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Characterization of Potential Pathogenic *Cladosporium* Exposure Risks from Heating, Ventilation and Air Conditioning (HVAC) in Two Cities, China

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Summary

Cladosporium is widespread in Heating, Ventilation and Air Conditioning (HVAC). Some species in which are responsible for the majority of fungal infections or allergy in humans. To assess the exposure risks of *Cladosporium* in HVAC to human health, the colony distribution, population constituent and pathogenic species identification of *Cladosporium* were investigated in current study. We selected two cities, Beijing and Nanjing, representing the North and South of China respectively, for the study. Three kinds of public places including hospitals, hotels and super markets were selected and 108 air samples from 18 sampling sites were collected from air supply outlets of HVAC in summer, 2012. The collecting plates were incubated at 28°C for 2-3 weeks. Purified fungal colonies were identified at the genus and species level based on morphological and DNA sequences. Internal transcribed spacer ITS1 and ITS2 region, partial gene sequences of the translation elongation facter1-α gene (EF-1α) and the actin gene (ACT) were used for phylogenetic analysis. Among all genera detected, *Cladosporium* was identified to have the widest prevalence (92.3%) from HVAC, which was detected from 100 of 108 air samples. 9 species of *Cladosporium* were identified viz. *C. breviramosum*, *C. cladoporioides*, *C. cucumerinum*, *C. gossypiicola*, *C. macrocarpum*, *C. perangustum*, *C.* sp., *C. sphaerospermum* and *C. tenuissimum*. Among of them, *C. cladosporioides* had the highest concentration and frequency, considered as predominant species in HVAC. There were three species, *C. cladoporioides*, *C. sphaerospermum* and *C. macrocarpum,* distributed as opportunistic pathogen to human reported before. The total frequency of the three species was 76.29% of this genus. What's more, *C. cladoporioides* and *C. sphaerospermum* were detected in both cities with relatively high concentration and frequency. The frequency and prevalence rate in the South were higher than those in the North, suggesting that HVAC in the South was more susceptible to *Cladosporium* contamination.

Keywords: HVAC; Supply air; *Cladosporium*; Health risk; Public place

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Introduction

Heating, Ventilation and Air Conditioning (HVAC) are commonly used in residential, commercial or industrial buildings to maintain comfortable and ambient temperature. To a large extent, indoor air quality (IAQ) depends on the supply air from HVAC. However, supply air is susceptible to microbial contamination. Fungi as an important contamination source attracted great attention in recent years [1-4]. Contamination fungi not only transport into the rooms through HVAC, but also can grow on air filters or pipes that may be released into indoor environment. They may lead to adverse health issues including infectious diseases, respiratory pathologies and allergic reactions [5,6].

The continuous contact with airborne fungi through inhalation generally caused no particular effects for human health.

However, certain fungi and their metabolites are well-known agents that contribute to human diseases. *Cladosporium*, which is considered as an ubiquitous genera, could be frequently isolated in air samples taken throughout the year and distributed in different countries [7,8]. *Cladosporium* species are human opportunistic pathogens except for some cases of immune-compromised patients, but they are unlikely to have equal effects on health outcomes [9]. This genus comprises over 20 species as pathogens and allergen with clinical importance [7]. For instance, *C. cladosporioides*, which is widely distributed in the air, is reported to occasionally infect the lung, skin, eyes and brain of human, causing allergic mycoses [10].

Different geographical locations may be conducive to the presence of different fungi species. The climatic environment surrounding the buildings that equipped with HVAC impacts the background fungal concentrations [11,12]. The examination and characterization of typical fungal distributions at different geographical locations can be helpful for the clinical targeted prevention of allergic diseases.

Fungal spores, especially those of ascomycetes that include airborne fungi, comprise a large proportion of PM2.5 [13]. Particulate Matters (PM) is considered as one of the main evaluation indexes of air quality, mainly containing PM10 (particles with diameter of 10 μm or less) and PM2.5 (particles with diameter of 2.5 μm or less) [14]. PM10 contain sulfide, oxynitride, automobile exhaust and cooking-generated particles et al. PM2.5 is a complex mixture of dust, pollen, bacteria and fungi [15,16]. The size of the particle is a main determinant of where in the respiratory tract the particle will rest when inhaled [17]. Because of their small sizes, particles on the order of 10 μm or less (PM10) can penetrate into the deepest part of the lungs such as the bronchioles, and p Number of colonies articles smaller than 2.5 μm, PM2.5, tend to penetrate into the gas exchange regions of the lung such as alveolus.

