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Assessment of Hospital Indoor Airborne Microflora and Its Potential Risks for Nosocomial Infections Using Hi Airflow™ Sampler

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ABSTRACT
The aim of this study was to assess the diversity and distribution of bacterial and fungal pathogens in hospital indoor air using a specialized air sampler device (HiAirflow™ Sampler) that collects air samples onto agar media plates for microbial analysis. The study sampled air from 10 different wards and locations within a hospital using four different culture media plates: Nutrient agar, Malt extract agar, Pseudomonas agar, and Blood agar. The study identified and counted the bacterial and fungal colonies on each media plate using cultural and morphological characteristics as well as antimicrobial sensitivity testing. The study revealed that the hospital air contained a variety of microorganisms, including bacteria and fungi, some of which are associated with nosocomial infections. The study also showed that some of these microorganisms were resistant to certain antibiotics, which could complicate treatment. The study recommended that hospital air quality should be regularly monitored and controlled to prevent and reduce the risk of nosocomial infections in healthcare settings. The study also proved that HiAirflow™ Sampler is a convenient and efficient instrument for microbial air monitoring in controlled environments.

Keywords: *Hospital indoor airborne microflora; Nosocomial infections; HiAirflow™ sampler; Microbial air monitoring, Antimicrobial activity*.

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INTRODUCTION

Hospital indoor airborne microflora, which consists of microorganisms suspended in the air in hospital settings, can pose significant risks to patients, visitors, and healthcare workers [1]. These microorganisms can include bacteria, viruses, fungi [2] and other particles [3] that can originate from various sources within the hospital environment, such as patients, healthcare workers, contaminated surfaces, and ventilation systems. Inhalation, direct contact, and indirect contact are common routes of transmission for these airborne microorganisms, leading to respiratory tract infections, surgical site infections, and other infections, especially in immunocompromised individuals [4]. Patients with respiratory infections, wounds, or other infectious diseases can release microorganisms into the air through coughing, sneezing, or talking. Healthcare workers and visitors can also be sources of microorganisms through shedding of skin cells or carrying microorganisms on their clothing or personal items [5]. Contaminated surfaces, such as bed linens, curtains, and medical equipment, can also release microorganisms into the air when disturbed. Poorly designed or maintained ventilation systems can contribute to the spread of airborne microorganisms in hospitals. Hospital acquired infections (HAIs), also known as nosocomial infections, are infections that patients acquire during their stay in a healthcare facility, including hospitals. HAIs can be caused by a variety of microorganisms, including those present in hospital indoor airborne microflora. Factors such as

