$ 0.013	0.15 $\pm$ 0.04
Building 3	25.4 $\pm$ 0.4	7.85 $\pm$ 0.06	0.27 $\pm$ 0.03	0.6 $\pm$ 0.1	0.006 $\pm$ 0.006	0.08 $\pm$ 0.01

**Note.** Building 3 serves as a control. Data are shown as mean  $\pm$  standard deviation.

### Occurrence of Total Bacteria, *Legionella* spp., *Mycobacterium* spp., and Its Species *M. avium*

The frequencies of detection (FOD) of *Legionella* spp., *Mycobacterium* spp., and *M. avium* in the three hospital buildings were 100%, 100%, and 76%, respectively (Figure 1 and Supplementary Table S3, available in [www.besjournal.com](http://www.besjournal.com)). The total bacteria, *Legionella* spp., and *Mycobacterium* spp. were detected in all water samples taken from 3 different buildings, ranging from  $(0.70\text{--}8.03) \times 10^6$ ,  $(0.11\text{--}1.30) \times 10^5$ ,

and  $(0.56\text{--}12.09) \times 10^4$  gene copies/L, respectively. *M. avium* was only detected in water samples of building 1 and building 2, while no *M. avium* was found in building 3. The FOD of *M. avium* (91.7%) in building 1 was higher than that in building 2 (80.0%), and the FOD in all samples was 76.0% (19 out of 25). There was a significant difference in the number of *M. avium* gene copies between the automatic faucet and manual faucet ( $P < 0.01$ ). Different levels of total bacteria and *Legionella* gene copies were observed among the three buildings ( $P < 0.05$ ).



**Figure 1.** Enumeration of 16S rRNA genes, *Legionella* spp., *Mycobacterium* spp., and *M. avium* in water samples collected from three hospital buildings. (A) 16S rRNA genes; (B) *Legionella* spp.; (C) *Mycobacterium* spp.; (D) *M. avium*. The bottom, middle, and top line of the box correspond to the 25%, 50%, and 75% percentiles, respectively. Data beyond the end of the whiskers are outliers. Building 1 is used for outpatient service, building 2 for respiration department and wards, and building 3 is an office building free of medical services.

### Correlations between Microorganisms and Water Quality Parameters

The correlations between the water quality parameters and the gene copies of total bacteria and opportunistic pathogens are shown in Table 2. In all samples, significant negative correlations were found between the number of total bacterial 16S rRNA gene copies and total chlorine ( $r = -0.650$ ,  $P < 0.01$ ), and between the number of *M. avium* gene copies and total chlorine ( $r = -0.705$ ,  $P < 0.01$ ). Positive correlations were observed between the number of total bacterial 16S rRNA gene copies and temperature ( $r = 0.508$ ,  $P < 0.01$ ), pH ( $r = 0.535$ ,  $P < 0.01$ ), and nitrite ( $r = 0.475$ ,  $P < 0.05$ ). Correlation analysis was also performed between target organisms. The number of gene copies of *Mycobacterium* ( $r = 0.554$ ), *M. avium* ( $r = 0.689$ ) showed positive correlations with 16S rRNA gene numbers, respectively ( $P < 0.01$ ).

### Microbial Community Composition in the Hospital Water System

Illumina sequencing of 16S rRNA gene amplicons was performed to characterize microbial community compositions in the hospital water system (Figure 2). A total of 1,427,942 sequences were obtained from 25 water samples. These sequences were clustered into 3,182 OTUs at a 97% similarity level. The relative abundances of the most dominant phyla across the 25 samples were Proteobacteria (26.20%–90.22%), Actinobacteria (0.84%–42.63%), Bacteroidetes (0.85%–29.33%), Cyanobacteria (1.30%–14.24%), Firmicutes (0.04%–26.34%), Acidobacteria (0.32%–2.38%), Planctomycetes (0.15%–3.58%), Nitrospirae (0.04%–5.34%), Chlamydiae (0.04%–1.12%), and Gemmatimonadetes (0.05%–1.23%). At the class level, Alphaproteobacteria, Betaproteobacteria, Actinobacteria, Gammaproteobacteria, Chloroplast, Clostridia, Saprospirae, Acidimicrobiia, Cytophagia, Deltaproteobacteria, Flavobacteriia, Nitrospira, and

Bacilli were the dominant classes in these samples (the sum of relative abundance > 1%). Novosphingobium, Mycobacterium, and Sphingomonas were the most prevailing genera in all the water samples, the combined relative abundances of which were 11.21%, 8.36%, and 7.17%, respectively. Other major genera in the samples were Schlegelella, Pseudomonas, Sediminibacterium, Hyphomicrobium, Bradyrhizobium, and Nitrospira (the relative abundance > 1%). MINE analysis identified 22 statistically significant associations between *Mycobacterium* spp. and other microbial genera under a false detection rate of 0.05 (Supplementary Table S4 available in www.besjournal.com); whereas no significant association was found between *Legionella* spp. and other microbial genera. The highest maximal information coefficient (MIC) between *Mycobacterium* spp. and other microbial genera was with *Methylosinus* (MIC = 0.9427, TIC = 0.7910), followed by *Sediminibacterium* (MIC = 0.8411, TIC = 0.3093), *Polaromonas* (MIC = 0.8325, TIC = 0.2773) and *Limnohabitans* (MIC = 0.7983, TIC = 0.4796).

The main differences between the microbial community in phylum and class (top 20) in different groups are shown in Supplementary Tables S5–S6, available in www.besjournal.com. For the three buildings, the significant differences in abundance (top 20) were observed in Verrucomicrobia ( $P < 0.01$ ), Gemmatimonadetes ( $P < 0.01$ ), Synergistetes ( $P < 0.01$ ) at the phylum level, and in Deltaproteobacteria ( $P < 0.01$ ), Planctomycetia ( $P < 0.01$ ), Holophagae ( $P < 0.05$ ) and Sphingobacteriia ( $P < 0.01$ ) at the class level. For the two types of faucets, the relative abundances of microorganisms (top 10) were different. The relative abundances of Firmicutes (phylum,  $P < 0.01$ ), Verrucomicrobia (phylum,  $P < 0.01$ ), Clostridia (class,  $P < 0.01$ ), and Gammaproteobacteria (class,  $P < 0.05$ ) in samples taken from manual faucets were higher than those