In recent decades, the problems from *Cladosporium* contamination have attracted many researchers. In spite of their obvious importance, knowledge about the characteristics of *Cladosporium* from HVAC in public places is still limited. In order to determine the potential hazards of *Cladosporium* from HVAC in public places, an investigation was carried out in two geographical locations: Beijing and Nanjing, as typical metropolis in the North and South of China. The aim of this survey was to quantify the distribution and concentrations of the different species of *Cladosporium* flora from HVAC in public places. We focus on the potential pathogenic *Cladosporium* species from HVAC.

Materials and Methods

Sampling strategy and sites

Supply air samples from HVAC were collected in Beijing (E116o46' N39o92') and Nanjing (E118o46' N32o03') in 9 days (2012.8.23-2012.9.4). Indoor Particulate Matters (PM) was measured at the breathing zone height for each sampling site for 3-5 times (Table 1). The data of the outdoor temperatures and relative humidity (RH) during the sampling period were obtained from China Meteorological Administration (Table 2). A total of 108 (50 from Beijing, 58 from Nanjing) HVAC supply air samples were collected from 18 public places, including 9 (3 markets, 3 hotels and 3 hospitals)in Beijing and 9 (3 markets, 3 hotels and 3 hospitals) in Nanjing, respectively. We selected randomly floors and locations as sampling sites.

Sampling of fungi

Samples were collected by the Andersen 6-stage impactor (with aerodynamic cut-size diameters of 7.0, 4.7, 3.3, 2.1, 1.1 and 0.65 μm) located at 50 cm from air supply outlets, drawing air at a flow rate of 28.3 L/min for 5 min. The sampler was swabbed by 70% ethanol before every collection. Microorganisms were collected on Sabouraud's Agar in Petri-dishes located on all

Table1: PM of indoor sampling sites in survey.

City	Sampling site	Indoor		Outdoor	
		PM[a] 10 (mg/m³)	PM 2.5 (mg/m³)	PM 10 (mg/m³)	PM 2.5 (mg/m³)
Beijing (North)	Hospital 1	0.011	0.011	0.02	0.019
	Hospital 2	0.066	0.062	0.072	0.064
	Hospital 3	0.022	0.02	0.041	0.037
	Hotel 1	0.057	0.08	0.107	0.133
	Hotel 2	0.477	0.305	0.711	0.609
	Hotel 3	0.225	0.076	0.353	0.214
	Market 1	0.125	0.12	0.351	0.297
	Market 2	0.072	0.069	0.118	0.08
	Market 3	0.04	0.038	0.088	0.067
Nanjing (South)	Hospital 4	0.033	0.051	0.077	0.079
	Hospital 5	0.041	0.058	0.071	0.083
	Hospital 6	0.058	0.083	0.09	0.092
	Hotel 4	0.043	0.035	0.065	0.046
	Hotel 5	0.044	0.03	0.072	0.05
	Hotel 6	0.054	0.055	0.089	0.091
	Market 4	0.043	0.035	0.069	0.057
	Market 5	0.029	0.041	0.048	0.061
	Market 6	0.017	0.022	0.033	0.051

Table 2: The meteorology of sampling cities, Beijing and Nanjing.

Sampling City	Date	Max Temp. (°C)	Min Temp. (°C)	RH (%)
Beijing (North)	2012.8.23	30	16	58
	2012.8.24	27	22	56
	2012.8.27	32	21	47
	2012.8.28	34	22	52
	2012.8.30	26	15	94
	2012.9.4	29	15	52
Nanjing (South)	2012.8.21	31	26	80
	2012.8.22	31	24	77
	2012.8.23	29	22	70
	2012.8.24	29	22	68
	2012.8.26	30	24	90

* Data from China Meteorological Administration web (http://www.cma.gov.cn/en2014/)

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impactor stages. All samples were transported to the lab within 3 hours and then incubated for 7 days at 25°C. Concentrations were calculated as colony forming units per cubic meter of air (CFU/m^3).