overcrowding, inadequate ventilation, poor hand hygiene, contaminated surfaces, and improper use of PPE can contribute to the spread of microorganisms and increase the risk of HAIs [6]. Transmission of hospital indoor airborne microflora can occur through inhalation, direct contact, and indirect contact [7]. Inhalation is a common route of transmission, where microorganisms suspended in the air can be inhaled into the respiratory tract, leading to respiratory infections [8]. Direct contact with contaminated surfaces or individuals can also result in transfer of microorganisms to the skin or mucous membranes, leading to infections [1]. Indirect contact can occur when microorganisms settle on surfaces and are then picked up by hands or objects, facilitating their spread to other areas of the hospital. To mitigate the risks associated with hospital indoor airborne microflora and HAIs, hospitals implement various control measures [9]. These may include ensuring proper ventilation and air exchange rates, using air filtration systems, implementing regular cleaning and disinfection protocols for surfaces, promoting hand hygiene among healthcare workers, isolating patients with infectious diseases, and providing appropriate PPE. Monitoring and surveillance of indoor air quality and microbiological testing of air samples can also help identify potential sources of airborne microorganisms and guide control measures [10]. Guidelines and regulations from national and international organizations such as the World Health Organization (WHO), Centers for Disease Control and Prevention (CDC), and Occupational Safety and Health Administration (OSHA), as well as local health department regulations provide guidance to hospitals on managing hospital indoor airborne microflora and preventing HAIs. Compliance with these guidelines and regulations is crucial in maintaining good indoor air quality and reducing the risk of airborne infections in healthcare facilities. In conclusion, hospital indoor airborne microflora can pose risks to patients, visitors, and healthcare workers, and can contribute to the spread of HAIs. Implementing appropriate control measures and adhering to guidelines and regulations are essential in mitigating these risks and maintaining a safe environment in hospitals [11]. The HiAirflow™ Sampler from HiMedia is a specialized air sampling device used for microbial air monitoring in controlled environments, such as cleanrooms, pharmaceutical manufacturing facilities, hospitals, and food processing areas. It collects air samples onto agar media plates or other suitable media for subsequent microbial analysis [12]. This Sampler is a portable instrument that utilizes a high-performance air pump to draw in a known volume of air through a calibrated inlet. This captures airborne particles onto the agar surface of the petri dish or other media. Its adjustable sampling flow rates allow users to optimize sampling parameters based on their specific requirements. Notably, the Sampler offers efficient sampling and is capable of collecting a wide range of airborne microorganisms, including bacteria, yeast, molds, and other particles. It is compatible with a wide range of agar media, which makes it versatile and suitable for general microbial monitoring, identification of specific microorganisms, or testing for environmental monitoring compliance. The sampler is designed for easy operation, with user-friendly controls and clear indicators for sampling flow rate and duration. It is lightweight and portable, which makes it convenient for field sampling or use in different locations. It may include a built-in data logging feature to record sampling parameters, such as flow rate, sampling time, and location, for traceability and documentation purposes. The HiAirflow™ Sampler is typically constructed with durable materials to withstand the rigors of environmental monitoring in various settings. It may also feature a high-quality HEPA filter to prevent contamination of the sampled air and protect the agar media from external contaminants. The sampler is designed to meet relevant industry standards and guidelines, such as ISO 14698 for microbial monitoring of cleanrooms and controlled environments. It may also be calibrated to ensure accurate and reliable sampling results [13]. In summary, the HiAirflow™ Sampler is a reliable air sampling instrument that is widely used in various industries for microbial air monitoring. Its features, including efficient sampling, versatile media compatibility, ease of use, data logging, robust construction, and compliance with relevant standards, make it a valuable tool for environmental monitoring and quality control in controlled environments. In this study air samples have been collected with the help of HiAirflow™ Sampler from 10 different locations of a hospital using four types of culture media plates: Nutrient agar for isolation of normal flora, Malt extract agar for isolation of fungus, *Pseudomonas* agar for isolation of *Pseudomonas aeruginosa* and Blood agar for fastidious pathogens. The number of colonies on each media from their respective location has been counted and further analysis has been done. The significance of this study is to assess the risk of nosocomial infections or hospital-acquired infections that can affect the patients and staff in the hospital environment. The study also concluded that several pathogenic organisms were isolated from the hospital environment that can cause nosocomial infections. These infections can be treated by using appropriate antibiotics. The study also suggested that infection prevention and control measures should be followed to prevent and control the spread of nosocomial infections in healthcare settings. The study also concluded that HiAirflow™ Economical V3.1 is a handy and easy-to-use instrument that can support up to 90 mm Petri plates for sampling. It has three sampling types: manual, programmed, and programmed sequence which allow researchers to choose their desired protocol

for collecting samples. It also has a date and timer setup that help to recognize previously saved data by date and time.

MATERIALS AND METHODS

STUDY DESIGN AND MEDIA PREPARATION

The study was carried out in August 2021. To isolate specific pathogenic organisms from the indoor hospital environment, four different culture media were prepared, N. agar, Malt extract agar, *Pseudomonas* agar, and Blood agar. The sampling process (figure 1) was carried out using a specialized air sampler, which was designed to draw air from the environment and deposit it onto the culture media plates. The media plates were exposed on the head of the sampler, and a manual program was initiated to control the flow rate and duration of air sampling. The volume of air sampled for this study was set at 500 liters, with a flow rate of 120 L/min, resulting in a sampling time of 4 minutes and 10 seconds for each location. At each specific location, all four different media plates were exposed for sampling, resulting in a total sampling time of 16 minutes and 40 seconds at each location.