**Table 2.** Correlations between the water quality parameters and the gene copies of total bacteria and opportunistic pathogens

Item	Temperature	pH	Chlorine	Nitrate	Nitrite	Ammonia	Total bacteria
Total bacteria	0.508 <sup>b</sup>	0.535 <sup>b</sup>	-0.650 <sup>b</sup>	-0.735 <sup>b</sup>	0.475 <sup>a</sup>	-0.112	1.000
<i>Legionella</i>	-0.058	0.207	-0.154	-0.280	-0.181	0.379	0.140
<i>Mycobacterium</i>	0.269	0.308	-0.367	-0.470 <sup>a</sup>	0.313	-0.012	0.554 <sup>b</sup>
<i>M. avium</i>	0.416	0.701 <sup>b</sup>	-0.705 <sup>b</sup>	-0.814 <sup>b</sup>	0.385	0.006	0.689 <sup>b</sup>

**Note.** <sup>a</sup>Significance < 0.05, <sup>b</sup>Significance < 0.01.



in samples taken from automatic faucets.

To evaluate the shared OTUs in different building taps in this hospital, Venn diagrams were constructed (Supplementary Figure S1, available in www.besjournal.com). There were 122, 347, and 271

shared OTUs present in water samples collected from building 1, building 2, and building 3, respectively. Five hundred and forty shared OTUs were found in the three buildings, with the 10 most abundant genera identified as *Novosphingobium*,

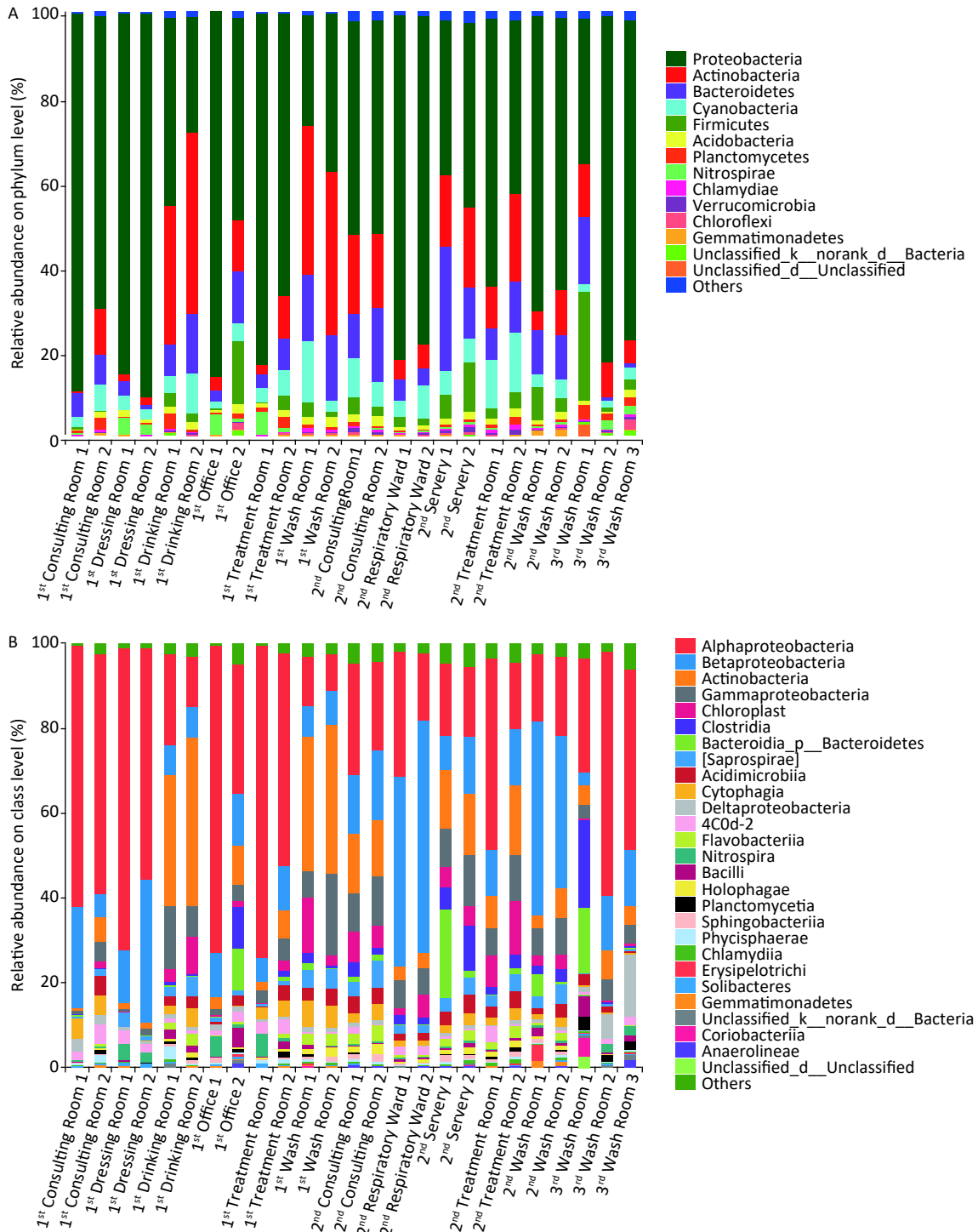


Figure 2. Relative abundance of bacterial phyla (A) and class (B) at each sampling site.

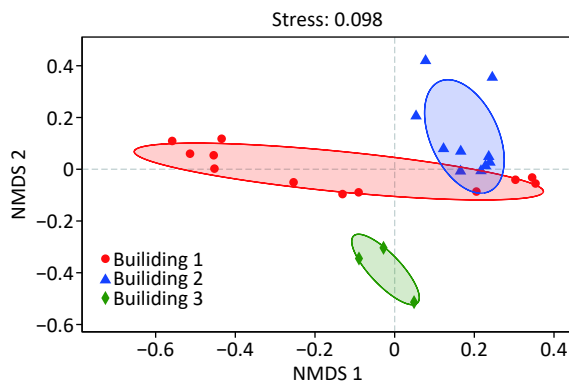
*Mycobacterium*, *Sphingomonas*, *Pseudomonas*, *Sediminibacterium*, *Hyphomicrobium*, *Bradyrhizobium*, *Nitrospira*, *Sphingopyxis*, and *Erythromicrobium*.

