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Distribution characteristics and analysis of fungal aerosol concentration and particle size in air-conditioned wards in Wuhan, China

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Abstract

Fungal contamination in the air of hospital wards can affect the health of medical staff, patients, and caregivers. Through systematic analysis of the concentration, types, and particle size distribution characteristics of fungi in the air of wards in Wuhan, China, in 2023, it was found that there was no significant correlation between the concentration of fungi in the air of wards and the disease type and personnel density. The main influencing factors were temperature, humidity, and seasonal changes. The distribution characteristics of fungal particle size in the wards of various departments in winter and summer showed a roughly normal distribution, with the percentage of particle size gradually increasing from stage I to stage III. The proportion from stage III to stage V was generally the highest, while the proportion from stage V to stage VI gradually decreased. There was no significant difference in the median diameter of airborne fungal conidia between different departments in winter and summer, and the median diameter of fungal conidia was less than 3.19 μm. The dominant fungal genus in the wards during winter and summer were basically the same, and there was no significant difference compared to wards in other inpatient buildings.

The current study indicates that more attentions should be paid to the increasing of filtration efficiency of fungal conidia particle sized from 1.1~4.7 μm, and appropriate antifungal and sterilization drugs, equipments and methods should be selected in the maintenance of daily hygiene, including the operation and management of the air conditioning systems in the inpatient wards.

Key words

inpatient ward, fungal aerosol, dominant genera, distribution characteristics

INTRODUCTION

Bioaerosols maintain high atmospheric concentrations due to natural events, human activities, and specific conditions in places like hospitals. In hospital environments, surgical procedures and ventilation systems significantly increase bioaerosol generation and spread. The size and distribution of these particles are critical for determining their deposition within the respiratory system [1].

The deposition of bioaerosol particles in the respiratory system is closely related to their particle size distribution characteristics. The particles with a particle size over 10 μm are almost completely deposited in the nasopharynx, about 10% of particles with a particle size of 2.0~5.0 μm are deposited in the bronchial area, and those with a particle size less than 2.0 μm are mainly deposited in alveolar tissue. Specifically, when the particle size of bioaerosols is within the range of 1.0~2.0 μm, about 50% of the particles are deposited in the alveoli, and the smaller the particle size, the greater the deposition amount. If the particle size of bioaerosols is smaller, their surface area is larger, and they can adsorb more heavy metals and volatile organic harmful substances, resulting in greater toxicity [2–5].

Fungi in the air usually exist in the form of conidia, which are one of the main components of bioaerosols. The distribution of fungal conidia in the air is wide, the number is huge, and there is significant taxonomic diversity among fungal conidia. Some of them can cause human fungal infectious diseases, such as fungal skin diseases and fungal visceral diseases. In recent years, due to the overuse of antibiotics, hormones, immunosuppressants, and anti-cancer drugs, the body's immune system has been weakened, and fungal infections have increased significantly [6]. Most of the infections are secondary, and are difficult to detect in the early stages. Fungi such as *Candida* spp., *Aspergillus* spp., and *Cryptococcus* spp. are prone to developing drug resistance and are difficult to cure. Fungal pathogenicity is related to its biological characteristics, local stimuli, destructive substances produced, fungal toxins, and carcinogenic effects. In addition, there are various types of fungi, and some fungi

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have strong invasiveness. After entering the body, they grow very quickly, especially in infected environments [7–10]. The nutritional requirements for the growth and reproduction of fungi are not high, and their tenacious reproductive ability is also one of the pathogenic mechanisms.

In recent years, research on indoor air fungal pollution has received great attention, with the majority focused on the types and concentration changes of indoor and outdoor air fungi, while neglecting the particle size and distribution characteristics of airborne fungal aerosols [11–12].

Compared to ordinary buildings, hospitals are public service places with special significance due to their different functions and user groups [13]. Pathogenic microorganisms in the air of hospitals are relatively concentrated, and long-term exposure to environments with high levels of airborne microorganisms can lead to many health problems. This applies especially to patients who have a relatively weak resistance to infection, and in whom the health impact will be more severe [14–16].