COLLECTION OF AIR SAMPLES

Samples were collected from 10 different wards and locations within the hospital, including the outpatient department (OPD), the intensive care unit (ICU) on the 5th floor, the lobby of the 5th floor, the COVID positive ward, the waiting hall on the ground floor, the waiting area on the 4th floor, the general ward on the 3rd floor, the waiting area on the 2nd floor, the women's general ward on the 1st floor, and the operation theatre on the 1st floor. These locations were strategically chosen to represent different areas of the hospital, where the risk of airborne microorganism transmission may vary. The sampling process was conducted over two days, with only 5 locations covered on the first day due to logistical constraints. After collection, to ensure the integrity of the samples, all petri plates were carefully transported to the research laboratory of Gujarat University immediately after collection. The laboratory was located approximately 10 kilometers away from the hospital, and the samples were transported using appropriate measures to maintain their viability and prevent cross-contamination.

Upon arrival at the laboratory, the petri plates were carefully examined for any signs of contamination or damage. Subsequently, the plates were incubated at 37°C, which is the optimal temperature for the growth of most pathogenic microorganisms, for a period of 24 to 48 hours. This allowed for the growth of well-isolated colonies on each culture medium, which could then be further analysed.

ISOLATION AND IDENTIFICATION OF PATHOGENS

After the incubation period, the colonies on the culture media plates were carefully examined for their morphology, colour, and other characteristics, which can provide preliminary information about the type of microorganisms present in the air samples. Primary identification has been done on the basis of cultural and morphological characteristics to identify the specific pathogenic organisms present in the samples. Further antimicrobial sensitivity test was performed by Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standard Institute (CLSI) [14]. This information was crucial for understanding the potential risks posed by these microorganisms in the hospital environment and implementing appropriate control measures to minimize their spread.

RESULTS

ISOLATION OF PATHOGENS

After the incubation period, plates were observed for the growth of bacterial and fungal pathogens as shown in figure 2. The total number of colonies were counted and listed according to their location and media. As seen in table 1 the bacterial isolates were observed more in numbers as compared to fungal isolates. More than 300 colonies of bacteria were isolated on Nutrient agar media from five different locations including, the ICU on the 5th floor, waiting hall on the ground floor, waiting area on the 4th floor, waiting area on the 2nd floor and operation theatre 1st floor. Fungi were seen on the plate of malt extract agar from all the locations, in which the highest number of fungal isolates were obtain from the women's general ward on the 1st floor whereas the lowest number of fungi was isolated from operation theatre located on first floor. The number of colonies isolated on all four media from all ten locations is mentioned in table 1. The results in table 1 show that the number of colonies isolated on each medium varies greatly across different locations, indicating the presence of varying amounts and types of microorganisms in the air of each location. For Nutrient agar, the number of bacterial colonies ranges from 16 to >300, while fungal colonies were not detected in any of the locations except for location 6 where 4 fungal colonies were observed. For Blood agar, the number of bacterial colonies ranges from 8 to 111, and the number of fungal colonies ranges from 2 to 14. For Pseudomonas agar, the number of bacterial colonies ranges from 1 to 19,

while fungal colonies were not detected in any of the locations. For Malt extract agar, the number of bacterial colonies ranges from 1 to 47, while fungal colonies range from 1 to 30.
Overall, the table 1 suggest that the air in hospitals contains a diverse range of microorganisms, including bacteria and fungi, which can pose a potential risk to patients and healthcare workers. Further investigation and implementation of appropriate measures are needed to control and reduce the spread of these microorganisms in hospital environments.