CFU/m^3 was calculated as:

(Number of colonies×1000) / (Sampling time × Velocity of air flow rate)

Frequency was calculated as:

(Isolates fungi number)×100 / (Total fungi isolates number)

Identification

Microscopic observations of isolates were made from colonies cultivated for 7-14 d at 25°C on MEA. To study conidial development and branching patterns of conidia chains, squares of transparent adhesive tape were placed on conidiophores growing in the zone between the colony margin and 2 cm inwards.

Fungal colonies were established on agar plates, and genomic DNA was isolated as previously described [8]. To obtain resolution at species level for Cladosproium, the internal transcribed spacer ITS1 (5′-GGCGCATGGATAAACTATCC-3′) and ITS2 (5′-GATTATCCATTTGCCCCCAC-3′) regions were supplemented with partial gene sequences of the translation elongation factor 1-α gene (EF-1α) using the primers EF1-728F (5′-CATCGAGAAGTTCGAGAAGG-3′) and EF1-986R (5′-TACTTGAAGGAACCCTTACC-3′), and the actin gene (ACT)using the primers ACT-512F (5′-ATGTGCAAGGCCGGTTTCGC-3′) and ACT-783R (5′-TACGAGTCCTTCTGGCCCAT-3′)

PCR amplification program: 94°C for 5 min, 40 cycles of 94°C for 45 s, 48°C for ITS1(52°C for TEF and ACT) for 30 s, 72°C for 90 s and a final step at 72°C for 6 min. All amplicons were sequenced as described by Carbone et al. [18]. Basic local alignment search tool (BLAST) was used to identify the closest affiliated sequences in GenBank.

Phylogenetic analysis

Phylogenetic analyses were conducted to support phylogenetic species recognition and to better understand the evolutionary history of isolates, using the combined dataset of ITS, EF-1α and ACT sequences. 3 strains were randomly selected from each species to build phylogenetic tree (Table 3). Reference sequences obtained from GenBank were aligned using Clustal X (Table 4) [19]. Alignments were manually adjusted where necessary with MEGA version 5 [20]. Cladistics analyses using the Maximum Likelihood method were performed with the same program [21]. The Maximum Likelihood tree was constructed with Kimura 2-parameter model, including transitions and transversions and with pairwise deletion of gaps. Clade stability was assessed in a bootstrap analysis with 1000 replicates. In the phylogenies presented acceptable supported was interpreted as those branches with bootstrap support >70%.

Statistical analysis

Microsoft Excel® software was used for data entry and management. The SPSS Version 16.0 (SPSS, Standard Version) were used to perform one-way analysis of variance (ANOVA) (p<0.05). The original fungal concentrations were transformed by natural logarithm to approximate a normal distribution for the following analysis. The airborne fungal concentrations were presented as geometric mean (GM). The data were analyzed using descriptive statistics including median, mean and frequency. In addition, data analyses were conducted using non-parametric statistics, which do not require distributional assumptions (normal distribution). Spearman's rank (r) correlation test was used to determine the linear relationships between the two geographical locations.

Results

Cladosporium has the highest prevalence rate from HVAC

We firstly identified the fungi genera by morphological analysis supported by molecular biological technique. There were 99 genera detected in two cities. We list 28 genera of them as Table 5, which were same in two cities. *Cladosporium* was detected in 100 out of the 108 air samples with the highest prevalence rate of 92.3%, which was the most prevalent genus from HVAC among all of the fungi identified. There were 44 samples with *Cladosporium* detected in the North, while 56 in the South. The prevalence rate of *Cladosporium* in the South (96.6%) was significantly higher than the one in the North (80.0%) (p<0.05), suggesting that *Cladosporium* has wider distribution in the South than the North (Table 5).

Table 3: Information of stains used in phylogenetic tree.