In this study, one general ward was selected from each of the paediatric, cardiology, and respiratory departments of a comprehensive hospital in Wuhan, China. Seven rounds were conducted of on-site tests and air microbial sampling in winter and summer. The fungal concentration, particle size, dominant fungal genera, and distribution characteristics in the air of the air-conditioned wards were systematically analyzed. The aim was to provide basic data for daily hygiene maintenance, air conditioning system design, and operation management of the wards.

MATERIALS AND METHOD

Sampling locations and times. The location of the testing sampling was the internal medicine building of a hospital with a total of 20 floors in Wuhan in 2023. In order to explore whether there is a correlation between the types and concentrations of microorganisms and the types of diseases, three general wards were chose in the Cardiology, Paediatrics and Respiratory departments, which were located vertically and overlapping in the Internal Medicine Building, respectively on the third, fifth, and twelfth floors. The area of each ward was approximately 20m². Two sampling points were set up in each ward, and the sampling instruments were arranged on the diagonal of the ward. Before sampling, the air conditioning in the three wards had been running for more than 24 hours. For the needs of comparison and analysis, a meeting room with the air-conditioner turned-off had been added as the ward control group. The pedestrian area at the entrance of the first floor of the internal medicine building was chosen as the outdoor sampling point in winter, and adjusted it to the fresh air inlet of the air conditioning system on the fifth floor in summer. During sampling, the distance between the sampler and the ground was 1.35 meters.

Considering the movement of patients and medical staff in the wards, the sampling time in winter and summer was set at two typical periods, namely, 09:00 12:00 and 14:30 – 18:00. The specific sampling dates in summer were 30 June, 5 and 10 July 2023, with a total of three rounds of sampling. The winter testing was performed in December, on 16, 20, 24, and 28, 2023, with a total of four rounds of sampling. The sampling instrument used was the six-stage impact air microbiology sampler (JWL-6)(Beijing Testing Instrument Co., Ltd.). Before sampling, the sampler was disinfected and sterilized with alcohol to avoid affecting the test results. During the sampling operation, the researcher wore a face mask to avoid the influence of people talking and coughing near the sampler; personnel activities near the sampling point were also minimized. Some technical parameters of the sampler are shown in Table 1.

Table 1. Technical parameters of the sampler

Stage	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI
Capture range/μm	>7.0	4.7~7.0	3.3~4.7	2.1~3.3	1.1~2.1	0.65~1.1
Aperture/mm	1.18	0.91	0.71	0.53	0.34	0.25
Effective interception of particle size/μm	8.2	6.0	3.0	2.0	1.0	0.65

Sampling, cultivation, and identification of fungi. Sabouraud agar was used for cultivation. The formula was as follows: 10g of peptone, 40g of glucose, 20g of agar, and 1000ml of distilled water. PH: 5.5~6.0.

The flow rate of the sampler was set to 28.3 L/min, and the each sampling time was 5 minutes. During sampling, the temperature, humidity, wind speed, personnel density were recorded at the sampling point. After sampling, the culture plate was quickly remove and covered, numbered in order, and then wrapped tightly. The culture plates were taken to the laboratory and placed in a constant temperature incubator at 26 °C for 4–6 days.

Observation and identification of cultured fungi were carried out using a 400x oil lens microscope. The relative humidity in the laboratory was 45~55%, and the temperature – 20~26 °C. Fungal species were identified based on their respective identification keys. According to Samson and Pitt (2000), 'Integration of Modern Taxonomic Methods For *Penicillium* and *Aspergillus* Classification' (CRC Press), *Penicillium* species were distinguished by their swift growth and a change in colony colour from white to blue-green or olive-gray when cultured on plates. They featured bottle-shaped conidiophores that develop into brush-like structures.

