Detection of microorganisms in hospital air before and during the SARS-CoV-2 pandemic

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Abstract. – **OBJECTIVE:** Microorganisms present a global public health problem and are the leading cause of hospital-acquired infections. Therefore, it is essential to study the prevalence of microorganisms in hospital environments. The conclusion from such a study can contribute to identify the areas most likely to be contaminated in a hospital and appropriate measures that can decrease the exposure risk.

MATERIALS AND METHODS: The prevalence of microorganisms in hospital air was examined in different departments by obtaining air samples with an impactor before and during the SARS-CoV-2 pandemic. A total of 2145 microorganisms were identified, and the corresponding data were jointly analyzed by area, sampling period, and concentration.

RESULTS: The most frequently detected microorganisms in hospital air were Staphylococcus, Micrococcus, Neisseria, and fungi, and the more polluted departments were the hemodialysis department, respiratory department, treatment room, and toilet. Significant differences were found between the concentration of bacteria and fungi before and during the pandemic, which could be related to multiple environmental conditions. Furthermore, SARS-CoV-2 was negative in all the air samples.

CONCLUSIONS: Overall, this study confirmed the existence and dynamic characteristics of airborne microorganisms in a hospital. The results contribute to the adaptation of specific measures which can decrease the exposure risk of patients, visitors, and staff.

Key Words:
   Hospital microorganisms, COVID-19, Public health, SARS-CoV-2.

Introduction

Atmospheric pollution is one of the world's biggest challenges today due to the potential threat to human health and safety. Various elements can be detected in the air, of which microorganisms, such as bacteria, fungi, and viruses may be the most harmful to human health[1,2]. Poor ventilation conditions and hygiene can lead to a higher concentration of pathogenic microorganisms indoors than outdoors. Since humans spend most of their time indoors, poor indoor air quality could significantly increase the risk of human diseases[3].

Hospitals are a major source of airborne microbial contamination, and infections acquired by patients during a hospital stay are defined as hospital-acquired infections (HAIs) or nosocomial infections[2,4-6]. Several conditions have been identified that can influence the frequency and development of HAIs: type of microorganism, immune status of the patient, and environmental factors, such as temperature and humidity. The pathogens can spread transversely within the hospital, especially where there are large numbers of susceptible people, and pose a great threat to public health[7-10]. Furthermore, HAIs are not only associated with increased morbidity and mortality, but also with a substantial socioeconomic burden[11].

The average concentration of bacteria in hospital air is 75-1194 CFU/m^3, indicating a relatively high concentration of microorganisms in hospitals[12]. Long-term exposure to these high concentrations of microbial aerosols could reduce people's immunity, resulting in an increase in their susceptibility to diseases. This is especially significant in hospitalized patients who are more vulnerable due to illness or surgery[13,14]. In addition, in light of the current SARS-CoV-2 pandemic, the spread of pathogenic microorganisms in hospitals requires additional research[15,16]. The WHO guidelines stipulate that the main route of SARS-CoV-2 transmission is direct or indirect person-to-person transmission. However, scholars are still debating whether other routes, including airborne transmission, are also significant, especially in

the indoor environment[17]. Since most studies[18-20] have reported different results, this debate has not been resolved yet.

To safeguard people's health in China, the Ministry of Health released a national indoor air quality standard in 2002. According to this standard, the bacteria concentration should be limited to 2500 CFU/m^3 or less in public indoor spaces[21]. To adhere to the national standards and ensure the health and general well-being of the patients, visitors, and personnel, hospitals have strict disinfection protocols. The Chinese standard "Hygienic standard for disinfection in hospitals" (GB 15982-2012) was launched in 2012[22]. However, following the SARS-CoV-2 pandemic, frequent updates which contain stricter requirements have been released[23]. Research[20,24] has established that SARS-CoV-2 could be transmitted through bioaerosols in the air because it can survive in it for several hours and maintain infectious. However, additional investigation is still needed, mainly related to the viability of the virus[20,25,26].