Strain No.	Latin name	Area	Public place
L-1-21	*C. cucumerinum*	North	Market
NB51-4	*C. cucumerinum*	South	Market
NF51-15	*C. cucumerinum*	South	Market
J-2-1-24	*C. macrocarpum*	North	Market
ND52-9	*C. macrocarpum*	South	Market
GYDS-2-1	*C. perangustum*	North	Hotel
Z-1305-1-5	*C. perangustum*	North	Hotel
XGM-6066-1-14	*C. perangustum*	North	Hotel
NF41-12	*C. breviramosum*	South	Market
NC11-26	*C. breviramosum*	South	Hospital
FT-1-1-2	*C. breviramosum*	North	Hospital
NA51-1	*C. tenuissimum*	South	Hotel
NE21-14	*C. tenuissimum*	South	Hotel
BA-1-2-1	*C. tenuissimum*	North	Hospital
NB21-4	*C. cladosporioides*	South	Market
J-1-1-1024	*C. cladosporioides*	North	Market
NC41-1	*C. cladosporioides*	South	Hospital
NC42-22	*C .gossypiicola*	South	Hospital
NC11-5	*C .gossypiicola*	South	Hospital
ND52-3	*C .gossypiicola*	South	Market
DF-1-1025-6	*C. sphaerospermum*	North	Hotel
NC42-15	*C. sphaerospermum*	South	Hospital
NG32-10	*C. sphaerospermum*	South	Hospital
307-1-1	*Cladosporium.* sp	North	Hospital
ND32-19	*Cladosporium.* sp	South	Market
NC42-1	*Cladosporium.* sp	South	Market

Table 4: Strains used in phylogenetic analysis.

Taxon name	Collection	GenBank Accession No.		
		ITS	EF-1α	ACT
C. breviramosum	ATCC 76215	AF393683	-	-
	ATCC 64696	AF393684	-	-
C. cladosporioides	CBS 170.54	AY213640	FJ936162	HM148490
	CBS 112388	NR_119839	HM148244	HM148490
C .cucumerinum	CBS 177.54	KR912311	HM148322	HM148562
	CBS 176.54	JQ781722	HM148322	HM148567
C. gossypiicola	ATCC 38026	AF393702	HM148431	HM148680
	CNU 081002	KC339773	HM148431	HM148680
C. macrocarpum	CBS 181.54	NR_119657	EF679457	EF679534
	FF JaC 4	KM396371	EF679456	EF679533
C. perangustum	CBS 167.54	HM148124	HM148368	HM148613
	CBS 126365	HM148123	HM148367	HM148612
C. sphaerospermum	CBS 193.54	DQ780343	EU570261	EF101380
	CBS 102045	DQ780351	EU570262	EF101378
C. tenuissimum	CBS 262.80	HM148201	HM148446	HM148691
	CBS 117.79	HM148200	HM148445	HM148690
Toxicocladosporium irritans	CBS 185.58	EU040243	KJ564345	JN116660

Table 5: Prevalence (%) of *Cladosporium* from HVAC air samples.

Samples type	No.			%[a]		
	North	South	Total	North	South	Total
Acremonium	1	2	3	2	3.4	2.8
Alternaria	11	14	25	22	24.1	23.1
Arthrinium	4	6	10	8	10.3	9.3
Ascomycete	1	2	3	2	3.4	2.8
Ascomycota	6	5	11	12	8.6	10.2
Aspergillus	26	40	66	52	69	61.1
Chaetomium	2	4	6	4	6.9	5.6
Cladosporium	**44**	**56**	**100**	**88**	**96.6**	**92.6**
Cochliobolus	3	5	8	6	8.6	7.4
Coriolopsis	1	4	5	2	6.9	4.6
Emericella	2	1	3	4	1.7	2.8
Epicoccum	3	1	4	6	1.7	3.7
Fusarium	2	9	11	4	15.5	10.2
Gibberella	3	4	7	6	6.9	6.5
Leptosphaerulina	4	2	6	8	3.4	5.6
Magnaporthe	1	1	2	2	1.7	1.9
Neosartorya	1	4	5	2	6.9	4.6
Paecilomyces	4	11	15	8	19	13.9
Penicillium	29	36	65	58	62.1	60.2
Peniophora	1	1	2	2	1.7	1.9
Phanerochaete	2	16	18	4	27.6	16.7
Phoma	2	2	4	4	3.4	3.7
Pleosporales	2	5	7	4	8.6	6.5
Schizophyllum	1	5	6	2	8.6	5.6
Scopulariopsis	2	1	2	4	1.7	1.9
Tilletiopsis	7	19	26	14	32.8	24.1
Trametes	1	5	6	2	8.6	5.6
Trichoderma	1	5	6	2	8.6	5.6
Total samples	50	58	108	100	100	100