IDENTIFICATION AND ANTIMICROBIAL SUSEPTIBILITY TESTING

On basis of the cultural and morphological characteristics, 9 different pathogens were identified, namely *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Enterococcus faecalis, Pseudomonas sp., Burkholderia pseudomallei* and *Porphyromonas gingivalis* and their antimicrobial susceptibility test has been performed. Antibiotics used were, Cephalothin (30 µg), Clindamycin (2 µg), Co-Trimoxazole (25 µg), Erythromycin (15 µg), Gentamicin (10 µg), Ofloxacin (1µg), Penicillin-G (10 µg), Vancomycin (30 µg).
Table 2 displays the results of the antimicrobial sensitivity testing carried out on different bacterial isolates. The isolates were subjected to different antibiotics, and the results of the tests were recorded as S (Sensitive) or R (Resistant). The antibiotics tested were CEP (Cefepime), CD (Clindamycin), COT (Co-Trimoxazole), E (Erythromycin), GEN (Gentamicin), OF (Ofloxacin), P (Penicillin-G), and VA (Vancomycin). *E. coli* was resistant to CEP but sensitive to CD, COT, E, GEN, OF, P, and VA. *K. pneumoniae* was sensitive to CEP, CD, COT, S, and VA but resistant to E and OF. *S. aureus* was sensitive to all antibiotics tested. *S. pneumoniae* was resistant to CEP and COT but sensitive to CD, E, GEN, OF, P, and VA. *S. pyogenes* was sensitive to all antibiotics tested. *E. faecalis* was sensitive to CEP, CD, COT, E, GEN, OF, and P but resistant to VA. *Pseudomonas sp.* was sensitive to all antibiotics tested. *B. pseudomallei* was resistant to CEP and CD but sensitive to COT, E, GEN, OF, P, and VA. *P. gingivalis* was resistant to CEP, CD, and COT but sensitive to E, GEN, OF, R, and VA.
In conclusion, the table provides valuable information on the antimicrobial sensitivity patterns of the different bacterial isolates tested. The information can be used to guide the choice of antibiotics for the treatment of bacterial infections caused by these isolates. However, it is important to note that the sensitivity patterns may vary depending on the geographical location, the prevalence of resistant strains, and the testing methods used. Therefore, it is recommended to perform antimicrobial sensitivity testing periodically and to update the sensitivity patterns regularly.

DISCUSSION

This manuscript describes a study conducted to investigate hospital indoor airborne microflora and its potential implications for nosocomial infections. Hospital indoor airborne microflora refers to the collection of microorganisms suspended in the air of hospital settings that pose risks to patients, visitors, and healthcare workers. These microorganisms can lead to respiratory tract infections, surgical site infections, and other infections, particularly in immunocompromised individuals. Hospitals employ various control measures, such as air filtration and ventilation systems, cleaning protocols, hand hygiene practices, isolation precautions, and personal protective equipment (PPE), to mitigate these risks. However, monitoring and surveillance of indoor air quality and microbiological testing of air samples are also crucial to identify potential sources of airborne microorganisms and guide control measures.
The objective of this study was to assess the diversity and distribution of bacterial and fungal pathogens in hospital indoor airborne microflora using a specialized air sampler device (HiAirflow™ Sampler) that collects air samples onto agar media plates or other suitable media for subsequent microbial analysis. Additionally, the study aimed to identify specific pathogenic organisms present in the air samples using cultural and morphological characteristics as well as antimicrobial sensitivity testing.
Similar study has been conducted by Latika Bhatia and Ritu Verma [15] on the quality and quantity of airborne microflora in two major hospitals in Sagar city, India. The researchers used different sampling and isolation techniques to collect and identify the microorganisms present in the air of four different wards of each hospital. They also compared the microbial load at different times of the day. They found that the general ward had the highest microbial load and the ICU had the lowest in both hospitals, and that the afternoon had the highest counts compared to the morning and evening. The most common fungal isolate was *Aspergillus sp*. and the most common bacterial isolate was *Staphylococcus sp*. The researchers concluded that the high level of airborne microflora could pose a health risk for patients and staff, especially those with compromised immunity, and that proper ventilation and disinfection measures should be implemented to reduce the microbial load.
In another study by F.O. Ekhaise and his coworkers [16] investigated the airborne microflora in two major hospitals in Benin City, Nigeria, using the settled plate technique. The paper aimed to establish a standard

