



Original Research Article

Bioaerosol assessment of indoor air in hospital wards from a tertiary care hospital

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ABSTRACT

Introduction & Objective: Exposure to microorganisms suspended in the air of both occupational and residential indoor environments is associated with a wide range of adverse health effects with major public health impacts. The quality of indoor air is one of the most significant factors affecting the health and well being of people. So the present study was conducted to assess bacteriological and fungal concentration of the indoor air of a teaching tertiary care institute hospital.

Materials and Methods: The present study was carried for a period of three months from June 2018 to August 2018. Air sampling was performed with passive air sampling (settle plate's methods) according to the 1/1/1 scheme (a Petri dish with a diameter of 9 cm was placed for 1 hour, 1 meter above the floor, and about 1 meter away from the walls). Each ward Petri dishes was exposed for 60 min in the morning and afternoon. Bacteria and fungi was collected on nutrient Agar, Blood Agar and Sabouraud Dextrose Agar(SDA). To obtain the appropriate surface density for counting and to determine the load with respect to time of exposure, the sampling times were set at 60 min in the morning (at 10.00-11.00 AM) and afternoon (2:00-3.00 PM). Both quantitative and qualitative analyses was conducted.

Observation: The results indicate that the bacterial CFU/m³ air has been recorded in the range of 65.52 CFU/ m³ to 1179 CFU/ m³ at 60 min exposure. The results indicate that the fungal CFU/m³ air has been recorded in the range no growth to 262 CFU/ m³ at 60 min exposure. Gram Positive Bacteria were isolated more than Gram Negative Bacteria with predominance of *Staphylococcus aureus*. Whereas, the fungal isolates includes dominance of *Candida* spp followed by *Aspergillus* spp.

Conclusion: This study revealed that hospital buildings were being ventilated by the aid of natural ventilation system which may increase the possibility of entrance of pollutants from unhygienic external environment. Modern built environment can be a potential source of bioaerosols. Bio-aerosol monitoring in hospitals can be used for tracking of nosocomial infections, identify the source and spread of airborne microorganisms to control hospital associated infections (HAI).

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1. Introduction

Exposure to microorganisms suspended in the air of both occupational and residential indoor environments is associated with a wide range of adverse health effects with major public health impacts. The quality of indoor air is one of the most significant factors affecting the health and well being of people.^{1,2}

The air inhaled by people is abundantly populated with microorganisms which are also called bioaerosols. Bio-aerosols are airborne particles that are living (bacteria, viruses and fungi) or originate from living organisms.^{1,2}

Bioaerosols are ubiquitous, highly variable, complex, natural or man-made in origin. The sampling and analysis of airborne microorganisms has received attention in recent years due to concerns with mould contamination in indoor environments, the threat of bioterrorism and the occurrence of associated health effects, including infectious diseases, acute toxic effects, allergies and cancer.¹⁻³

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In many environments such as hospitals, the presence of bio aerosols can compromise normal activities. Infectious aerosols tend to be extremely small ($<5 \mu\text{m}$) and can, therefore, remain suspended and viable in the air stream over long periods of time, resulting in extremely high risk of airborne infection in confined places.^{4,6}

Nosocomial infection is a serious and widespread problem with many of the infections associated with person to person contact with an estimated 1 in 10 patients acquiring an infection during a hospital stay. While many of these infections are associated with person-to-person contact, there is increasing evidence that some infections are transmitted by the airborne route.^{4,6}

It has been calculated that the airborne route of transmission may account for as much as 10–20 % of all endemic nosocomial infections. Supervision on biological bio-aerosol in hospitals can provide information for epidemiological investigation of hospital infections, research on microorganisms present in the air, development and qualitative control, as well as information about their current status.^{7–9}

Quantitative and qualitative estimations of levels of microorganisms in the air reveal its value as an index for environmental hygiene and as an index related to human health. In hospitals, the problem of *Staphylococcus aureus* and *pseudomonas aeruginosa* and candida spp is a global public health problem, but it is particularly serious in resource limited countries.^{10–12} For the present study, we used settle plates technique^{5,8,10} to estimate bacterial load in the indoor air of wards.

Passive air sampling uses “settle plates”, which are standard Petridishes containing culture media, which are exposed to the air for a given time in order to collect biological particles which “sediment” out and are then incubated.^{10,11}

According to some authors,^{12–14} passive sampling provides a valid risk assessment as it measures the harmful part of the airborne population which falls on to a critical surface, such as in the surgical cut or on the instruments in operating theatres.

In addition, active air sampling is applicable when the concentration of microorganisms is not very high. However, where the building and environmental conditions of the hospital are very poor; we suspect that there will be very high concentration of microorganisms.^{13–16}

So the present study was conducted to assess bacteriological and fungal concentration of the indoor air of a teaching tertiary care institute hospital.