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Concentrations of *Cladosporium* from HVAC

The total fungal concentration from HVAC ranged from 50.9 to 1659.7 CFU/m^3, and the GM of total fungal concentration was 389.7 CFU/m^3. The *Cladosporium* concentration level was within a range of 4.7-254.4 CFU/m^3. The GM *Cladosporium* concentration was 77.9 CFU/m^3, accounting for 23.0% of total fungi concentration. In terms of two cities, the *Cladosporium* concentration ranged from 4.7 to 254.4 CFU/m^3 in the Northand 7.8-134.2 CFU/m^3 in the South. The GM *Cladosporium* concentration was 95.5 CFU/m^3 in the North and 60.2 CFU/m^3 in the South, respectively. From the data above, the North had a higher *Cladosporium* concentration than the South (p<0.05). However, the GM frequency of *Cladosporium* in the North was significantly lower than the South (p<0.05) (Table 6).

The Species of *Cladosporium* from HVAC

By morphological and molecular identification (Figure 1), we detected 9 species of *Cladosporium* from HVAC including viz. *C. cladoporioides*, *C. sphaerospermum*, *C. tenuissimum*, *C. perangustum*, *C.* sp., *C. gossypiicola*, *C. cucumerinum*, *C. macrocarpum*, and *C. breviramosum*, in descending order of GM concentration (Table 7). Among them, 8 species were detected in the North, and 7 in the South. 6 species were detected in both of the two areas including *C. cladoporioides*, *C. cucumerinum*, *C. perangustum*, *C.* sp., *C. sphaerospermum*, and *C. tenuissimum*. With the highest frequency (67.81%) overall, *C. cladosporioides* was revealed to be the predominant species of *Cladosporium* within the 9 species. Furthermore, our results demonstrated that *C. cladosporioides* GM concentration in the North was significantly higher than the South (p<0.05). Besides *C. cladosporioides*, the GM concentrations of *C. gossypiicola*, *C. macrocarpum*, *C. sphaerospermum*, *C. tenuissimum* in the North were higher than the one in the South.

Cladosporium distributed differently in three kinds of public places

We next sought to investigate the distribution of the 9 species of *Cladosporium* detected in three kinds of public places. As the results indicated, 9, 8 and 6 species were detected in hospitals, hotels and markets respectively. It was discovered that *Cladosporium* from the hospitals and hotels' HVAC had higher concentration than markets'. Besides *C. cladosporioides* which has the highest overall prevelance, *C. sphaerospermum* was another species had relatively higher concentration and frequency than the other 7 species. Additionally, compare to the *Cladosporium* concentrations in the hospitals and hotels, except for *C. cladosporioides*, the other 8 species had lower concentration in the markets (Table 8).

Discussion

Contamination from HVAC has been an increasing public health concern in the past several decades. Several studies reported that some fungi had high prevalence rate in HVAC including *Penicillium*, *Aspergillus*, *Alternaria*, *Aureobasidium* and *Cladosproium* [22-25]. However, reports that compare the prevalence of these fungi are still not available thus makes it hard to determine the most dominant one. We firstly reported here that *Cladosporium* was the most distributed contaminant fungi from HVAC in public places with the highest prevalence rate (92.3%) in all supply air samples. At species level within the *Cladosproium*, *C. cladoporioides* had the highest concentration and frequency (52.80 CFU/m^3, 67.81%) indicating they were were the predominant species from supply air in HVAC.

