CONTAMINATION AND TRANSMISSION OF MYCOBACTERIA IN INDOOR ENVIRONMENTS OF PUBLIC BUILDINGS

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SUMMARY

Objectives: The aim of this study was to detect *Mycobacterium tuberculosis* complex, *M. avium* subsp. *avium* and *M. intracellulare*, Mycobacterium contamination and to explore the aerosol transmission of mycobacteria in public buildings in China.

Methods: A total of 552 environmental samples, namely 165 aerosol, 199 water, 70 air duct dust, and 118 soil samples, were collected from 39 public buildings and analysed using nested polymerase chain reaction.

Results: The positivity rate of *Mycobacterium tuberculosis* complex, *M. avium* subsp. *avium* and *M. intracellulare* in air samples were 0.6% and 1.8%, respectively. There was significant difference in the positivity rate of Mycobacterium aerosol among the three types of public building ($\chi^2 = 6.108$, $p = 0.047$). No positive results of *Mycobacterium tuberculosis* complex and *M. avium* and *M. intracellulare* were obtained from cooling, tap, shower, or fountain water. The positivity rate of Mycobacterium for water samples was 31.7% (63/199). The positivity rate of *Mycobacterium tuberculosis* complex, *M. avium* subsp. *avium* and *M. intracellulare*, Mycobacterium in soil samples were 1.1%, 34.6% and 43.6%, respectively. There was significant difference in the positivity rate of *M. avium* and *M. intracellulare* ($\chi^2 = 47.219$, $p < 0.001$) and Mycobacterium ($\chi^2 = 33.535$, $p < 0.001$) in the different origins of soil samples.

Conclusions: Mycobacteria are widespread in public buildings. *Mycobacterium tuberculosis* complex, *M. avium* and *M. intracellulare* were simultaneously present in the air ducts of central air conditioning systems and indoor air in public buildings, which indicates that aerosol transmission is a potential route.

Key words: mycobacteria, public building, indoor environment

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INTRODUCTION

Mycobacteria can be divided into three groups: *Mycobacterium tuberculosis* complex (MTC), *Mycobacterium leprae*, and nontuberculous mycobacteria (NTM). *Mycobacterium tuberculosis* is considered the primary pathogen of tuberculosis in humans. Other members of the MTC associated with human TB infection with the highest prevalence in various parts of Africa (1). NTM are opportunistic pathogens and ubiquitous environmental microorganisms (2). The incidence of NTM pulmonary disease appears to be increasing throughout the world (3). NTM vary substantially in their ability to cause disease. Of the NTM, the *Mycobacterium avium* complex (MAC) species are the most common cause of human and animal disease globally (4). The MAC comprises *M. avium* and *M. intracellulare*, which account for over 70% of nontuberculous mycobacterial disease (5).

Mycobacteria have been isolated from various water and soil environments and constitute a substantial public health risk (6). Human infection occurs through the inhalation of aerosol containing mycobacteria (3). The contamination source of the aerosol may be natural water, showerheads and water taps, hot tubs and spas, fountains and pools, humidifiers, and flowerpots, as well as heating, ventilating, and air conditioning systems (7). Some studies have suggested that environmental exposure is a likely risk factor for the development or reestablishment of disease related mycobacteria (8).

In China, few studies have investigated the presence of mycobacteria in various environments. Methods for detecting mycobacteria include culturing, polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay. Environmental samples, especially air samples, are difficult to collect and detect because of low concentrations of microorganisms; consequently, high-sensitivity detection methods are necessary to reduce the rate of false negatives (1). PCR is an effective tool for rapid detection in environmental samples and overcomes some of the limitations of culturing, which include the necessity for special culture media and the exceedingly slow growth.

In this study, water, soil, dust, and indoor aerosol samples were collected from public buildings. MTC, *M. avium* subsp. *avium* and *M. intracellulare*, Mycobacterium were detected through nested PCR. The first purpose of this study was to confirm the presence and source of mycobacteria contamination in public buildings in

China, and the second was to explore whether indoor aerosol and the air ducts of central air conditioning systems in public buildings are possible transmission routes of mycobacteria.