for future reference and to assess the potential health risks of hospital indoor air. The paper found that the microbial load of the hospital air varied from wards to wards and from time to time, with the highest counts recorded in the children ward and the female ward in the evening. The paper also identified six bacterial and four fungal genera, some of which are known to cause infections and allergies. The paper concluded that human activities, ventilation systems, and environmental factors influenced the microbial population in the hospital air. The paper suggested that regular monitoring and control of hospital air quality is important for preventing cross infection and ensuring a healthy indoor environment.

In 2019 Arzu Kunwar and coworkers [17] evaluate the bacteriological quality of indoor air in various hospitals in the Kathmandu district and to determine the antibiotic susceptibility patterns of the bacteria that were isolated. The researchers found that the indoor air quality in all eight hospitals was subpar, receiving C- and D-grades in accordance with the European Union Guidelines to Good Manufacturing Practices. Out of the hospitals surveyed, *S. aureus* was the most common bacterium found in seven of them. Additionally, *Streptococcus sp.*, *Bacillus sp*., *E. coli*, *Proteus sp*., and *Pseudomonas sp*. were also identified. The susceptibility of the isolated *S. aureus* to gentamicin and ofloxacin was found to be high, whereas *Pseudomonas sp.* was highly susceptible to ceftriaxone and ofloxacin. However, Imipenem was found to be ineffective against *Pseudomonas sp.*

These findings have significant implications for the control and prevention of nosocomial infections in hospital settings. High bacterial load in indoor air can pose a potential health risk for patients, staff, and visitors, particularly those who are immunocompromised or have respiratory problems. To minimize the transmission and resistance of nosocomial pathogens, the researchers recommended regular monitoring of air quality, proper sanitation of hospital environment and equipment, and rational use of antibiotics.

Further research is needed to identify the sources and routes of bacterial contamination in indoor air and to evaluate the impact of air quality on health outcomes. Overall, this study highlights the importance of maintaining indoor air quality in hospitals to minimize the risk of nosocomial infections and promote the health and safety of patients, staff, and visitors.

Research by Suchithra Sudharsanam and colleagues [18] aimed to assess the indoor air quality in hospitals in South Chennai, India, by sampling the bioaerosols and identifying the predominant bacteria and fungi present. The study used the Petri plate gravitational settling method to collect air samples from various wards of three different hospitals. The study found that Gram-positive cocci, especially *Staphylococci* and *Micrococci*, were more common than Gram-negative bacilli, such as *Klebsiella sp.* and *Pseudomonas sp.* The researchers also isolated fungi such as *Aspergillus niger* and *Aspergillus flavus.* The study concluded that the bacterial counts were high in some wards, especially intensive care units and postoperative wards, and that some of the microorganisms isolated could cause nosocomial infections. The study recommended regular monitoring of indoor air quality in hospitals and preventive measures to maintain a healthy environment for patients and staff.

Table 1 Total number of colonies on culture media plate

Locations	Nutrient agar		Blood agar		*Pseudomonas* agar		Malt extract agar	
	Bacterial	Fungal	Bacterial	Fungal	Bacterial	Fungal	Bacterial	Fungal
1	33	-	111	8	01	-	-	12
2	>300	-	08	02	-	-	-	12
3	16	-	70	02	19	-	-	25
4	113	-	21	14	-	-	-	09
5	>300	-	44	-	09	-	-	15
6	>300	-	75	-	03	04	-	18
7	115	-	20	02	19	-	-	30
8	>300	-	50	-	16	01	-	09
9	111	-	108	-	03	02	-	47
10	>300	-	14	-	12	-	-	01

*__Locations__, **1)** Outpatient department (OPD), **2)** the intensive care unit (ICU) on the 5th floor, **3)** the lobby of the 5th floor, **4)** the COVID positive ward, **5)** the waiting hall on the ground floor, **6)** the waiting area on the 4th floor, **7)** the general ward on the 3rd floor, **8)** the waiting area on the 2nd floor, **9)** the women's general ward on the 1st floor, and **10)** the operation theatre on the 1st floor