According to previous references, *Cladosporium* species have unequal effects on health outcomes [26-28]. We identified and assessed the potential risk to human diseases of different *Cladosporium* species detected from HVAC according to their distribution. *C. cladoporioides, C. sphaerospermum* and *C. macrocarpum* detected in our investigation have been reported to be allergenic especially asthma due to their spores or hyphal fragments [23,29]. The total frequency of the three species was 76.29% of this genus. Notably, *C. cladoporioides* and *C. sphaerospermum* were detected in both cities with relatively high frequency. The sensitization of the rest species has not been tested, and more clinical tests need to be done. Apart from these three species, *C. herbarum* is another dominant sensitizing fungus in air-spora. Previous literatures showed the existence of *C. herbarum* on floors, in carpets, HVAC insulation, filters and fans [30,31]. Zhang reported that *C. herbarum* were isolated from atmospheric air in Beijing [32]. Interestingly, we didn't detect *C. herbarum* in our investigation which might due to the small sample size in our study. ASHRAE (American Society of Heating Refrigerating and Air-conditioning Engineers) guidelines pointed out that the operating criterion for air-conditioning system application and cleaning should be designed to reduce fungal contaminant [33]. Although the exact load of fungal spores to initiate major infections or allergy is unknown, our data suggest that HVAC in public places should be meticulously maintained and frequently monitored to minimize the chance of fungi growing especially those potentially pathogenic species.

Currently, though fungal concentration is one of the most important factors causing sensitization, there are no federal standards or recommended guidelines from related organizations (e.g, American Society of Heating, Refrigerating and Air-Conditioning Engineers, ASHRAE; World Health Organization, WHO; The U.S. Department of Health and Human Services,

Table 6: The *Cladosporium* concentrations from HVAC.

Fungal species	Area								
	North				South				Total
	Min.	Max.	GM[a]	Median	Min.	Max.	GM[a]	Median	GM[a]
Total fungi (CFU/m^3)	66	1659.7	574.5	862.8	50.9	355.5	204.9	203.2	389.7
***Cladosporium* (CFU/m^3)**	4.7	254.4	95.5	50.1	7.8	134.2	60.2	71	77.9
***Cladosporium* Frequency (%)**	2.1	65.5	16.6	33.8	5.3	57.1	29.4	31.2	23

[a]Geometric mean (GM)

HHS) for airborne concentrations of fungi spores. The research focusing on the threshold of certain fungal concentration which might have potential pathogenic effects is also heavily lacking. A relatively early study done by Gravesen [34] pointed out that the concentration threshold for evoking allergic symptoms was 3000 spores/m^3 for *Cladosporium*. Despite our results were far less (4.71 to 254.42 CFU/ m^3) than Gravesen's suggestion, we cannot ignore their potential harmfulness especially considering the accumulative effects from HVAC in indoor environment with continual supply air. Meanwhile, certain individuals may react at lower concentrations due to their higher sensitivity.

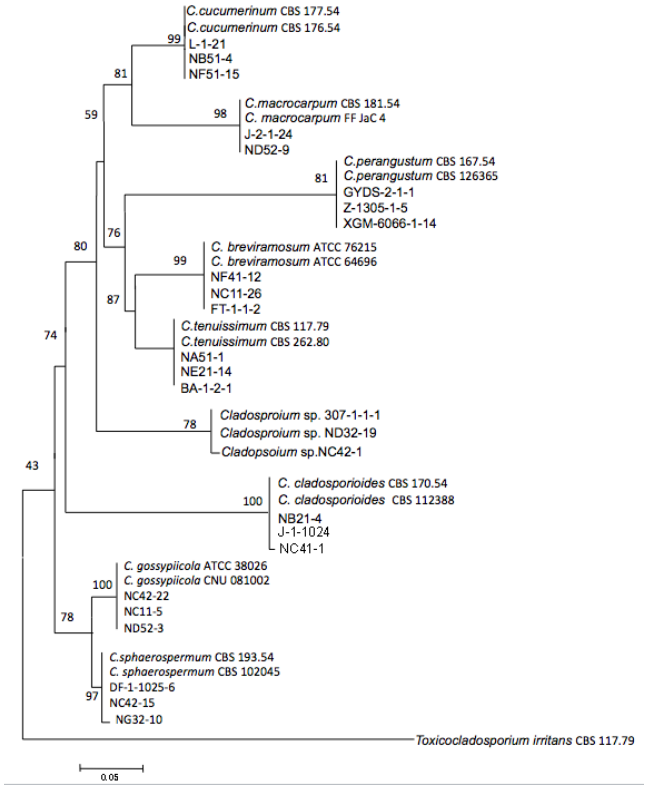

Figure 1 Maximum likelihood trees based on combined data set of ITS, EF-1αand ACT sequences, showing the relationship of species in *Cladosproium*. *Toxicocladosporium irritans* was chosen as out-group. The bootstrap values were obtained after a bootstrap test of 1000 replications.