Although the number of pathogenic microorganisms can reflect the spread of microbial aerosols in the hospital to a certain extent, the difference in bacterial and fungal density in the hospital air before and during the SARS-CoV-2 pandemic has not been thoroughly studied. This is the first study in which airborne bacteria, fungi, and the presence of SARS-CoV-2 were simultaneously detected in various departments of a hospital. The main objectives in this study were as follows: (1) evaluating the concentration and diversity of bacterial and fungal bioaerosols before and during the pandemic, while simultaneously measuring the temperature and relative humidity; (2) detection of SARS-CoV-2 virus in indoor air; and (3) analyzing the differences between detected results in different departments and sample periods. The air samples were first obtained with an impactor. Then these samples were cultured to identify potential bacterial and fungal pathogens in a hospital. In addition, a quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assay was used to detect SARS-CoV-2. The results contribute to establishing more targeted measures that can improve the safety of public areas and reduce HAIs.

Materials and Methods

Sampling Location

This study was performed in the Yan'an Hospital in Kunming, China, during 2019-2020. Yan'an hospital is a level 3 hospital of 52,000 m^2 with 1498 beds. The first confirmed case of SARS-CoV-2 in Kunming was reported in February 2020. The air samples were collected before the start of the pandemic in the winter of 2019 and again during the pandemic in 2020 from nine locations in the hospital, including the intensive care unit (ICU), newborn department (ND), hemodialysis department (HD), orthopedics department (OD), respiratory department (RD), treatment room (TR), toilet (TL), warehouse (WH), and outpatient hall (OH).

Air Sampling

Air samples were collected with a portable impactor (MAS100; Merck, Darmstadt, Hesse, Germany) which had a flow rate of 100 L·min^{-1} during five randomly selected days[27]. The collection took place prior to scheduled cleaning and lasted five to ten minutes. To simulate the breathing zone, air sampling was conducted with the impactor at a height of 1.5 m above the floor and 1 m away from the wall. The sampling locations were determined based on the evaluation methods as stipulated in the Chinese standard, GB 15982-2012[22]. Indoor areas of ≤ 30 m^2 were sampled in three locations on the inner, middle and outer diagonals. Indoor areas ≥ 30 m^2 were sampled in five locations at the four corners and the center. After each sampling, the impactor was disinfected with cotton wipes immersed in 70% ethanol.

A total of 180 samples, 20 from each location, were collected for further analysis. The temperature and relative humidity were simultaneously recorded by using a TES-1365 humidity temperature meter (TES, Taipei, Taiwan, China) with a data logging interval of 1 min.

Microbial Identification

All samples were immediately processed after arrival at the laboratory. Petri dishes with tryptic soy agar (TSA) were incubated at 37°C for 24-48 hours to assess the bacteria present in the air samples. Fungi were evaluated using malt extract agar supplemented with the antibiotic chloramphenicol (0.05%). These plates were incubated for 7 days at 25 C ± 1°C. The colonies on each plate were optically enumerated and calculated as colony-forming units per cubic meter (CFU/m^3). Samples of each culture medium were obtained in triplicate to increase the sampling accuracy. The mean values, standard deviation (SD), and total concentration were calculated for each sample and location.

TSA plates containing bacterial colonies were initially categorized according to the morphological characteristics: shape, size, and color. Then, the bacterial phenotype was further identified based on microscopic appearance and results of biochemical tests, including morphology, motility, reaction to Gram-staining, catalase activity, formation of endospores, and production of oxidase. According to these characteristics, the identified bacteria strains were divided into the following morphological groups: Gram-positive cocci, Gram-negative cocci, Gram-positive rods, and Gram-negative rods. The malt extract agar plates containing fungal colonies were also initially categorized morphologically by their color and shape of spores. To identify the dominant colonies, wet-mount slides with lactophenol blue were prepared and observed under a fluorescence microscope of 400× magnification (Olympus BX53, Tokyo, Japan).

SARS-CoV-2 Identification

The detection of SARS-CoV-2 in the indoor air samples was conducted by RT-PCR. The samples were first ultra-centrifuged for 1.5 h at 110,000 x g and 4°C. The RNA was extracted using the Vazyme viral RNA/DNA Mini Kit (Vazyme Biotech Co., Ltd, Nanjing, Jiangsu Province, China) following the manufacturer's protocol and collected in elution buffer. The specific primer and probe RT-PCR targeting the SARS-CoV-2 N gene and orf1a/b were detected using a Fosun COVID-19 RT-PCR Detection Kit (Shanghai Fosun Long March Medical Science Co., Ltd; Shanghai, China) to identify SARS-CoV-2 in the samples. PCR amplification was performed with a RT-PCR kit (BGI Biotechnology, Wuhan, Hubei Province, China) according to manufacturer's instructions. Amplification cycling was run under the following conditions: one cycle at 55°C for 10 min, one cycle at 95°C for 2 min, followed by 45 cycles of 95°C for 15 s, and 60°C for 30 s. Air samples were considered as positive if the Ct (cycle threshold) value was ≤38. All sample analyses were performed in triplicate.