Microbial community richness and diversity indices in all the water samples are shown in [Supplementary Table S7](#), available in [www.besjournal.com](http://www.besjournal.com). The coverage of all samples was above 99.7%, showing that sequencing depth was sufficient in this work. There were significant differences in the Sobs, ACE and Chao1 indexes in three buildings ( $P < 0.05$ ), indicating the variation of the richness and diversity of the microbial community in different buildings.

NMDS analysis of the samples based on Bray-Curtis distance matrices is illustrated in [Figure 3](#). The result showed that the samples were clustered into three groups by different buildings. However, the samples of washroom and drinking room water collected from building 1 clustered closer to those of building 2, suggesting higher community similarity to building 2 water samples than the rest in building 1. ANOSIM analysis also demonstrated the difference in microbial community structures between different buildings ( $r = 0.44$ ,  $P = 0.001$ ).

#### Relationships between Water Quality Properties and Microbial Community Composition

The relationships between water quality properties and microbial community composition were assessed with RDA ([Figure 4](#)). RDA1 axis showed a significant correlation with temperature, chlorine, and nitrate ( $P < 0.05$ ), and the RDA2 axis showed a significant correlation with temperature ( $P$



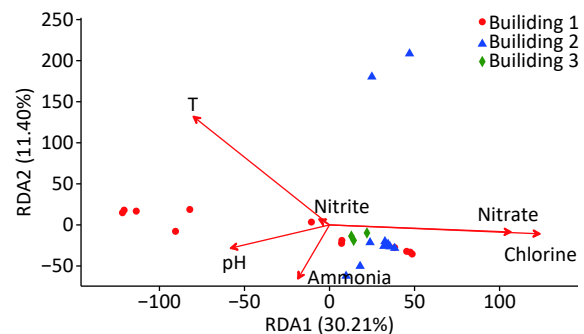
**Figure 3.** NMDS analysis of bacterial communities in the hospital water system, color-coded by sampling building. NMDS ordination was derived from pairwise Bray-Curtis distances matrices.

$< 0.05$ ). Over 41.6% of the variation in bacterial community composition can be explained by the RDA1 axis and RDA2 axis. Similar to NMDS ([Figure 3](#)), the RDA results showed that the samples from building 3 tightly clustered into a group, and the samples from building 2 had the similar trend except that two samples were taken from the respiratory ward (i.e., points located in the upper of the figure); however, the samples collected from building 1 dispersed without obvious clustering. Total chlorine, nitrate, and temperature were the major environmental driver factors, showing a significant correlation with the bacterial community composition ( $P < 0.05$ ).

#### DISCUSSION

This research revealed a high prevalence of *Legionella* spp. and *Mycobacterium* spp. in water faucets and provided insight into these opportunistic pathogens and the broader bacterial community composition in a respiratory disease hospital water system, which may have implications for reducing microbial contamination risks of hospital water supplies.

*Legionella* spp. and *Mycobacterium* spp. were ubiquitously found in water supply systems, such as drinking water treatment trains, premise plumbing, water reservoirs, water storage tanks, water heaters and showerheads<sup>[28-33]</sup>. Because of the complexity of hospital water systems and the vulnerability of hospitalized patients, many of the infections associated with opportunistic pathogens have been reported in hospital environments<sup>[34]</sup>. However, to our knowledge, there have been few reports on a large-scale specialized respiratory system hospital in



**Figure 4.** Redundancy analysis (RDA) of the bacterial community in the hospital water samples. RDA correlation plot showing variance in bacterial community composition explained by the water quality properties.



China using culture-independent methods.

In this study, *Legionella* spp. and *Mycobacterium* spp. were ubiquitously present (100%) in the hospital water system, while *M. avium* was only found in buildings for medical services. The finding was similar to one previous study on secondary water supply systems in Shanghai, demonstrating the ubiquity of *Legionella* and mycobacterial genera and the absence of *M. avium* in public and residential buildings<sup>[35]</sup>. Another one-year survey of opportunistic pathogens in the tap-water (including hospital, hotel, school, public office, and private dining room) of one northern city of China also showed high detection rates of *Legionella* spp. and *Mycobacterium* spp. (i.e., 100% FOD in 44 samples) by qPCR<sup>[36]</sup>. However, lower FOD of *Legionella* was also reported in private hospitals and university hospitals in Italy, which used chlorine dioxide as disinfection rather than chloramine<sup>[37,38]</sup>.

NTM are environmental mycobacteria other than *Mycobacterium tuberculosis* and *Mycobacterium leprae*. Species such as *M. avium*, *M. intracellulare*, *M. mucogenicum*, *M. kansasii*, *M. goodii*, *M. fortuitum*, and *M. chelonae* are frequently reported opportunistic pathogens for humans. In this study, the high FOD of *M. avium* in tap water was in contrast to another study isolating NTM from patients in the same hospital during 2008–2012, demonstrating *M. kansasii* (45.0%), *M. intracellulare* (20.8%), *M. chelonae/abscessus* (14.9%) as the most frequently identified clones, with *M. avium* only accounting for 3.6% of clinical isolates<sup>[39]</sup>. Although we did not investigate other mycobacterial species in water samples and biofilm samples in the present study, it is likely that the existence of biofilms in the water systems can harbor a high level of opportunistic pathogens and potentially release them into the water. Different analytic methods employed in these two studies might also account for the differences. Our work applied molecular techniques with advantages of lower detection limits and higher specificity, while the study employed conventional culture methods, which was considered as the gold standard for identifying live pathogens<sup>[39]</sup>. It is also possible that the composition of *Mycobacterium* spp. in this hospital may demonstrate temporal changes.

Ecological interactions among bacteria are ubiquitous and complex in water, the pattern of which is determined by the bacterial population and water quality parameters<sup>[40]</sup>. As shown in [Supplementary Table S4](#), MINE analysis identified 22 statistically significant associations between

*Mycobacterium* spp. and other microbial genera, such as *Methylosinus*, *Sediminibacterium*, *Novosphingobium*, *Bdellovibrio*, *Staphylococcus*, *Flavobacterium*, and *Ralstonia* ( $P < 0.05$ ). Similar to our previous study, the associations between *Mycobacterium* spp. and four bacteria genera including *Novosphingobium*, *Flavobacterium*, *Ralstonia*, and *Arcobacter* were found, suggesting their antagonistic or synergistic relationships in water systems<sup>[41]</sup>. In addition, *Flavobacterium* can produce some metabolites and components, such as dimethyl polysulfides, which result in the odor in tap water<sup>[42]</sup>. Notably, a positive significant association was identified between *Mycobacterium* spp. and pathogenic staphylococcus, which may increase virulence potential in water systems. On the contrary, a negative significant association was found between *Mycobacterium* spp. and *Bdellovibrio*. *Bdellovibrio* is a parasite or predator of other bacteria including some pathogens, which may act as natural inhibitors of bacterial growth in man-made water systems<sup>[43]</sup>.