Temperature and humidity are important factors in influencing the amount of fungus spores in the air. Schoch [35] reported that environmental RH typically conduces to *Cladosporium* growth more than temperature. Under normal RH range 50-95%, over 80% RH is more suitable for *Cladosporium* to thrive [30,36]. The average temperatures of our investigation samples were at 30˚C in the South and 28˚C in the North, and the humidity were 77% and 60% in two areas respectively. As expected, the prevalence rate of *Cladosporium* in the South was higher than that in the North. Meanwhile, according to the correlation analysis between *Cladosporium* and temperature (or RH) outdoor (Table 9), almost all *Cladosporium* species had a higher correlation coefficient with RH than temperature. Our results confirmed that *Cladosporium* growth received greater effect from RH than temperature. In addition, RH in the South is more suitable for *Cladosporium* growth than that in the North. The South RH was closer to 80% compared to that of the North which matched the results in our study that *Cladsporium* has a wider prevalence rate in the South. Therefore, our results suggest that the areas in the South are more widely contaminated than those in the North. Surprisingly, *Cladosporium* concentration is higher in the North than the South. Since the HVAC discharges atmospheric air currents into the indoor environment directly from the outdoor air sources [36,37], this seemingly discrepancy might be caused by a higher atmospheric background of *Cladosporium* concentrations in the North. We believe more information about the environmental factors such as the city atmosphere, construction and so on would be helpful to better understand the observation.

The Spearman correlation coefficient analysis between *Cladosporium* concentration and indoor PM10 and PM2.5

Table 7: Concentration and frequency of *Cladosporium* species from HVAC.

Fungal species	Area											
	North					South					Total	
	Min. (CFU/ m^3)	Max. (CFU/ m^3)	GM[a] (CFU/ m^3)	Median (CFU/ m^3)	Frequency (%[b])	Min. (CFU/ m^3)	Max. (CFU/ m^3)	GM[a] (CFU/ m^3)	Median (CFU/m^3)	Frequency (%[b])	GM[a] (CFU/ m^3)	Frequency (%[b])
C. breviramosum	0	0	0	0	0	0.35	0.35	0.35	0.35	0.58	0.18	0.22
C. cladoporioides	0.35	105.09	69.43	52.55	72.7	0.35	70.61	36.16	35.31	60.03	52.8	67.81
C. cucumerinum	0.35	1.41	0.22	0.71	0.23	0.71	4.24	1.17	2.12	1.94	0.7	0.89
C. gossypiicola	0.35	7.77	6.82	3.89	7.14	0	0	0	0	0	3.41	4.38
C. macrocarpum	0.35	0.35	0.35	0.35	0.37	0	0	0	0	0	0.18	0.22
C. perangustum	0.35	8.13	3.52	4.07	3.68	0.35	8.13	5.41	4.07	8.98	4.47	5.73
C. sp.	0.35	1.77	1.18	0.89	1.24	0.35	9.43	7.56	4.72	12.55	4.37	5.61
C. sphaerospermum	0.35	19.79	7.04	9.9	7.37	0.35	16.36	5.83	8.18	9.68	6.44	8.26
C. tenuissimum	0.35	7.07	6.93	3.54	7.26	0.35	14.24	3.75	7.12	6.22	5.34	6.86
Total Cladosporium	4.71	254.42	95.49	50.1	100	7.77	134.23	60.23	71	100	77.86	100

[a]Geometric mean (GM)
[b]% of the total *Cladosporium* (Geometric mean)

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Table 8: *Cladosporium* distribution in three kinds of public places.