Both quantitative and qualitative analyses was conducted. The quantitative analysis was mainly conducted to determine bacterial/fungal load or number of bacteria/fungi in the indoor air. Qualitative analysis was conducted to identify specific species of bacteria.

2. Material and Methods

2.1. Study design

Cross-sectional study was conducted to assess the bacteriological/fungal concentration and to identify specific species of bacteria in the indoor air of tertiary care teaching hospital.

2.2. Sampling procedures^{10,17–20}

1. Air sampling was performed with passive air sampling (settle plate's methods) according to the 1/1/1 scheme (a Petri dish with a diameter of 9 cm was placed for 1 hour, 1 meter above the floor, and about 1 meter away from the walls or any major obstacles).
2. Air samples were taken from various randomly selected wards of the hospital, namely surgery, emergency, orthopedic, general ward, obstetric, medical ward, TB ward, etc which provides patient care services at the time of data collection.
3. In each ward Petri dishes was exposed for 60 min in the morning and afternoon. To minimize dilution of air contaminants, openings like doors and windows were closed including the mechanical ventilators during sampling. In addition, the movement of people during sampling was restricted to avoid air disturbance and newly emitted microorganisms.
4. Bacteria and fungi was collected on nutrient Agar, Blood Agar and Sabouraud Dextrose Agar (SDA).
5. To obtain the appropriate surface density for counting and to determine the load with respect to time of exposure, the sampling times were set at 60 min in the morning (at 10.00-11.00 AM) and afternoon (2:00-3.00 PM).

2.3. Air sample analysis

1. Both quantitative and qualitative analyses was conducted.
2. The quantitative analysis was mainly conducted to determine bacterial/fungal load or number of bacteria/fungi in the indoor air.
3. To determine the load, exposed culture medias/ air samples were taken to the laboratory and incubated at 37°C for 24 h. After 24 h incubation period, bacterial and fungal load was enumerated as colony forming units (CFU) and CFU/m^3 was determined by the formula $N = 5a * 10^4 (bt)^{-1}$ ^{17–19} where
 N = microbial CFU/m^3 of indoor air;
 a = number of colonies per Petri dish;
 b = dish surface (cm^2); and
 t = exposure time (minutes).
4. Qualitative analysis was conducted to identify specific species of bacteria and fungi by standard microbiological techniques.

Table 1: Bacteriological concentration of indoor air

S. No.	Departments/wards	CFU/m ³		Colonies per plate	
		Morning (10.00-11.00 AM)	Afternoon (2:00-3.00 PM)	Morning (10.00-11.00 AM)	Afternoon (2:00-3.00 PM)
1.	TB chest	1179	982	90	75
2.	Medicine (Male)	655	655	50	50
3.	Medicine (Female)	786	655	60	50
4.	ICCU	1048	786	80	60
5.	NICU	524	327	40	25
6.	Surgery (Male)	327	327	25	25
7.	Surgery (Female)	655	655	50	50
8.	ENT	524	524	40	40
9.	Casualty	786	786	60	60
10.	Gynaecology	131	131	10	10
11.	Obstetric	131	131	10	10
12.	Orthopaedics (Male)	262	262	20	20
13.	Orthopaedics(Female)	196	196	15	15
14.	Nephrology unit	65	65	05	05
15.	Ophthalmology	393	393	30	30
16.	Paediatrics	524	524	40	40
17.	Central clinical lab	786	655	60	50
18.	Operation theatres (General, surgery, minor, Gynaecology, etc)	No growth		NA	

Table 2: Fungal concentration of indoor air

S. No.	Departments/wards	CFU/m ³		Colonies per plate	
		Morning (10.00 -11.00 AM)	Afternoon (2:00-3.00 PM)	Morning (10.00 -11.00 AM)	Afternoon (2:00-3.00 PM)
1.	TB chest	157	65	12	05
2.	Medicine (Male)	65	65	05	05
3.	Medicine (Female)	131	52	10	04
4.	ICCU	131	65	10	05
5.	NICU	No Growth		NA	
6.	Surgery (Male)	No Growth		NA	
7.	Surgery (Female)	39	39	03	03
8.	ENT	No Growth		NA	
9.	Casualty	262	196	20	15
10.	Gynaecology	23	26	02	02
11.	Obstetric	No Growth		NA	
12.	Orthopaedics (Male)	65	65	05	05
13.	Orthopaedics (Female)	39	26	03	02
14.	Nephrology unit	No Growth		NA	
15.	Ophthalmology	131	104	10	08
16.	Paediatrics	26	39	02	03
17.	Central clinical lab	104	65	08	05
18.	Operation theatres (General, surgery, minor, Gynaecology, etc)	No growth		NA	