Statistical Analysis

The data were analyzed with SPSS 20.0 4 (IBM Corp., Armonk, NY, USA). Differences between bacterial and fungal concentrations in different departments were analyzed using parametric (one-way ANOVA) and nonparametric statistical methods (the chi-square test). The t-test was used to analyze the statistical difference in concentrations from the same sampling locations before and during the SARS-CoV-2 pandemic. All results are presented as the mean value (±SD). A statistical significance level of $p < 0.05$ was applied during the statistical analyses.

Results

Overview of Microbiological Detection Results

A total of 180 samples were collected during the two sample periods, in which 2145 microorganisms were isolated and defined as positive results. Of the 2145 detected microorganisms, 87% (1868/2145) were bacteria and 13% (277/2145) fungi. These microorganisms included the following phenotypes: 72% Gram-positive cocci (1345), 16% Gram-negative cocci (296), 8% Gram-positive rods (149) ,4% Gram-negative rods (78), and fungi 13% (277), as shown in Figure 1. The most common isolated genera of microorganisms were *Staphylococcus, Micrococcus, Neisseria,* and the fungi *Penicillium*.

Detection of Microorganisms in Different Departments

The existence of microorganisms in the air was determined to assess the relationship between the concentration of bacteria and fungi in different hospital locations before and during the SARS-CoV-2 pandemic.
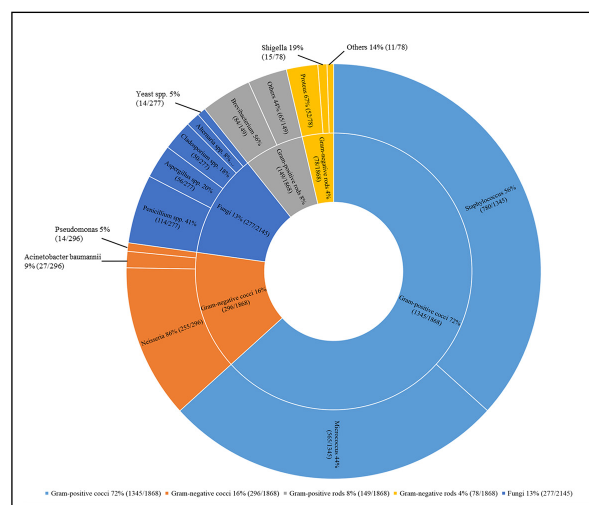

Figure 1. The types and number of microorganisms detected in the air samples during two sample periods across all 9 departments (N=2415).

Concentration of Bacteria

The mean ± SD of bacteria concentration in different hospital departments before and during the SARS-CoV-2 pandemic is summarized in Figure 2. The number of airborne bacteria before the pandemic had a range of 246.66–1343.33 CFU/m^3, while it ranged from 99.50 to 541.11 CFU/m^3 during the pandemic. The t-test results showed a statistically significant difference (t = 2.13, p = 0.009) between the mean concentration of airborne bacteria before (531.15±29.2) and during the pandemic (293.33±16.89). As shown in Figure 2, HD, RD, TR, and TL were the places with the highest concentration of bacteria detected in the air samples of both sample periods. There were two places with the lowest concentrations of bacteria in air samples: ICU (289.67±39.53 CFU/m^3) and ND (396±23.30 CFU/m^3). The results of the one-way ANOVA indicated a significant difference in bacterial concentration among different departments of the hospital (F = 11.03, p = 0.004).