	Hospital		Hotel		Market	
	GM (CFU/m³)	Frequency (%)	GM (CFU/m³)	Frequency (%)	GM (CFU/m³)	Frequency (%)
C. breviramosum	0.2	0.2	0.3	0.3	0	0
C. cladoporioides	62.7	69.2	65.3	65.6	30.4	70
C. cucumerinum	0.4	0.4	1.7	1.7	0	0
C. gossypiicola	3.6	4	2.9	2.9	3.7	8.5
C. macrocarpum	0.5	0.6	0	0	0	0
C. perangustum	7.7	8.5	5.4	5.4	0.3	0.7
C. sp.	2.1	2.3	8.2	8.2	2.8	6.5
C. sphaerospermum	8.1	8.9	6.3	6.3	4.9	11.2
C. tenuissimum	5.3	5.8	9.4	9.4	1.3	3
Total Cladosporium	90.7	100	99.5	100	43.4	100

Table 9: Spearman correlation coefficient (r) between 9 species and sampling cities' outdoor relative humidity (RH) and temperature (Temp).

Fungal species	Temp (°C)	RH (%)
C. breviramosum	0.471	0.501*
C. cladoporioides	0.290*	0.343**
C. cucumerinum	0.592	0.694
C. gossypiicola	0.208	0.586
C. macrocarpum	0.374	0.311
C. perangustum	-0.478*	-0.192
C. sp.	0.044	0.706**
C. sphaerospermum	0.447	0.771*
C. tenuissimum	0.136	-0.176
Cladosporium	0.201	0.274*

* Significant difference ($p<0.05$)
** Extremely significant difference ($p>0.01$)

suggested *Cladosporium* concentration had a moderate yet significant correlationship with PM2.5 (r=0.35, $p<0.05$) but not PM10 (r=0.047). According to the indoor PM10 and PM2.5 data measured in the current study, PM10 and PM2.5 in the North (0.122 and 0.087) sampling sites were significantly higher than those in the South (0.040 and 0.045) ($p<0.05$). This suggests that *Cladosporium* could affect IAQ by arising PM2.5 concentration, while this is indeed correlated with the higher spore concentration we observed in the north. It is of great interests to perform further assays in future to analyze the PM2.5 composition and determine to what degree *Cladosporium* contamination is contributing to the PM2.5 levels.

We characterized the distribution of allergic fungi from HVAC in current report. *C. cladosporioides* (r=0.343) and *C. macrocarpum* (r=0.311) showed low correlation with outdoor RH in spearman correlation coefficient analysis which might attribute to the relatively narrow range of the temperature and outdoor RH during our sampling period. They were also within the optimum range for *C. cladosporioides* and *C. macrocarpum*. A highly correlated relationship between *C. sphaerospermum* (r=0.771, $p<0.05$) and outdoor RH was found. Indeed, compared to North, the concentration and frequency of *C. sphaerospermum* were higher in the South where higher RH and temperature were recorded. As a conclusion, in summer, *C. cladosporioides* and *C. macrocarpum* have no humidity preference for distribution in two cities, but *C. sphaerospermum* is more suitable to grow in the South.

The patterns of other species of *Cladosporium* are contingent. Their distribution, frequency and concentration might also depend on a number of more direct surrounding environmental factors. For example, *C. cucumerimum* parasitizes on leaves, stems and fruit of *Cucurbitaceae*. The *Cucurbitaceae* distribution area around buildings affects *C. cucumerimum* concentration in HVAC. These species have not been reported as pathogenic to humans clinically.

The three types of public places covered in our study are all high traffic public areas. However, hospitals are special public place usually paired with health-related issues. There are a number of immunocopromised patients such as organ transplant patients and postoperative patients who are more susceptible to fungi-induced infection and allergy. It should be noted that both total fungi abundance and total concentration of the three allergic *Cladosporium* species were significantly higher in hospital than the other two places. Despite its low concentration, the pathogenic species *C. macrocarpum* was detected exclusively in hospital, suggesting more attention is necessary in monitoring and controlling fungi contamination in hospitals.

In summary, we revealed the population characteristics of *Cladosporium* at species level in HVAC of public places. This work provides the theoretical basis for prevention and the necessity of controlling the exposure risk caused by pathogenic fungi in HVAC.

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