Chloramine has replaced free chlorine as a disinfectant in municipal water systems in Shanghai, because it provides many benefits, including higher stability, lower production trihalomethanes, and haloacetic acids, and improves maintenance of disinfectant residual in distribution pipes<sup>[44,45]</sup>. Chloramine has been known to penetrate biofilm more strongly compared to chlorine, while it less effectively inactivated microorganisms near the biofilm surface<sup>[46]</sup>. It is reported that mycobacterial FODs in a chloraminated water potable system was higher than other chlorinated systems<sup>[47]</sup>. Donohue et al.<sup>[48]</sup> also found that the levels of NTM in chloraminated water were statistically higher than those in chlorinated water. *M. avium* was reported as one of the most frequently detected NTM in many water systems. Mycobacteria can be categorized into fast-growing species (colony formation 3–7 d, including *M. abscessus*, *M. chelonae*, and *M. fortuitum*) and slow-growing species (colony formation > 7 d, including *M. avium*, *M. intracellulare*, *M. xenopi*, and *M. kansasii*)<sup>[49,50]</sup>. As a slow-growing mycobacterium, *M. avium* was more disinfectant-resistant than fast-growing species<sup>[51]</sup>.

According to another survey by Coniglio et al.<sup>[52]</sup>, monochloramine was associated with a lower prevalence of *Legionella* colonization in complex piping systems. However, in our study, the occurrence of *Legionella* was 100%. Higher *Legionella* prevalence is likely associated with relatively low disinfectant levels in the present work.

About 30% (7/25, 28.0%) of samples had total chlorine concentrations lower than 0.5 mg/L, which is the disinfectant concentration recommended for the prevention of *Legionellosis* by the American Society of Heating, Refrigeration, and Air Conditioning Engineering<sup>[53]</sup>. In addition, there are many other factors influencing the growth and survival of *Legionella* species. For example, the free-living amoeba can serve as hosts for *Legionella* spp., providing an intracellular environment to protect from disinfectants in water systems<sup>[54]</sup>.

Our study showed that the number of *M. avium* gene copies in water samples collected from the automatic faucet was higher than that collected from the manual faucet ( $P < 0.01$ ). Faucets were likely to be an important source of healthcare-associated infections. One outbreak of *M. mucogenicum* bacteremia was reported to be associated with contaminated electronic faucets in a pediatric hematology-oncology department<sup>[55]</sup>. Studies also demonstrated that *Legionella* species and *Pseudomonas aeruginosa* had a similar trend with higher FODs in automatic/sensor (no-touch)/electronic faucets<sup>[56,57]</sup>. Some possible reasons included: low water flow, materials of electronic faucets internal components, bacterial colonization of internal magnetic valves and retrograde contamination from the faucet outlet<sup>[58]</sup>.

Hospital water systems are complex ecosystems that colonize trillions of microorganisms interacting with each other and with their environment. Nitrate was one of the major drivers in microbial composition across all samples in this study (Figure 4). High relative abundances of *Nitrospira* has also been noted in our study. It has been reported that nitrification can occur in a chloraminated system, which can promote the growth of nitrifying bacteria<sup>[59]</sup>. Nitrification can also reduce water quality, leading to inadequate disinfectant residual and shift of microbial community structures<sup>[28]</sup>.

Proteobacteria, one of the bacterial phyla with highest abundance in environmental water samples, was the most abundant phylum of gram-negative bacteria, mainly including five classes, viz Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria<sup>[60-62]</sup>. Proteobacteria widely existed in water systems, which is consistent with previous studies<sup>[35,63]</sup>. For example, Vaz-Moreira et al.<sup>[63]</sup> reported that Alphaproteobacteria predominated in tap water, while Gamma dominated in source water. The ubiquitous tap water genera comprised Proteobacteria of the class

Alpha- (Blastomonas, Brevundimonas, Methylobacterium, Sphingobium, Sphingomonas), Beta- (Acidovorax, Ralstonia), and Gamma- (Acinetobacter and Pseudomonas). At phylum level, the relative abundances of Verrucomicrobia ( $P < 0.01$ ) and Gemmatimonadetes ( $P < 0.01$ ) were noted among the three hospital buildings. Verrucomicrobia and Acidobacteria classified as 'sensitive taxa' could act as biomarkers to distinguish samples between source and tap water according to a study by Han et al.<sup>[1]</sup>, which assessed the impact of source water on tap water bacterial communities in 46 drinking water supply systems in China.

Several bacterial genera with a relatively high abundance were known to contain pathogenic species. *Mycobacterium* was (8.36%) the second most prevailing genus in all the water samples. *Mycobacterium* contained a series of pathogenic species, such as *M. abscessus*, *M. chelonae*, *M. avium*, *M. gordonae*, and *M. kansasii*. According to some studies, NTM infection in hospitals should consider the possibility of patient colonization or environmental contamination. In particular, *M. gordonae* derived from contamination of water supply systems or other environmental sources have been associated with the pseudo-outbreaks of hospital infection<sup>[18,64]</sup>. In the *Pseudomonas* genus, some species, such as *P. aeruginosa*, can cause serious infections in immunocompromised patients with cancer, severe burns, and cystic fibrosis<sup>[65]</sup>. 30% to 50% of nosocomial infections by *P. aeruginosa* were caused by contaminated water in ICU wards, and the types of faucets and connecting pipes materials are important factors influencing the prevalence of *P. aeruginosa*<sup>[66]</sup>.