Concentration of Fungi

Figure 3 represents the mean ± SD of fungi sampled from the air in different hospital departments before and during the SARS-CoV-2 pandemic. The number of airborne fungi before the pandemic ranged from 88.11-207.62 CFU/m^3, while it ranged from 28.75 to 134.91 CFU/m^3 during the pandemic. Results of the t-test indicated a statistically significant difference (t = 2.16, p = 0.002) between the mean concentration of airborne bacteria before (148.89±26.28) and during the pandemic (72±10.35). The largest concentration of fungi was also found in the HD, TL, and
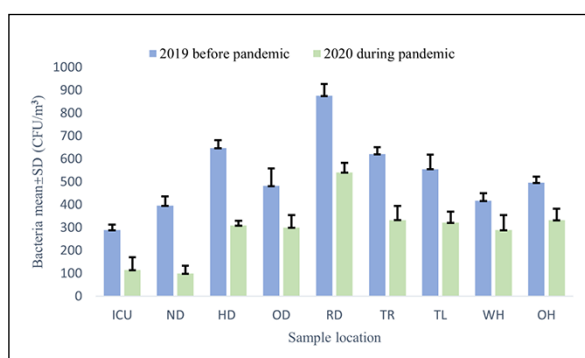
Figure 3. The mean ± SD of fungi in indoor air of different departments before and during the SARS-CoV-2 pandemic. Intensive care unit (ICU), newborn department (ND), hemodialysis department (HD), orthopedics department (OD), respiratory department (RD), treatment room (TR), toilet (TL), warehouse (WH), and outpatient hall (OH).
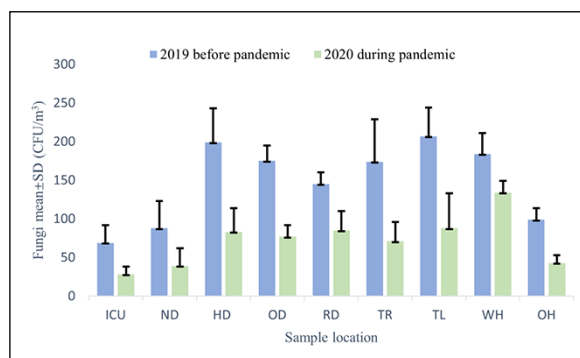
WH. This result was similar to the concentration of bacteria, however the differences in fungi concentration between these locations were smaller than that of bacteria. Notably, the largest concentration of fungi during the pandemic was found in the WH, while most bacteria were sampled in the RD.

Detection of SARS-CoV-2 and Environmental Conditions

During the air sampling, the average temperature and relative humidity in the different departments were 23.72°C and 38.61%, respectively (Table I). The results of the one-way ANOVA showed no statistically significant differences between the temperature (F = 0.017, p = 0.90) and relative humidity (F = 0.063, p = 0.81) before and during the pandemic. Furthermore, air samples taken from the nine departments during the pandemic were all negative for SARS-CoV-2 (Table I). Table I also shows the type of microorganisms that were identified the most in the air samples. *Staphylococcus* was the microorganism with the highest proportion among all sampled departments. It is worth noting that almost none of the sampled departments had exactly the same three types of dominant microorganisms.

Discussion

Many types of microorganisms can be suspended in the air, including viruses, bacteria, fungi, and their by-products. In hospitals, these microor-

Figure 2. The mean ± SD of bacteria in indoor air of different departments before and during the SARS-CoV-2 pandemic. Intensive care unit (ICU), newborn department (ND), hemodialysis department (HD), orthopedics department (OD), respiratory department (RD), treatment room (TR), toilet (TL), warehouse (WH), and outpatient hall (OH).

Table I. The detection results of microorganisms and SARS-CoV-2 in air samples were taken from different departments of the hospital during the SARS-CoV-2 pandemic in 2020. Intensive care unit (ICU), newborn department (ND), hemodialysis department (HD), orthopedics department (OD), respiratory department (RD), treatment room (TR), toilet (TL), warehouse (WH), and outpatient hall (OH).

Sampling location	Staff (n)	Temp (°C)	RH (%)	SARS-CoV-2	Top three microorganisms		
ICU	26	23.3	41	-	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Micrococcus luteus*
ND	20	24.7	34.3	-	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Micrococcus luteus*
HD	12	23.5	29.6	-	*Staphylococcus epidermidis*	*Micrococcus luteus*	*Neisseria*
OD	9	24.1	40.6	-	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Penicillium spp.*
RD	8	24.5	42.8	-	*Staphylococcus aureus*	*Micrococcus lylae*	*Neisseria*
TR	6	25.2	37.2	-	*Staphylococcus epidermidis*	*Micrococcus luteus*	*Staphylococcus aureus*
TL	2	23.1	43	-	*Staphylococcus aureus*	*Micrococcus luteus*	*Bacillus spp.*
WH	4	22.1	42.4	-	*Staphylococcus aureus*	*Micrococcus luteus*	*Neisseria*
OH	25	23.0	36.6	-	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Micrococcus luteus*

ganisms may come from outdoor air, and patients and visitors who spread it through coughing, sneezing, and respiration[28]. As a result, microbial aerosols in hospitals pose a threat to the health of patients and medical staff. Therefore, minimizing the spread of aerosols containing pathogens in the hospital air is essential to protect the health of patients and medical staff.