Table 3: Microbiological profile

S. No	Departments/wards	Media used	Microbiological profile
1.	TB chest	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Micrococci, Staphylococcus aureus, GPB, Candida
2.	Medicine (Male)	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	GPC, GPB, Aspergillus
3.	Medicine (Female)	Blood Agar Nutrient Agar Sabouraud's dextrose AGAR	Staphylococcus aureus, Candida, Aspergillus, Bacillus
4.	ICCU	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Staphylococcus aureus, Candida
5.	NICU	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Staphylococcus aureus, GPB
6.	Surgery (Male)	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	E.Coli, Staphylococcus aureus,
7.	Surgery (Female)	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Staphylococcus aureus, Candida, GPB
8.	ENT	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Staphylococcus aureus, GPB, Micrococci
9.	Casualty	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Staphylococcus aureus, Klebsiella spp, Candida, Aspergillus, Pseudomonas
10.	Gynaecology	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Staphylococcus aureus, Candida, Diptheroids
11.	Obstetric	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Staphylococcus aureus, GPB
12.	Orthopaedics (Male)	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Staphylococcus aureus, Candida
13.	Orthopaedics(Female)	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Staphylococcus aureus, Candida
14.	Nephrology unit	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Staphylococcus aureus, Coccobacilli
15.	Ophthalmology	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Staphylococcus aureus, Candida, Clostridium
16.	Paediatrics	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	Staphylococcus aureus, Candida, Aspergillus
17.	Central clinical lab	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	GPB, GPC, Klebsiella, Staphylococcus aureus, Candida
18.	Operation theatres (general, surgery, minor, Gynaecology, etc)	Blood Agar Nutrient Agar Sabouraud's dextrose Agar	NA

GPB-gram positive bacilli, GPC-gram positive cocci

3. Observation and Results

The present cross-sectional study was conducted to assess the bacteriological/fungal concentration and to identify specific species of bacteria in the indoor air from a tertiary care teaching hospital for period of 3 months (June 2018 to August 2018)

3.1. Quantitative Analysis of Bioaerosol Concentration (load)

3.1.1. Bacterial load

The results indicate that the highest bacterial CFU/m³ air has been recorded at 10.00 am in TB chest ward which is 1179 CFU/ m³ at 60 min exposure, while the lowest bacterial CFU/m³ air has been recorded at 10.00 am in Nephrology ward which is 65.52 CFU/ m³ at 60 min exposure. Details of bacterial load CFU/m³ air for different health care wards are shown in Table 1.

3.1.2. Fungal load

The results indicate that the highest Fungal CFU/m³ air has been recorded at 10.00 am in casualty which is 262 CFU/ m³ at 60 min exposure, while no fungal growth CFU/m³ air has been recorded at Nephrology, Surgery (male), ENT, & Obstetric wards.

Details of fungal load CFU/m³ air for different health care wards are shown in Table 2.

In present study, air in the critical area of operating rooms were free of bioaerosols highlighting proper ventilation, fumigation and disinfectants in such areas.

3.2. Qualitative analysis of Bioaerosol (Microbiological Profile)

In the present study, hospital indoor air contains diverse range of microorganisms. Loads of Gram Positive Bacteria were higher than Gram Negative Bacteria. Amongst all bacterial isolates *Staphylococcus aureus* was identified in almost all health care wards except for Medicine (Male) ward. Whereas *Candida* spp was predominant fungal isolate identified in almost all health care wards except for NICU, surgery (Male), ENT and nephrology wards respectively.

The microbiological profile in the health care facility showed variety of pathogenic micro-organisms with the following allocation.

1. Gram positive cocci (*Staphylococcus aureus* predominant followed by coagulase negative staphylococcus)
2. Gram negative bacilli (*Klebsiella* spp, *E. coli* and *Pseudomonas aeruginosa*)
3. Fungus (*Candida* spp predominant followed by *Aspergillus* spp)

Details of microbiological results from settle plates is shown in Table 3.

4. Discussion

Microbiological quality assessment of indoor air study is one the most important investigation to evaluate and determine microbial indoor air contamination. The information on the indoor microbial concentration of airborne bacteria and fungi is necessary to measure indoor air quality control and health hazards associated with it.

The bioaerosol concentration in the indoor environment of our tertiary care hospital, is estimated with the use of settle plate technique was ranged between 23-1179 CFU/m³. This range of bioaerosol load is much lesser than that reported from JIMA²⁰ university specialized hospital and Gondar teaching hospital, northwest Ethiopia²¹ in which it was estimated between 2123-9733CFU/m³ & 480-1468CFU/m³ respectively. According to these standards, the microbiological load of our tertiary care teaching hospital is considered as 'low'.