## CONCLUSION

Our results showed a ubiquitous presence of *Legionella* spp., *Mycobacterium* spp., and *M. avium* in the pulmonary hospital drinking water system, implying the hospital water system is an important reservoir for opportunistic pathogens. High-throughput sequencing indicated that a shared community of bacteria, mainly including *Novosphingobium*, *Mycobacterium*, and *Sphingomonas*, was present constantly in the three hospital buildings. The relative abundances of Firmicutes (phylum), Verrucomicrobia (phylum), Clostridia (class), and Gammaproteobacteria (class) in samples taken from manual faucets were higher than that those of samples taken from automatic faucets. Disinfectant residuals, nitrate, and temperature could significantly affect opportunistic

pathogens and microbial diversity in a hospital water system. Our study also showed different pathogen contamination levels and variations of microbial community structures in different hospital buildings, implying their significant role in shaping the drinking water microbiome and determining the pathogen occurrence rates. Further research needs to focus on the survival mechanisms of opportunistic pathogens, such as *M. avium*, in hospital water systems and approaches to reduce risks of microbial contamination.

### CONFLICTS OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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**Supplementary Table S1.** Sampling information in this study

<b>Building</b>	<b>Location</b>	<b>Faucet type</b>	<b>Sampling month</b>
1 <sup>st</sup>	Consulting room	Automatic faucet	April, 2018
1 <sup>st</sup>	Consulting room	Automatic faucet	April, 2018
1 <sup>st</sup>	Dressing room	Automatic faucet	April, 2018
1 <sup>st</sup>	Dressing room	Automatic faucet	April, 2018
1 <sup>st</sup>	Drinking room	Manual faucet	March, 2018
1 <sup>st</sup>	Drinking room	Manual faucet	March, 2018
1 <sup>st</sup>	Office	Manual faucet	April, 2018
1 <sup>st</sup>	Office	Manual faucet	April, 2018
1 <sup>st</sup>	Treatment room	Automatic faucet	April, 2018
1 <sup>st</sup>	Treatment room	Automatic faucet	April, 2018
1 <sup>st</sup>	Wash room	Automatic faucet	March, 2018
1 <sup>st</sup>	Wash room	Automatic faucet	March, 2018
2 <sup>nd</sup>	Consulting room	Manual faucet	March, 2018
2 <sup>nd</sup>	Consulting room	Manual faucet	March, 2018
2 <sup>nd</sup>	Respiratory ward	Manual faucet	March, 2018
2 <sup>nd</sup>	Respiratory ward	Manual faucet	March, 2018
2 <sup>nd</sup>	Servery	Manual faucet	March, 2018
2 <sup>nd</sup>	Servery	Manual faucet	March, 2018
2 <sup>nd</sup>	Treatment room	Manual faucet	March, 2018
2 <sup>nd</sup>	Treatment room	Manual faucet	March, 2018
2 <sup>nd</sup>	Wash room	Manual faucet	March, 2018
2 <sup>nd</sup>	Wash room	Manual faucet	March, 2018
3 <sup>rd</sup>	Wash room	Manual faucet	May, 2018
3 <sup>rd</sup>	Wash room	Manual faucet	May, 2018
3 <sup>rd</sup>	Wash room	Manual faucet	May, 2018

**Supplementary Table S2.** Physiochemical parameters of water samples collected from the hospital water system

Location	Temperature (°C)	pH	Chlorine (mg/L)	NO <sub>3</sub> -N (mg/L)	NO <sub>2</sub> -N (mg/L)	NH <sub>4</sub> -N (mg/L)
1 <sup>st</sup> Consulting room 1	22.1	7.62	0.45	1.4	0.009	0.19
1 <sup>st</sup> Consulting room 2	20.3	7.67	0.76	1.4	0.006	0.19
1 <sup>st</sup> Dressing room 2	23.5	7.52	0.35	1.3	0.005	0.14
1 <sup>st</sup> Dressing room 1	24.9	7.58	0.06	1.5	0.007	0.10
1 <sup>st</sup> Drinking room 1	20.3	7.51	0.84	2.0	0.002	0.17
1 <sup>st</sup> Drinking room 2	17.3	7.57	0.85	1.5	< 0.001	0.18
1 <sup>st</sup> Office 1	22.6	7.67	0.57	0.8	0.004	0.17
1 <sup>st</sup> Office 2	27.5	7.62	0.77	1.9	0.004	0.16
1 <sup>st</sup> Treatment room 1	23.9	7.66	0.55	0.3	0.009	0.17
1 <sup>st</sup> Treatment room 2	21.6	7.69	0.74	0.7	0.009	0.16
1 <sup>st</sup> Wash room 1	18.5	7.24	0.77	1.9	0.004	0.16
1 <sup>st</sup> Wash room 2	17.2	7.58	0.84	2.7	0.002	0.17
2 <sup>nd</sup> Consulting room 1	22.0	7.26	0.57	1.8	0.046	0.08
2 <sup>nd</sup> Consulting room 2	19.8	7.31	0.85	1.9	< 0.001	0.09
2 <sup>nd</sup> Respiratory ward 1	27.4	7.32	0.76	2.0	0.006	0.12
2 <sup>nd</sup> Respiratory ward 2	25.2	7.37	0.83	1.8	0.006	0.12
2 <sup>nd</sup> Servery 1	15.9	7.51	0.89	2.6	0.003	0.18
2 <sup>nd</sup> Servery 2	13.9	7.56	0.91	2.9	0.005	0.17
2 <sup>nd</sup> Treatment room 1	17.2	7.57	0.85	1.8	0.005	0.17
2 <sup>nd</sup> Treatment room 2	15.1	7.62	0.88	2.0	0.009	0.16
2 <sup>nd</sup> Wash room 1	14.8	7.17	0.84	1.9	< 0.001	0.20
2 <sup>nd</sup> Wash room 2	12.7	7.23	0.93	2.3	< 0.001	0.18
3 <sup>rd</sup> Wash room 1	25.7	7.91	0.30	0.6	0.006	0.07
3 <sup>rd</sup> Wash room 2	24.9	7.84	0.27	0.5	0.006	0.09
3 <sup>rd</sup> Wash room 3	25.6	7.79	0.24	0.7	0.005	0.08



**Supplementary Table S3.** Occurrence and concentrations of total bacteria, *Legionella* spp., *Mycobacterium* spp., and *M. avium* in the hospital water system