Meanwhile, *Aspergillus* species were distinguished by their vesicular conidiophores and conidia that are typically spherical to oval in shape. As described by Woudenberg et al. (2015), '*Alternaria* section *Alternaria*: Species, *formae speciales* or pathotypes?' (Westerdijk Fungal Biodiversity Institute), Alternaria species on a culture plate were characterized by conidia that are typically large and dark, often with short beaks and fine septations. According to Dugan (2017), 'The Identification of Fungi' (APS Press), *Cladosporium* species under the microscope are characterized by dark-pigmented, multi-cellular conidia with a pebbled surface texture. *Trichoderma* exhibited vibrant green conidia, round to ellipsoidal shape, densely clustered on branched conidiophores. *Rhizopus* was identified by its large, rhizoid-like hyphae and conidia that burst to release particles, forming a distinctive black mass. These morphological characteristics are key markers for identifying these fungal species. Colonies that did not produce conidia on the initial culture medium and displayed no signs of conidia formation after two weeks of inoculation and cultivation, were systematically classified as asexual [17].

Calculation of airborne fungal aerosol concentration and median diameter. A total of 687 effective data of fungal culture plates were obtained from winter and summer sampling, and the fungal concentration was calculated according to Eq. (1):

$$C = \frac{N \times 1000}{t \times 28.3} \quad (1)$$

where, C (cfu/m³) is the fungal concentration in the air, N is the number of all colonies in the culture plate, and t (min) is the sampling duration. The number of 1,000 means dilution factor and 28.3 is the air flow rate of the sampler (L/min).

According to Eq.(2), one can calculate the percentage of airborne particles of each stage in the sampler to the total number, and accumulate them in the order of stage VI to stage I to calculate the cumulative percentage of each stage. Then, based on the effective interception particle size and cumulative percentage of each stage in the sampler, the logarithmic regression equation can be obtained. When the cumulative percentage reaches 50%, the corresponding effective interception particle size value is the median diameter.

$$P = \frac{n}{N} \times 100\% \quad (2)$$

Here, P is the percentage of airborne particles of each stage, n denotes the number of colonies of each stage, and N is the total number of colonies of six stages.

RESULTS

Firstly, SPSS was used to perform the Shapiro-Wilk test (W-test) on the microbial concentrations of two sampling points in the same ward. The results showed that both sampling data in the ward followed a normal distribution (P>0.05). Independent sample t-test was then used to perform a difference test on the data from two different sampling points. The results showed that there was no significant difference between the two sampling data in the ward (P>0.05), which indicated that the distribution of airborne microbe in the same ward was approximately uniform.

Fungal concentration. In winter, the average fungal concentration in the air of the Cardiology Ward, sampled in four rounds, was the highest, at 343 cfu/m³. The average fungal concentrations in the Paediatrics, respiratory departments, and outdoor sampling point were similar, at 312 cfu/m³, 346 cfu/m³, and 342 cfu/m³, respectively. The outdoor sampling point in winter was set at the entrance of the Internal Medicine Building, with a larger flow of people. In summer, the concentration of fungi in the air sampled at each sampling point was lower than that in winter, and the average fungal concentration in the air of cardiology ward, sampled in three rounds, was also the highest – 179 cfu/m³. The average fungal concentration in the Respiratory Department, outdoors, Conference Room, and Paediatrics decreased sequentially, at 209 cfu/m³, 191 cfu/m³, 183 cfu/m³, and 152 cfu/m³, respectively. In summer, the outdoor sampling point was set at the fresh air inlet of the air conditioning system on the fifth floor, excluding the influence of passing pedestrians.

When compared to other studies in similar hospital environments, the obtained fungal concentrations in the current study were found to be within a similar range. For instance, a study conducted in Iran reported fungal concentrations in hospital wards ranging from 20–150 cfu/m³, depending on the ward and season, which aligns with the presented findings [18]. These comparisons indicate that the fungal concentrations obtained in the current study are typical for hospital environments.