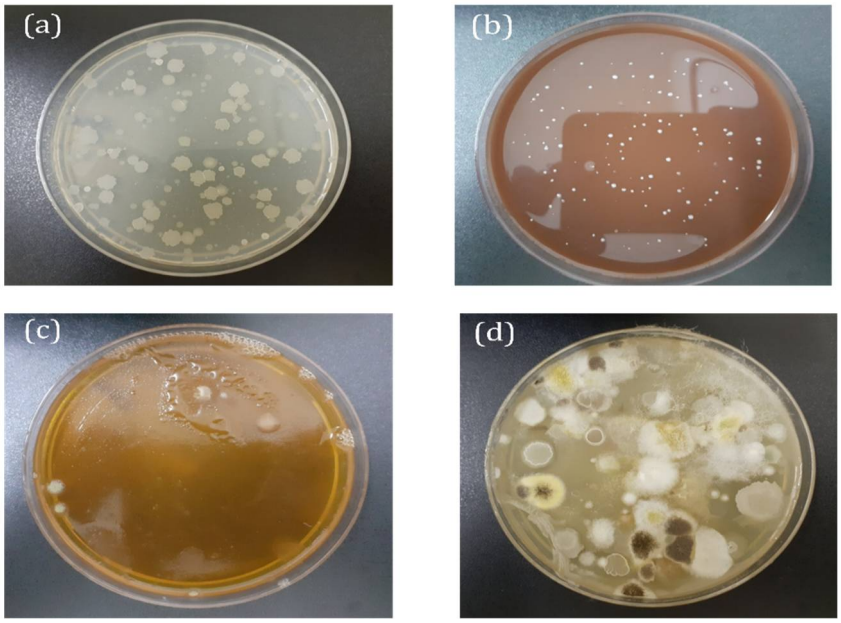
Table 2 Antibiotic sensitivity of isolated organisms

Organisms	Antibiotics							
	CEP	**CD**	**COT**	**E**	**GEN**	**OF**	**P**	**VA**
Escherichia coli	R	S	S	S	S	S	S	S
Klebsiella pneumoniae	S	S	S	R	R	S	S	S
Staphylococcus aureus	S	S	S	S	S	S	S	S
Streptococcus pneumoniae	R	S	S	R	S	S	S	S
Streptococcus pyogenes	S	S	S	S	S	S	S	S
Enterococcus faecalis	S	S	S	R	S	S	S	R
Pseudomonas sp.	S	S	S	S	S	S	S	S
Burkholderia pseudomallei	R	R	S	S	S	S	S	S
Porphyromonas gingivalis	R	R	R	S	S	S	R	S

*__S__ (sensitive), **R** (resistant), **CEP** (Cefepime), **CD** (Clindamycin), **COT** (Co-Trimoxazole), **E** (Erythromycin), **GEN** (Gentamicin), **OF** (Ofloxacin), **P** (Penicillin-G), and **VA** (Vancomycin)

Figure 1 Air sampling at hospital.

Figure 2 Isolation of organism on **(a)** Nutrient agar, **(b)** Blood agar, **(c)** *Pseudomonas* agar and **(d)** Malt extract agar.



CONCLUSION

Our study concluded that hospital indoor air contained a diverse range of microorganisms that posed a health risk to patients and staff with compromised immunity. The researchers recommended regular monitoring and control of hospital air quality, proper sanitation of the hospital environment, and

equipment to prevent and minimize the spread of nosocomial infections. Future research aims to investigate various aspects related to microbial load, air filtration, and control measures, including identifying the molecular and genetic characteristics of the isolated microorganisms and their resistance mechanisms to antibiotics. The results of this research are expected to have significant implications for healthcare facilities' design and operation, particularly in the context of preventing hospital-acquired infections.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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COMPETING INTEREST

The authors have declared that no competing interest exists.

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