We collected air samples from different departments of a hospital before and during the SARS-CoV-2 pandemic to evaluate the differences in concentration of airborne microorganisms. The results indicated that the most frequently detected airborne microorganisms were *Staphylococcus and Micrococcus*. These microorganisms are considered common types in hospitals and have been reported by other researchers as well[12,29-31]. Wu et al[32] found that airborne microorganisms in a nursing institution mainly included *Acinetobacter baumannii, Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus*, similar to the results of this study. *Staphylococcus* bacteria are usually abundantly found in indoor environments as they are related to dust and physiological shedding of human skin, which diffuses through the air[23].

The highest concentration of microorganisms was identified in the RD, consistent with a study by Sivagnanasundaram et al[2]. The reason may be that patients in RD usually carry more pathogens than patients in other departments. However, contrary to the results of Sivagnanasundaram et al[2] and Qudiesat et al[34], the number of microorganisms identified in the ICU was the least, similar to the results of Latika et al[2,33,34]. Generally, the ICU has high sanitary conditions and ventilation mode because of the care for the most fragile patients.

Although an abundance of bacteria and fungi were detected in the air samples, SARS-CoV-2 was negative in all of them. A study conducted in a hospital in Iran[35] also found that the air samples were negative for SARS-CoV-2, similar to the results of a study by Ong et al[24] in Singapore and our results. However, whether airborne transmission of SARS-CoV-2 is feasible is not entirely elucidated yet, as research has shown opposing results. For example, Chia et al[20] collected air samples from hospital rooms where COVID-19 patients were admitted and detected a small number of positive air samples. Their results showed a correlation between the environmental contamination of virus particles and the stage of disease. Hemati et al[36] also obtained air samples from hospital rooms occupied by COVID-19 patients and identified SARS-CoV-2 by RT-PCR in 6 out of 45 samples (13.33%); however, a viable virus was not detected. The available evidence strongly suggests that airborne transmission is plausible, but it might not be the main route of infection, and a definite conclusion cannot be drawn yet[37,38].

The results of this study indicate significant differences between the concentration of bacteria and fungi before and during the SARS-CoV-2 pandemic. Research[28,39] has shown that the concentration of bacteria and fungi in the indoor air of hospitals is mainly affected by human occupation, temperature, humidity, outdoor air, and insufficient disinfection. Since no significant differences in temperature and relative humidity were detected between the two sample periods, other reasons for the differences in air contamination are indicated. Taking protocols implemented after the start of the SARS-CoV-2 pandemic into consideration, we hypothesize that human occupation and disinfection measures may be the main factors that contribute to our findings. Hospitals in China have implemented strict rules on the number of visitors after the pandemic broke out, in addition to intensified disinfection measures[23,40,41]. Research[42] has shown that each hospital has a distinct environment where some types of bacteria are more present and hazardous than others. Since our results have also shown differences in microbial colonies between different hospital departments, it is recommended to implement more targeted and specific disinfection protocols. These measures, in addition to intensifying disinfection, could reduce the indoor concentration of airborne bacteria and fungi.

Limitations

This study has a few limitations. Firstly, it was conducted at a single hospital in China; thus, the results cannot be generalized to other hospitals or regions. Secondly, the methodology may influence the findings as the type of impactor and air sampling could affect the efficiency of physical collection and accuracy of measurement[43].

Conclusions

The results of this study confirm once again that the hospital environment is a dynamic microbial environment. It is influenced by specific factors that may significantly affect the airborne concentration of bacteria and fungi. The air samples collected during the SARS-CoV-2 pandemic indicated lower concentrations of bacteria and fungi. In addition, no evidence for airborne transmission of SARS-CoV-2 was found as all the air samples were negative. These outcomes could result from limitations in human activity and stricter and more frequent disinfection measures. They can be taken into consideration in recommendations for further mitigative measures to effectively reduce the exposure risk of patients, staff, and visitors. However, as more factors could be related, further comprehensive research conducted in multiple institutions is needed to increase the understanding of factors that influence the levels of airborne microorganisms.

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