MATERIALS AND METHODS

Study Sites

From June 2012 to September 2013, 39 public buildings located in four areas (Fengtai District of Beijing City, Nanjing City of Jiangsu Province, Changzhou City of Jiangsu Province, and Shenzhen City of Guangdong Province) in China were selected as study sites. The seven public buildings in Fengtai District comprised three supermarkets, two hotels and two hospitals. The nine public buildings in Nanjing City comprised four supermarkets, three hotels and two hospitals. The nine public buildings in Changzhou City comprised three supermarkets, three hotels and three hospitals. The fourteen public buildings in Shenzhen City comprised four supermarkets, six hotels and four hospitals.

These public buildings contained central air conditioning systems with air ducts that had not been cleaned and disinfected during the past year. The cooling towers and air ducts of the central air conditioning systems, fountains, water distribution systems, and flowerpots were randomly chosen as sampling sites.

Sample Collection

Cooling water and fountain water were collected from all public buildings. For hotels, tap water and shower water were also collected. A 500-mL water sample was collected in a sterile bottle that contained 0.25 mL of 0.1 equivalent l^{-1} sodium thiosulfate to neutralize disinfectants. Soil samples from flowerpots and fountains were collected in sterile 50-mL screw cap centrifuge tubes. The wipe sampling method was used to collect single-gram dust samples from the surfaces of the air ducts of air vents. The aerosol samples were collected using a biosampler (SKC, Eighty Four, PA, USA) operating at a flow rate of 12 L/min for 90 min. The impinger contained 20 mL of collection medium, which was 10 mmol L^{-1} phosphate-buffered saline and 0.01% (vol/vol) Tween-80. The aerosol samples were collected from the supply air outlets of central air conditioning systems and indoor air near flowerpots.

Nucleic Acid Extraction

The 500 mL water sample was filtered through 0.22-μm filters (Millipore, Billerica, MA, USA) and DNA was then extracted directly from the filters with the E.Z.N.A. water DNA kit (Omega, Guangzhou, China). DNA was extracted from a 1-g dust or soil sample by using the E.Z.N.A. soil DNA kit (Omega). For aerosol samples, 20 mL of collection medium was centrifuged at 8,000 ×g for 10 min, and 3 mL of collection solution was retained. DNA was extracted from 3 mL of concentrated collection medium with the E.Z.N.A. bacterial DNA kit (Omega). DNA was eluted in 100 μL of elution buffer (supplied in the kit) and stored at −20 °C prior to the nested PCRs.

A negative extraction control (purified glass beads) was included in each batch of samples for DNA extraction and was measured using nested PCR to exclude contamination in the process of DNA extraction.

Primer of Nested PCR

Primers of each nested PCR are listed in Table 1.

Nested PCR for *Mycobacterium Tuberculosis* Complex

The primer pair T1 and T2 was used to amplify a 567-bp product for the first PCR. The amplification conditions were 95 °C for 5 min, followed by 40 cycles of 94 °C for 1 min, 62 °C for 30 s, and 72 °C for 1 min, and a final 10 min incubation at 72 °C. The PCR product was used as the template of second round PCR. Primers T3 and T4 were used to amplify a 310-bp product through nested PCR. The amplification conditions were 95 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 56 °C for 30 s, and 72 °C for 1 min, and a final 10 min incubation at 72 °C.

The first and second PCR amplifications were performed in a 25-μL reaction mixture containing 2 μL of the template,

Table 1. *Primers used for nested PCR*

Target organism(s)	Primer	Oligonucleotide sequence	Product size (bp)	Target gene
Mycobacterium tuberculosis complex	T1	5'- GGCGGGACAACGCCGAATTGCGAA-3'	567	IS6110
	T2	5'- CGAGCGTAGGCGTCGGTGACAAAG-3'		
	T3	5'- TACTACGACCACATCAACCG-3'	310	
	T4	5'- GGGCTGTGGCCGGATCAGCG-3'		
Mycobacterium avium subsp. avium and Mycobacterium intracellulare	M1	5' - CACACTGCCCCACGACATC - 3'	842	DT1
	M2	5' - ACATCAGCGCGGAGTGGA - 3'		
	M3	5' - CACGAAGGAGTGGGTGCTC - 3'	504	
	M4	5' - GGGGAGGGTGTGAAGAACG - 3'		
Mycobacterium	M5	5'- CCCCACGATCACCAACGATG-3'	463	Hsp65
	M6	5'- CGAGATGTAGCCCTTGTCGAACC -3'		
	M7	5'- ACCAACGATGGTGTGTCCAT-3'	441	
	M8	5'- CTTGTCGAACCGCATACCCT-3'		

8 μL 2×GC-rich PCR mastermix, and 1 μL of each primer (10 μmol L^{-1}).