Target organism	Occurrence rate (%)				Concentration (gene copies/L)		
	Building 1	Building 2	Building 3	Total	Building 1	Building 2	Building 3
Total bacteria	100	100	100	100	$(1.02-8.03) \times 10^6$	$(0.70-3.40) \times 10^6$	$(2.34-2.84) \times 10^6$
<i>Legionella</i> spp.	100	100	100	100	$(0.44-1.09) \times 10^5$	$(0.11-1.30) \times 10^5$	$(3.69-4.46) \times 10^4$
<i>Mycobacterium</i> spp.	100	100	100	100	$(0.14-1.21) \times 10^5$	$(0.57-2.64) \times 10^4$	$(1.46-1.87) \times 10^4$
<i>M. avium</i>	91.7	80	0	76	$(ND-5.28) \times 10^4$	$(ND-9.10) \times 10^3$	ND

**Supplementary Table S4.** The MINE analysis between *Mycobacterium* and other microbial genera in the hospital water system

X var	Y var	MICe (strength)	TICe (presence of relationship)	MICe-p <sup>2</sup> (nonlinearity)	MASe (non-monotonicity)	MEVe (functionality)	MCNe (complexity)	Linear regression (p)
<i>Mycobacterium</i>	<i>Methylosinus</i>	0.9427	0.7910	0.2105	0.0469	0.9427	2.5850	0.8557
<i>Mycobacterium</i>	<i>Sediminibacterium</i>	0.8411	0.3093	0.2678	0.0937	0.8411	3.5850	0.7572
<i>Mycobacterium</i>	<i>Polaromonas</i>	0.8325	0.2773	0.7527	0.3929	0.8325	3.5850	0.2825
<i>Mycobacterium</i>	<i>Limnohabitans</i>	0.7983	0.4796	0.1878	0.1930	0.7983	3.3219	0.7813
<i>Mycobacterium</i>	<i>Gallionella</i>	0.7983	0.3781	0.5821	0.2022	0.7983	3.3219	0.4650
<i>Mycobacterium</i>	<i>Gemmatimonas</i>	0.7286	0.4796	0.2345	0.1060	0.7286	3.5850	0.7029
<i>Mycobacterium</i>	<i>Polynucleobacter</i>	0.7102	0.2516	0.6682	0.2770	0.7102	3.3219	0.2049
<i>Mycobacterium</i>	<i>Novosphingobium</i>	0.7090	0.2516	0.4944	0.1326	0.7090	3.5850	-0.4633
<i>Mycobacterium</i>	<i>Methyloversatilis</i>	0.7027	0.3851	0.5761	0.1588	0.7027	3.0000	-0.3557
<i>Mycobacterium</i>	<i>Synechococcus</i>	0.6994	0.3150	0.6749	0.1601	0.6994	3.0000	0.1567
<i>Mycobacterium</i>	<i>Enhydrobacter</i>	0.6840	0.4636	0.2253	0.1263	0.6840	3.0000	0.6773
<i>Mycobacterium</i>	<i>Bdellovibrio</i>	0.6656	0.4636	0.5334	0.1015	0.6656	3.3219	-0.3635
<i>Mycobacterium</i>	<i>Staphylococcus</i>	0.6614	0.4045	0.4145	0.1397	0.6146	3.5850	0.4968
<i>Mycobacterium</i>	<i>Opitutus</i>	0.6343	0.3851	0.6026	0.0782	0.6343	3.5850	0.1780
<i>Mycobacterium</i>	<i>Flavobacterium</i>	0.6227	0.3857	0.5102	0.1519	0.6227	3.5850	0.3354
<i>Mycobacterium</i>	<i>Ralstonia</i>	0.6155	0.2516	0.3426	0.0810	0.6155	3.5850	0.5224
<i>Mycobacterium</i>	<i>Fluviicola</i>	0.6146	0.4045	0.0333	0.0567	0.6146	3.5850	0.7624
<i>Mycobacterium</i>	<i>Arcobacter</i>	0.6094	0.2816	0.3572	0.1801	0.6094	3.5850	0.5022
<i>Mycobacterium</i>	<i>Crenothrix</i>	0.6041	0.3377	0.3639	0.1843	0.6041	3.3219	0.4901
<i>Mycobacterium</i>	<i>Mycoplana</i>	0.6041	0.3150	0.6039	0.2381	0.6041	3.3219	-0.0150
<i>Mycobacterium</i>	<i>Methylotenera</i>	0.6002	0.3150	0.3959	0.1895	0.6002	3.5850	0.4520
<i>Mycobacterium</i>	<i>Blautia</i>	0.5511	0.3093	0.5509	0.1539	0.5511	3.5850	0.0141

**Supplementary Table S5.** Significance test of difference about relative abundances between the samples collected from different buildings, showing the top 20 most abundant bacteria by phylum, class, and genus