The independent sample t-test was used to analyze the differences of data of each sampling point obtained in winter and summer, and the results showed that there was a significant difference (P<0.05), indicating that the season was a factor affecting the concentration of fungi in the air. However, in the same season, there was no significant difference in the air fungal concentration data between sampling points in different departments (P>0.05), indicating that the department of the hospital (disease type) was not a factor affecting the fungal concentration in the air of a general ward. In addition, the One-Way ANOVA was used to conduct a univariate analysis of the number of patients in a ward corresponding to the on-site sampling time, and it was found that the personnel density was not a significant factor affecting the fungal concentration in the air (P<0.05). At the same time, a univariate analysis was conducted on the temperature and humidity of the air in the ward during the sampling, and it was found that temperature and humidity were significant factors affecting the fungal concentration in the ward air (P>0.05).

Characteristics distribution of fungal particle size. Figure 1 showed the proportion of six stages of fungal aerosols in different sampling locations in winter and summer. It can be seen that the particle size distribution characteristics of fungi in the air of each ward were basically the same in winter and summer. The proportion of stages I–III gradually increased, while stages III–V had the highest overall. The proportion of stages V–VI gradually decreased, showing a normal distribution overall.

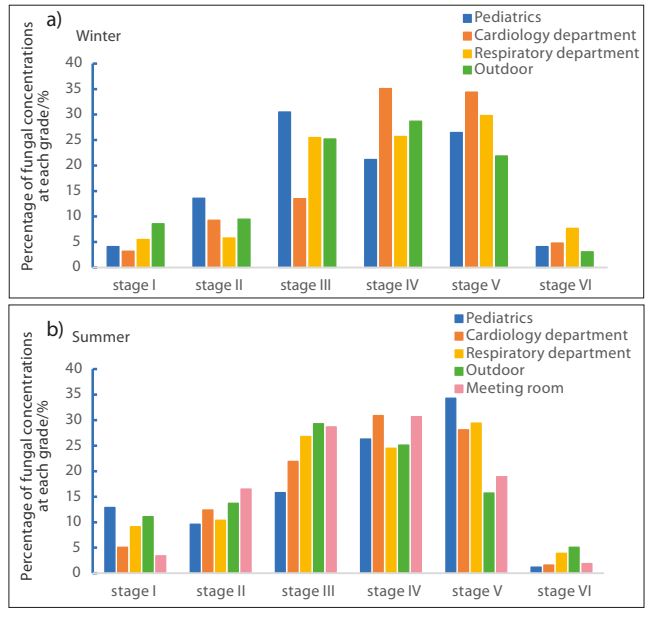

Figure 1. Percentage of fungal concentration at stages I–VI in wards of different departments. (a) Airborne fungal distribution characteristics in winter; (b) characteristics of airborne fungal distribution in summer

As shown in Fig. 1(a), the highest fungal concentration in the Cardiology Ward in winter was stage IV, accounting for 35.10%, with stages III–V accounted for 83.00%. The highest fungal concentration in the Paediatric Ward was stage III, accounting for 30.50%, and stages III–V accounted for 78.20%. The highest fungal concentration in the Respiratory Ward was stage V, accounting for 29.90%, and stages III–V accounted for 81.00%. The highest concentration of outdoor sampling point was stage IV, accounting for 28.70%, and stages III–V accounted for 75.80%.

As shown in Figure 1(b), the highest fungal concentration in the Cardiology Ward in summer was stage IV, accounting for 30.90%, while stages II–V accounted for 80.90%. The highest fungal concentration in the Paediatric Ward was stage V, accounting for 34.30%, and stages III–V accounted for 76.40%. The highest fungal concentration in the Respiratory Ward was stage V, accounting for 29.40%, while stages III–V accounted for 72.70%. The highest fungal concentration at the outdoor sampling point was stage III, accounting for 29.30%, while stages III–V accounted for 70.10%. The highest fungal concentration in the Conference Room was stage IV, accounting for 30.70%, while stages III – V accounted for 78.30%.