Nested PCR for *M. avium* subsp. *avium* and *M. intracellulare*

The primer pair M1 and M2 was used to amplify an 842-bp product for the first PCR. Primers M3 and M4 were used to amplify a 504-bp product through nested PCR. The first and second PCR amplifications were performed in a 25-μL reaction mixture containing 1 μL of the template, 12.5 μL of 2× Taq PCR Master Mix, and 0.2 μL of each primer (10 μmol L^{-1}). The amplification conditions for the first and second rounds of the PCRs were an initial denaturation step at 94 °C for 2 min, followed by 30 cycles of denaturation at 93 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s, and a final extension step at 72 °C for 5 min. The PCR products were diluted tenfold for use as templates in the second round of PCR.

Nested PCR for Mycobacterium

The primer pair M5 and M6 was used to amplify a 463-bp product for the first PCR. The first PCR amplifications were performed in a 25-μL reaction mixture containing 1 μL of the template, 12.5 μL of 2× Taq PCR Master Mix, and 0.5 μL of each primer (10 μmol L^{-1}).The amplification conditions were an initial denaturation step at 95 °C for 15 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 65 °C for 1 min, extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The PCR product was used as the template of second round PCR.

Primers M7 and M8 were used to amplify a 441-bp product through nested PCR. The second PCR amplifications were performed in a 25-μL reaction mixture containing 1 μL of the template, 12.5 μL of 2× Taq PCR Master Mix, and 0.2 μL of each primer (10 μmol L^{-1}). The amplification conditions were an initial denaturation step at 95 °C for 15 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 61 °C for 1 min, extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min.

Gel Electrophoresis

PCR products were identified through electrophoresis on a 1% agarose gel for 1 h at 100 V and visualized through GoodviewTM (TianGen, Beijing, China) staining under ultraviolet light.

Data Analysis

All data were analysed using the SPSS 16.0 for Windows statistical package. Contamination of mycobacteria in indoor environments of public buildings was summarized by frequency and percentage. The percent-positive results of different areas, public building types and origins were compared using the χ^2 test of row×column table. Differences were considered significant at $p < 0.05$.

RESULTS

A total of 552 samples, namely 165 aerosol, 68 cooling water, 55 tap water, 55 shower water, 21 fountain, 70 air-duct dust, and 104 flowerpot soil samples, as well as 14 soil samples from areas near fountains, were collected from 39 public buildings. Table 2 provides an overview of the distribution of the environmental samples.

Table 2. *Number and type of environmental samples (N = 552)*

Public building type	Air	Cooling water	Tap water	Shower water	Fountain water	Air duct dust	Soil from flowerpot	Soil nearby fountain	Total
Hotel	55	24	55	55	14	26	44	7	280
Supermarket	55	22	0	0	5	22	22	5	131
Hospital	55	22	0	0	2	22	38	2	141
Total	165	68	55	55	21	70	104	14	552

Table 3. *PCR results for aerosol samples in different areas (N = 165)*

Areas	Number of samples	Positive samples					
		Mycobacterium tuberculosis complex		M. avium subsp. avium and M. intracellulare		Mycobacterium	
		n	%	n	%	n	%
Fengtai District, Beijing City	30	0	0.0	1	3.3	4	13.3
Nanjing City, Jiangsu Province	40	0	0.0	0	0.0	4	10.0
Changzhou City, Jiangsu Province	45	0	0.0	2	4.4	13	28.9
Shenzhen City, Guangdong Province	50	1	2.0	0	0.0	8	16.0
Total	165	1	0.6	3	1.8	29	17.6

Contamination of Mycobacteria in the Air

Tables 3 and 4 show the PCR results for the aerosol samples in the different areas and public building types. No significant difference was observed in the positivity rate of Mycobacterium aerosol in the four areas ($\chi^2=6.019$, $p=0.111$). There was significant difference in the positivity rate of Mycobacterium aerosol among the three types of public building ($\chi^2=6.108$, $p=0.047$).