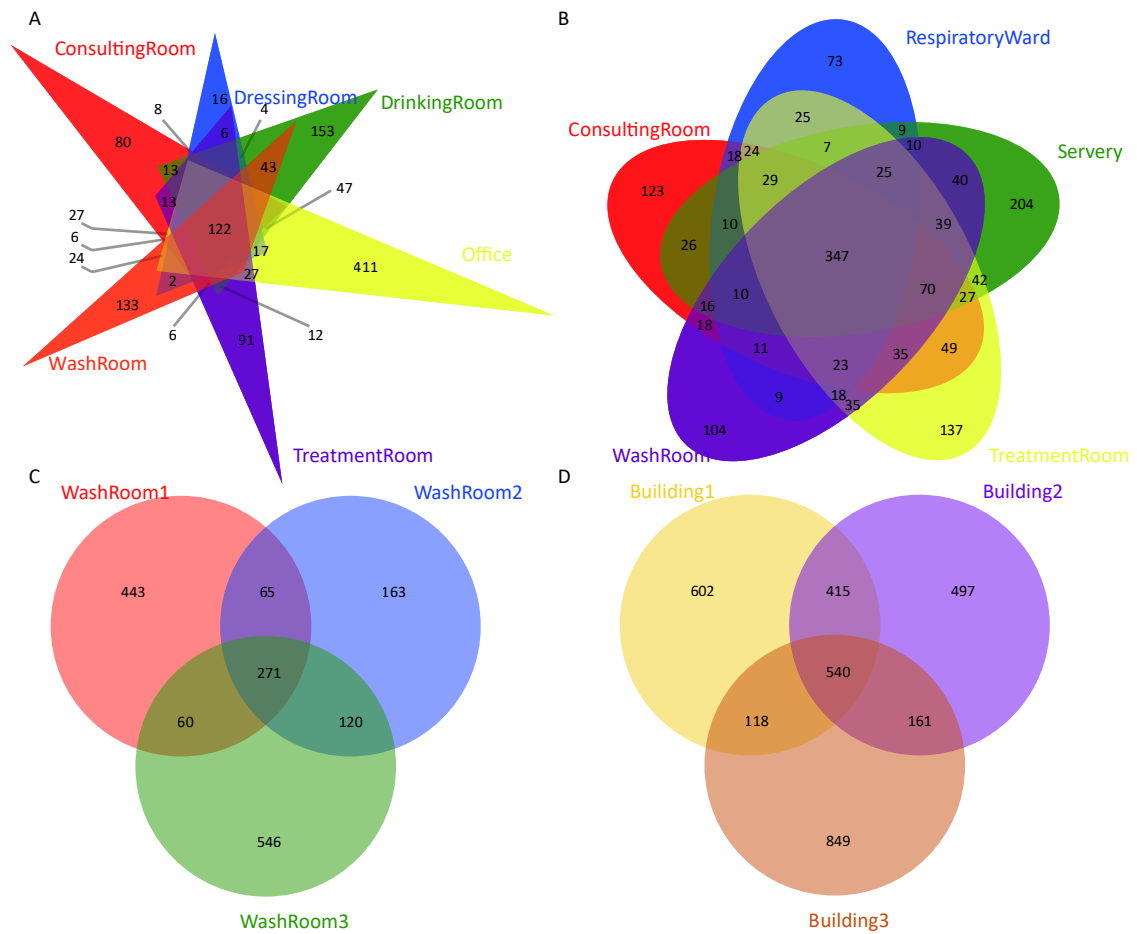
Phylum	Relative abundance of bacteria in three buildings (%)			Class	Relative abundance of bacteria in three buildings (%)			Genus	Relative abundance of bacteria in three buildings (%)		
	Building 1	Building 2	Building 3		Building 1	Building 2	Building 3		Building 1	Building 2	Building 3
Proteobacteria	62.62	57.88	63.89	Alphaproteobacteria	43.62	22.11	42.52	<i>Novosphingobium</i>	21.07	2.02	2.37
Actinobacteria	16.08	12.68	8.66	Betaproteobacteria	12.05	25.74	9.55	<i>Mycobacterium</i>	10.34	7.07	4.71
Bacteroidetes	7.89	11.96	5.87	Actinobacteria	13.97	9.62	5.33	<i>Sphingomonas</i>	7.89	6.50	6.51
Firmicutes	2.54	4.58	9.92	Gammaproteobacteria	5.73	8.67	4.33	<i>Schlegelella</i>	0.03	8.90	< 0.01
Cyanobacteria	5.03	7.05	2.10	Clostridia	1.38	3.43	7.44	<i>Hyphomicrobium</i>	0.41	0.60	5.46
Planctomycetes	1.20	0.68	2.44	Deltaproteobacteria	1.13 <sup>cf</sup>	0.61 <sup>bf</sup>	7.35 <sup>bf,cf</sup>	<i>Pseudomonas</i>	1.75	2.52	0.29
Acidobacteria	1.41	1.47	0.93	Chloroplast	2.78	5.51	0.34	<i>Sediminibacterium</i>	1.37	2.13	0.09
Nitrospirae	1.58	0.36	1.40	Acidimicrobia	1.96	2.97	1.70	<i>Nitrospira</i>	1.57	0.22	1.34
Chloroflexi	0.22	0.24	0.82	Saprospirae	2.15	3.50 <sup>bd</sup>	0.34 <sup>bd</sup>	<i>Bradyrhizobium</i>	1.44	0.76	0.60
Chlamydiae	0.31	0.57	0.35	Cytophagia	2.88	2.13	0.19	<i>Sphingopyxis</i>	0.26	1.78	0.42
Verrucomicrobia	0.12 <sup>af</sup>	0.57 <sup>af</sup>	0.48	Bacilli	1.08	0.65	2.26	<i>Erythronicrobium</i>	1.52	0.17	0.69
Gemmatimonadetes	0.25 <sup>af</sup>	0.36 <sup>af,bf</sup>	0.15 <sup>bf</sup>	Nitrospira	1.58	0.36	1.40	<i>Rhodococcus</i>	1.89 <sup>ad</sup>	< 0.01 <sup>ad</sup>	< 0.01
Armatimonadetes	0.01	0.64	0.04	Planctomycetia	0.47 <sup>cf</sup>	0.30 <sup>bf</sup>	2.27 <sup>bf,cf</sup>	<i>Flavobacterium</i>	0.41 <sup>ae</sup>	1.42 <sup>ae,be</sup>	0.03 <sup>be</sup>
Tenericutes	0.04	0.10	0.16	Flavobacteria	1.04	1.73	0.15	<i>Polynucleobacter</i>	0.22 <sup>af</sup>	1.50 <sup>af,be</sup>	0.06 <sup>be</sup>
Spirochaetes	0.07	0.04	0.14	Holophagae	0.56 <sup>ad</sup>	1.25 <sup>ad,bd</sup>	0.08 <sup>bd</sup>	<i>Janthinobacterium</i>	0.15 <sup>af</sup>	1.49 <sup>af,be</sup>	0.03 <sup>be</sup>
Synergistetes	< 0.01 <sup>af</sup>	0.23 <sup>af,be</sup>	0.02 <sup>be</sup>	Coriobacteria	0.03	0.02	1.47	<i>Lactobacillus</i>	0.16	0.33	1.18
Thermi	0.12	0.02	0.01	Sphingobacteria	0.45 <sup>ad</sup>	0.96 <sup>ad,bd</sup>	0.07 <sup>bd</sup>	<i>Rhodobacter</i>	1.00	0.18	0.46
Chlorobi	0.03	0.02	0.07	Phycisphaerae	0.72	0.37	0.17	<i>Methylosinus</i>	0.45	0.55	0.29
Elusimicrobia	0.04	0.02	0.02	Chlamydia	0.31	0.57	0.35	<i>Ruminococcus</i>	0.15	0.08	1.01
Deferribacteres	0.01	0.02	< 0.01	Anaerolineae	0.08	0.21	0.61	<i>Acinetobacter</i>	0.13 <sup>cf</sup>	0.07 <sup>bf</sup>	0.89 <sup>bf,cf</sup>

**Note.** <sup>a</sup> significant difference between building 1 and building 2, <sup>b</sup> significant difference between building 2 and building 3, and <sup>c</sup> significant difference between building 1 and building 3; <sup>d</sup>  $P < 0.05$ , <sup>e</sup>  $P < 0.01$ , and <sup>f</sup>  $P < 0.001$ .