Based on the distribution percentage of airborne fungal conidia size at each sampling point and the effective interception particle size of each stage, the median diameter of airborne fungal conidia in different wards in winter and summer could be calculated. The results are shown in Figure 2, from which it can be seen that the median diameter of airborne fungal conidia at each sampling point in winter was 2.71 μm (Paediatrics), 2.38 μm (Cardiology), 2.50 μm (Respiratory), 3.13 μm (outdoor) and 2.67 μm (Conference Room). The median diameter of fungal conidia in summer air was slightly larger than that in winter, with values of 2.71 μm (Paqediatrics), 2.70 μm (Cardiology), 3.20 μm (Respiratory), 3.19 μm (outdoor) and 2.83 μm (Conference Room).

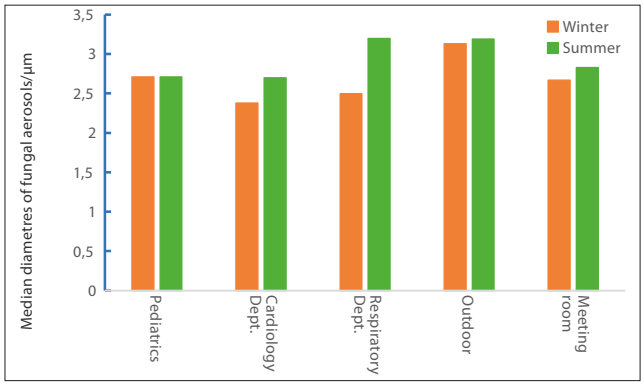

Figure 2. Median diameters of fungal aerosols in winter and summer

The calculation results showed that the median diameter of fungal conidia in the air at outdoor sampling points in winter and summer was slightly larger than that at other sampling points, which might be due to the filtration of larger dust particles by the air conditioning system in these wards. The median diameter of fungal conidia in the air of different wards was basically the same, indicating that personnel density and disease type had little effect on the median diameter. The median diameter of airborne fungal conidia in the same season was almost the same, and the particle size distribution characteristics of airborne fungi in different wards in winter and summer were also basically the same, indicating that seasons had little effect on the particle size of airborne fungal conidia. Due to the fact that most of the airborne fungal conidia sizes captured by the sampler were below stage III, the median diameter of airborne fungal conidia sizes was mostly less than 3.19 μm. Fungal conidia within this particle size range can enter the trachea or bronchi, and some can even enter the deep respiratory tract and even alveoli [18,19]. Therefore, the potential health hazards of small particle air fungal conidia to patients and medical staff should be taken seriously. When designing and operating air conditioning systems, dedicated filters should be added to improve the filtration efficiency for the airborne fungal conidia with particle size of 1.1~4.7 μm.

Characteristics of fungal colonies. A total of 914 fungal strains were cultured in various fungal culture plates obtained from the sampler in winter – 758 indoor strains and 156 outdoor strains; 22 genera of fungi were identified, including *Aspergillus, Penicillium, Fusarium, Candida, Trichoderma, Trichophyton, Cephalosporium, Epicoccum* and *Microsporum*. In summer, a total of 422 fungal strains were cultured in various culture plates of the sampler – 396 indoor strains and 26 outdoor strains. Six genera of fungi were identified, including *Aspergillus, Penicillium, Alternaria, Cladosporium, Trichoderma* and *Rhizopus*. Figure 3 shows the dominant genera and their proportions in the winter and summer samples.