Contamination of Mycobacteria in Water

Tables 5, 6 and 7 show the Mycobacterium PCR results for the water samples in different areas, public building types and sample origins, respectively. The positivity rate of Mycobacterium for water samples was 31.7% (63/199). No significant difference was observed in the positivity rate of Mycobacterium for the water samples among the three types of public building ($\chi^2=0.316$, $p=0.854$) or in different origins ($\chi^2=0.071$, $p=0.995$). There was significant difference in the positivity rate of Mycobacterium in the water samples of four areas ($\chi^2=37.098$, $p<0.001$).

Contamination of Mycobacteria in Soil

Tables 8, 9 and 10 show the Mycobacterium PCR results for the soil samples in different areas, public building types and sample origins. No significant difference was observed in the positivity rate of *M. avium* subsp. *avium* and *M. intracellulare* for the soil samples in the four areas ($\chi^2=4.069$, $p=0.254$) or among the three types of public building ($\chi^2=4.069$, $p=0.254$). No significant difference was also observed in the positivity rate of Mycobacterium for the soil samples in the four areas ($\chi^2=2.590$, $p=0.459$) or among the three types of public building ($\chi^2=0.851$, $p=0.653$). There was significant difference in the positivity rate

Table 4. *PCR results for aerosol samples in different public building types*

Types	Number of samples	Positive samples					
		Mycobacterium tuberculosis complex		M. avium subsp. avium and M. intracellulare		Mycobacterium	
		n	%	n	%	n	%
Hotel	55	0	0.0	0	0.0	12	21.8
Supermarket	55	0	0.0	0	0.0	4	7.3
Hospital	55	1	1.8	3	5.5	13	23.6

Table 5. *Mycobacterium PCR results for water samples in different areas*

Areas	Number of samples	Positive samples	
		n	%
Fengtai District, Beijing City	36	21	58.3
Nanjing City, Jiangsu Province	55	2	36.0
Changzhou City, Jiangsu Province	52	24	46.2
Shenzhen City, Guangdong Province	56	16	28.6

Table 6. *Mycobacterium PCR results for water samples in different public building types*

Public building type	Number of samples	Positive samples	
		n	%
Hotel	153	48	31.4
Supermarket	21	6	28.6
Hospital	25	9	36.0

Table 7. *Mycobacterium PCR results for water samples of different origins*

Sample origin	Number of samples	Positive samples	
		n	%
Cooling water	68	22	32.4
Tap water	55	17	30.9
Shower water	55	17	30.9
Fountain water	21	7	33.3

***Table 8**. PCR results for soil samples in different areas (N = 188)*

Areas	Number of samples	Positive samples					
		Mycobacterium tuberculosis complex		M. avium subsp. avium and M. intracellulare		Mycobacterium	
		n	%	n	%	n	%
Fengtai District, Beijing City	30	1	3.3	10	33.3	14	46.7
Nanjing City, Jiangsu Province	32	0	0.0	10	31.3	12	37.5
Changzhou City, Jiangsu Province	61	0	0.0	27	44.3	31	50.8
Shenzhen City, Guangdong Province	65	1	1.5	18	27.7	25	38.5
In total	188	2	1.1	65	34.6	82	43.6

***Table 9.** PCR results for soil samples in different public building types*

Types	Number of samples	Positive samples					
		Mycobacterium tuberculosis complex		M. avium subsp. avium and M. intracellulare		Mycobacterium	
		n	%	n	%	n	%
Hotel	78	2	2.6	32	41.0	37	47.4
Supermarket	48	0	0.0	12	25.0	19	39.6
Hospital	62	0	0.0	21	33.9	26	41.9

***Table 10.** PCR results for soil samples of different origins*

Sample origin	Number of samples	Positive samples					
		Mycobacterium tuberculosis complex		M. avium subsp. avium and M. intracellulare		Mycobacterium	
		n	%	n	%	n	%
Soil from flowerpot	104	1	1.0	57	54.8	64	61.5
Dust from air duct	70	1	1.4	3	4.3	12	17.1
Soil from fountain	14	0	0.0	5	35.7	6	42.9

of *M. avium* subsp. *avium* and *M. intracellulare* ($\chi^2 = 47.219$, $p < 0.001$) and Mycobacterium ($\chi^2 = 33.535$, $p < 0.001$) in different origins of soil samples.