**Supplementary Table S6.** Significance test of difference about relative abundances between the samples collected from different faucets, showing the top 20 most abundant bacteria by phylum and class

Phylum	Manual faucet	Automatic faucet	Class	Manual faucet	Automatic faucet
Proteobacteria	57.41	68.25	Alphaproteobacteria	28.49	48.49
Actinobacteria	14.36	12.71	Betaproteobacteria	19.02	13.40
Bacteroidetes	10.18	7.35	Actinobacteria	11.46	10.62
Cyanobacteria	5.65	5.14	Gammaproteobacteria	7.50 <sup>a</sup>	5.10 <sup>a</sup>
Firmicutes	5.68 <sup>b</sup>	1.19 <sup>b</sup>	Chloroplast	4.06	2.56
Acidobacteria	1.38	1.36	Cytophagia	1.69	3.46
Nitrospirae	0.80	1.64	Saprospirae	2.67	2.07
Planctomycetes	1.15	1.12	Clostridia	3.99 <sup>b</sup>	0.66 <sup>b</sup>
Chlamydiae	0.47	0.31	Acidimicrobiia	2.50	1.99
Verrucomicrobia	0.46 <sup>b</sup>	0.10 <sup>b</sup>	Deltaproteobacteria	1.90	1.18
Gemmatimonadetes	0.30	0.24	Nitrospira	0.80	1.64
Chloroflexi	0.38	0.13	Flavobacteriia	1.34	0.95
Armatimonadetes	0.38	0.02	Bacilli	1.31	0.50
Spirochaetes	0.05	0.10	Holophagae	0.89	0.53
Synergistetes	0.14 <sup>a</sup>	0.0 <sup>a</sup>	Planctomycetia	0.69	0.46
Tenericutes	0.10 <sup>a</sup>	0.02 <sup>a</sup>	Phycisphaerae	0.45	0.66
Thermi	0.09	0.01	Sphingobacteriia	0.74 <sup>a</sup>	0.33 <sup>a</sup>
Elusimicrobia	0.02 <sup>a</sup>	0.06 <sup>a</sup>	Chlamydiia	0.47	0.31
Chlorobi	0.04	0.02	Solibacteres	0.17	0.40
Deferribacteres	0.02	< 0.01	Acidobacteria	0.16a	0.38 <sup>a</sup>

**Note.** <sup>a</sup>  $P < 0.05$ , <sup>b</sup>  $P < 0.01$ .



**Supplementary Figure S1.** Venn diagrams showing the number of unique and shared OTUs in the hospital water system. (A) The number of unique and shared OTUs in building 1 (including consulting room, dressing room, drinking room, office, washroom and treatment room samples); (B) The number of unique and shared OTUs in building 2 (including consulting room, respiratory ward, servery, treatment room, and washroom samples); (C) The number of unique and shared OTUs in building 3 (three washroom samples); (D) The number of unique and shared OTUs among the three buildings.

**Supplementary Table S7.** Bacterial community richness and diversity indices in all the water samples

Sample	Coverage	Sobs	ACE	Chao	Shannoneven	Simpson	Shannon	Simpson
1 <sup>st</sup> Consulting room 1	0.9988	377	419	426	0.4875	0.0188	2.89	0.141
1 <sup>st</sup> Consulting room 2	0.9984	476	528	551	0.6162	0.0225	3.80	0.093
1 <sup>st</sup> Dressing room 1	0.9995	244	260	265	0.5214	0.0256	2.87	0.160
1 <sup>st</sup> Dressing room 2	0.9992	317	344	344	0.5031	0.0228	2.90	0.138
1 <sup>st</sup> Drinking room 1	0.9991	492	522	564	0.6684	0.0355	4.14	0.057
1 <sup>st</sup> Drinking room 2	0.9994	443	458	456	0.6520	0.0312	3.97	0.072
1 <sup>st</sup> Office 1	0.9979	430	509	527	0.4498	0.0104	2.73	0.223
1 <sup>st</sup> Office 2	0.9990	887	915	929	0.7244	0.0371	4.92	0.030
1 <sup>st</sup> Treatment room 1	0.9993	310	339	340	0.4527	0.0122	2.60	0.264
1 <sup>st</sup> Treatment room 2	0.9990	546	582	587	0.6478	0.0313	4.08	0.060
1 <sup>st</sup> Wash room 1	0.9991	442	475	489	0.6699	0.0408	4.08	0.055
1 <sup>st</sup> Wash room 2	0.9993	348	367	381	0.6404	0.0406	3.75	0.071
2 <sup>nd</sup> Consulting room 1	0.9987	606	637	647	0.7293	0.0607	4.67	0.027
2 <sup>nd</sup> Consulting room 2	0.9982	659	695	709	0.7072	0.0544	4.59	0.028
2 <sup>nd</sup> Respiratory ward 1	0.9989	538	581	596	0.4911	0.0102	3.09	0.183
2 <sup>nd</sup> Respiratory ward 2	0.9982	483	542	551	0.4742	0.0084	2.93	0.246
2 <sup>nd</sup> Servery 1	0.9982	704	734	751	0.7433	0.0725	4.87	0.020
2 <sup>nd</sup> Servery 2	0.9994	610	627	638	0.7681	0.0849	4.93	0.019
2 <sup>nd</sup> Treatment room 1	0.9976	676	734	736	0.6788	0.0502	4.42	0.030
2 <sup>nd</sup> Treatment room 2	0.9974	758	808	829	0.7060	0.0458	4.68	0.029
2 <sup>nd</sup> Wash room 1	0.9979	576	633	638	0.5531	0.0112	3.52	0.155
2 <sup>nd</sup> Wash room 2	0.9988	652	679	682	0.6500	0.0203	4.21	0.076
3 <sup>rd</sup> Wash room 1	0.9995	901	919	925	0.7346	0.0396	5.00	0.028
3 <sup>rd</sup> Wash room 2	0.9997	647	654	661	0.6244	0.0231	4.04	0.067
3 <sup>rd</sup> Wash room 3	0.9993	1,046	1,069	1,094	0.6587	0.0240	4.58	0.040