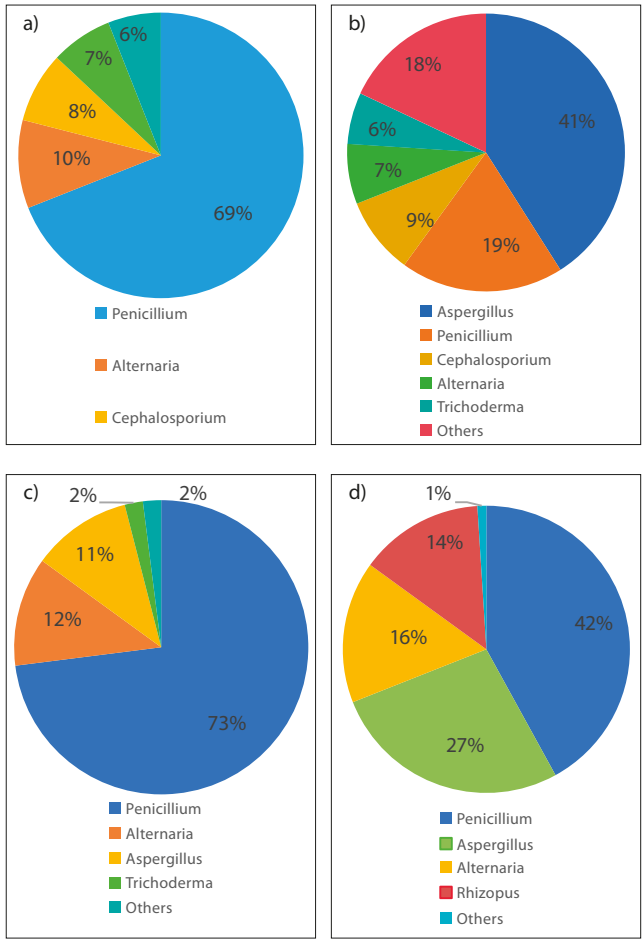

Figure 3. Dominant genera and their proportions in winter and summer samples. (a) Dominant indoor fungal genera in winter; (b) dominant outdoor fungal genera in winter; (c) dominant indoor fungal genera in summer; (d) dominant outdoor fungal genera in summer

From Figure 3 it can seen that the dominant genera of fungi in winter and summer were basically the same, but the proportion of each genus was different. The most common indoor fungus in winter was *Penicillium*, which accounted for 69.0% of the indoor fungal genera, followed by *Alternaria genus*, accounting for 10.0% of the total. The most common genus indoors in summer was also *Penicillium*, accounting for 73.0%, followed by *Alternaria* genus accounting for 12.0%, and then *Aspergillus*, with a percentage of 11.0%.

The personnel density and predominant disease type were recorded in each ward during both the winter and summer sampling periods. In winter, the Cardiology Ward had the highest personnel density, averaging 12 individuals per day (including patients, family members, and medical staff), while the Respiratory and Paediatrics Wards had average densities of 10 and eight individuals per day, respectively. In summer, the personnel density slightly decreased to 11 individuals per day in the Cardiology Ward and eight and seven individuals per day in the Respiratory and Paediatrics Wards, respectively. As shown in Table 2, the fungal concentrations in the Cardiology Ward were 343 cfu/m³ in winter (room temperature 26 °C, relative humidity 55%) and 179 cfu/m³ in summer (room temperature 26 °C, relative humidity 45%). The Respiratory Ward had fungal concentrations of 346 cfu/m³ in winter and 209 cfu/m³ in summer, while the Paediatrics Ward showed 312 cfu/m³ in winter and 152 cfu/m³ in summer. Statistical analysis showed no significant difference in fungal concentrations between wards with different disease types ($P>0.05$) or varying personnel densities ($P>0.05$). For instance, despite the higher personnel density in the Cardiology Ward, the fungal concentration in winter (343 cfu/m³) was not significantly higher than in the Paediatrics Ward (312 cfu/m³). Similarly, the Respiratory Ward, with a lower personnel density, had comparable fungal concentrations (346 cfu/m³in winter) to other wards.

These findings indicate that disease type and personnel density are not major factors influencing fungal concentrations in hospital wards. Studies have shown that the fungal concentrations in internet cafes, bars, schools, and hospitals in winter were 237 cfu/m³, 209 cfu/m³, 371 cfu/m³, and 138 cfu/m³, respectively, while the fungal concentrations in bars, residential areas, and hospitals in summer (before the rainy season) are 5012 cfu/m³, 3802 cfu/m³, and 49 cfu/m³, respectively [20]. This indicates that the sampling location and building type could significantly affect the indoor fungal concentration. These variations suggest that fungal concentrations are influenced by indoor temperature, humidity, and seasonal changes rather than disease type or personnel density.