DISCUSSION

The environmental samples were considered representative because they were distributed throughout the public buildings, which included three types of building located in four areas. The types of public building comprised hotels, supermarkets and hospitals. The environmental samples comprised aerosol, water, dust, and soil samples. All types of environmental samples were collected from each hotel. Samples of hospital were collected from outpatient department. Samples of supermarket were collected from crowded sites such as checkout lane, entrance, corridor between shelves, etc., so shower water were not collected from hospital and supermarket.

All three positive aerosol samples for MTC and *M. avium* subsp. *avium* and *M. intracellulare* were collected from a hospital. The positivity rate of Mycobacterium in hospital was higher than hotel and supermarket. The detection of pathogenic bacteria in aerosol is difficult because of the low concentration (9). To collect microorganism-contaminated aerosol, an impinger is generally considered to be more effective than a filter, bubbler or impactor, not only because it prevents the collected samples from drying but also because it prevents fungal contamination and overgrowth of other bacteria, thereby providing a suitable collection medium for the subsequent detection assay (10).

The positivity rate of Mycobacterium for water samples was similar in different areas, public building types and origins. No positive results of MTC and *M. avium* subsp. *avium* and *M. intracellulare* were obtained from cooling, tap, shower, or fountain water. The results were similar to those of Lahiri et al. (11) study, whereas other studies have described *M. avium* and *M. intracellulare* contamination of the aquatic environment (7, 12). Evidently, the aquatic environment, including warm water distribution systems, showers, household drinking water, swimming pools, and hot tub spas, is a contamination source for MAC infection (13). Further research is necessary to investigate *M. avium* and *M. intracellulare* contamination in the aquatic environment of public buildings in China.

The results show that Mycobacteria are widespread in soil in public buildings. Contamination of soil and dust in public buildings is a serious problem. Precautions should be taken to limit microbial contamination and reduce the risk of human infection. *Mycobacterium tuberculosis* complex, *M. avium* subsp. *avium* and *M. intracellulare* and Mycobacterium were detected in surface dust in the air ducts of central air conditioning systems, which indicates that mycobacteria may spread from soil to air ducts or other environments through aerosol.

Although some studies have shown that human infections may be caused by inhalation or aspiration of mycobacterial aerosols from contaminated environmental sources, the lack of direct evidence is one of the gaps in our understanding of the manner in which mycobacteria are transmitted (14). Our results demonstrate that mycobacteria are present in the indoor air of public buildings and air duct of central air conditioning system, which suggests that aerosol transmission is a potential route for mycobacterial infection. Mycobacterial contamination of the surface dust in the air ducts of central air conditioning systems was demonstrated by this study. Nested PCR and phylogenetic analyses have indicated that air ducts, which are an essential part of central air conditioning systems, are the most frequent route of transmission for *Legionella* (15). Therefore, to prevent human infections, more attention should be paid to the air ducts of central air conditioning systems as potential transmission routes.

CONCLUSION

This study investigated environmental mycobacteria contamination of public buildings in China. Analyses of water, soil, and indoor aerosol samples showed that Mycobacteria are widespread in public buildings. *Mycobacterium tuberculosis* complex, *M. avium* and *M. intracellulare* were simultaneously present in the air ducts of central air conditioning systems and indoor air in public buildings, which indicates that aerosol transmission is a potential route for mycobacterial infection. This study contributes to our understanding of contamination sources and transmission routes of mycobacteria in public buildings in China.

Conflict of Interests

None declared

Authors' Contribution

Zhang BaoYing and Liang XiaoJun contributed equally to this work.

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