Table 2. Fungal concentrations at various temperatures, humidity levels, and seasons in different hospital wards

Temperature (°C)	Fungal concentrations (cfu/m³)					
	Winter (Humidity 55%)			Summer (Humidity 45%)		
	Cardiology	Respiratory	Paediatrics	Cardiology	Respiratory	Paediatrics
20 °C	276	243	261	121	113	109
22 °C	309	259	286	139	148	110
24 °C	321	267	311	146	175	128
26 °C	343	346	367	179	209	152
28 °C	387	366	342	221	231	199
30 °C	398	387	378	257	253	234

DISCUSSION

This study found that the concentration of fungi in the air of wards in winter in Wuhan was higher than that in summer. This showed from the processing and analysis of data that the concentration of fungi in the air was closely related to environmental temperature and humidity. Seasonal variation of atmospheric fungal concentration in different regions might be due to the differences of regional climate [14–21]. The high temperature and heavy rainfall in Wuhan during summer could lead to the removal of fungal conidia from the air, which was not suitable for fungal reproduction and growth. In addition, the high temperature and humid climate conditions that occurred frequently in summer enhance the hygroscopicity of atmospheric aerosol particles, accelerated the wet deposition and removal process of fine particles, shortened the residence time of fungal conidia in the air, and led to a decrease of testing concentrations in summer [23–25].

Wuhan is known as the city of rivers, with the Yangtze River and Han River passing through, and lakes scattered throughout the city. Therefore, compared with other regions, the air in Wuhan in winter is relatively humid. Under the suitable temperature in air-conditioned wards, fungi can grow and reproduce on numerous hosts, resulting in a higher concentration of fungi in the air of wards in winter than in summer. The distribution characteristics of fungal particle size in hospital wards in the winter and summer seasons were basically the same, indicating that the season had no significant impact on the distribution of fungal conidia size in the air.

Through the detection of fungi in the air of winter and summer wards, it was found that the dominant genera were *Penicillium*, *Aspergillus, Cladosporium*, and *Alternaria*. After comprehensive analysis of existing literature, it was found that the dominant genera were basically consistent with those given in this study, excepting that the proportions of each genus varied in different regions and seasons. The above analysis indicated that the main fungal genera in the air were not affected by such factors as geographical location, building type, and season, but could affect the proportion of dominant fungal genera.

CONCLUSIONS

The fungal concentration in the air of wards in different departments was higher in winter than in summer. The fungal concentration in the air was not related to the disease type and personnel density, but could be influenced by such factors as indoor temperature, humidity, and seasonal changes.

The distribution characteristics of fungal particle size in wards of various departments were basically the same in winter and summer. The percentage of airborne fungal particle size showed a normal distribution from stages I – VI, with stages III – V accounting for the largest proportion. The fungal aerosols attached to the sampler in winter and

summer were mainly distributed in stages III – V (1.1 – 4.7 μm). The fungal concentration of stages III – V in winter accounted for 78.3% – 83.0% of the total concentration, and in summer it accounted for 70.1% ~80. 9%.

There was not much difference in the median diameter of airborne fungal conidia between wards of different departments in winter and summer. But the median diameter of airborne fungal conidia in most sampling points in summer is slightly larger than that in winter, and they all were less than 3.19 μm. The median diameter of fungal conidia in outdoor sampling points in winter and summer was larger than that in indoor sampling points. Therefore, when designing and operating the ward air conditioning system, it is necessary to increase the filtration effect of fungal conidia whose particle size is 1.1 – 4.7 μm.

The dominant genera of fungi in the air of wards in winter and summer were basically the same; however, the proportion of each genus was different. Overall, the dominant genera were *Penicillium, Aspergillus, Alternaria, Cladosporium, and Trichoderma* in order. Thus, appropriate antifungal and sterilization methods and equipment, in the daily hygiene of the ward and operation of the air conditioning system, should be selected in a targeted manner.

Institutional Review Board Statement. Ethical review and approval were waived for this study, due to the absence of sensitive data and to the processing of all personal information of the subjects involved in the study anonymously.

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