Though there is no standard uniform international estimation is available on the levels and acceptable microbial load in indoor air, the work conducted by WHO²² expert group on assessment of health risk of biological agents in indoor environment suggested that total microbial load should not exceed 1000CFU/m³. Whereas study conducted by sanitary standard of European commission²³ suggested that 50 CFU/m³ as 'very low', 100CFU/m³ as 'low' and range 200-500 CFU/m³ as 'high' and above 2000 CFU/m³ as 'very high load', considering this standards microbial load of our study is considered as 'high'.

Hospital indoor air contains a diverse range of microorganisms, airborne microbes were detected in hospitals by various workers.^{15,16,21,23} In study carried out by Jaffal et al,²⁴ *Staphylococcus aureus*, coagulase negative Staphylococci, Micrococci, Diptheriods, Gram Negative Bacilli, and *Bacillus* spp were the isolates identified from different wards of hospital similar results were obtained in our present study.

A study conducted by Sudharsanam et al²⁵ & Qudiesat et al²⁶ found high concentration of Gram Positive Cocci then Gram Negative Bacteria, our study also highlights the same findings as Gram Positive Bacteria were isolated more than Gram Negative Bacteria with predominance of *Staphylococcus aureus*. High concentration of Gram Positive Cocci in our study may attribute to their lower susceptibility to environmental stress as pointed out in study conducted by Borriello et al.²⁷ and to other factors such as improper ventilation and presence of increased number of occupants beyond room capacities.^{26–28}

In the present study, isolation of Gram Negative Bacteria was occasional and in lower concentration. One of the reasons may be their susceptibility to environmental stress.²⁸ *Klebsiella* spp was isolated in two wards, *E. coli* and *Pseudomonas aeruginosa* were isolated in one ward each and none, similar findings were seen in studies carried out by other researchers.^{27,28}

In our present study the fungal isolates includes dominance of *Candida* spp followed by *Aspergillus* spp. A study conducted by Sautour et al,²⁹ they found that most frequently detected airborne fungi were *Penicillium* spp & *Aspergillus* spp. A investigation carried out by Gorny et al,³⁰ the commonest fungal agents contaminating hospital rooms were found to be *Aspergillus* spp followed by *Penicillium* spp. Our study showed that there is higher incidence of isolation of *Candida* spp one of the reason of increased isolation of *Candida* spp in our study is due to overcrowding of patients and large numbers of visitors as pointed out in studies conducted by Sautour et al²⁹ & Gorny et al.³⁰

5. Conclusion and Recommendations

An assessment of airborne bacteria and fungi in indoor environment of hospital were experimentally investigated. Types and numbers of airborne micro-organisms were carried out at different hospital wards. The following conclusion & recommendations can be made:

1. Bacteria show higher growth comparing to fungi.
2. There is predominance of Gram Positive Cocci as compared to Gram Negative Bacilli.
3. This study revealed that hospital buildings were being ventilated by the aid of natural ventilation system which may increase the possibility of entrance of pollutants from unhygienic external environment. Many studies have indicated that insufficient ventilation system contributes to microbial loads of the wards.
4. Toilets and waste disposal sites should be located at significant distance away from hospital wards as they can act as potential source of infection. The study reveals that the microbial load in the hospital wards may be due to the presence of attached unhygienic toilets and poor waste management system.
5. Presence of increased incidence of *Staphylococcus aureus* and *Candida* spp in our hospital is a cause of concern as it is associated with increase incidences of noscomial infection in immune-compromised patients and children either as primary or secondary infections.
6. Apart from these infections, allergic reactions have also been reported following inhalation of fungal spores making it essential to pay attention to their presence in hospital air.
7. In present study, air in the critical area of operating rooms were free of any kind of bioaerosals, highlighting proper ventilation, fumigation and disinfectants in such areas.
8. Housekeeping activities (such as sweeping, using dry mops or clothes or shaking linen) can aerolise dust particles that may contain micro-organisms. Therefore, wet mopping should be preferred and recommended.
9. Fumigation at weekly intervals in hospital rooms may reduce load of airborne microbes. Bacillocid

a commercially available surface and environmental disinfectant that has good cleaning properties along with bactericidal, viricidal, sporicidal and fungicidal activity, should be sprayed or use in mopping in hospitals.

10. Given the cost constraints, settle plate technique (passive air sampling) can be used in hospitals with fewer technical facilities for preliminary assessment of indoor air quality. Further more, exposed plate method was found to capture microorganisms efficiently with little variation in duplicates samples, thereby suggesting its use in hospitals for preliminary assessment of indoor air quality and determine pathogenic microorganisms which can cause noscomial infections.

6. Limitation of the study

The study was carried out for small period of duration of 3 months, further long term studies can be carried out to strengthen the study findings and in turn allows proper evaluation for seasonal variation in airborne microbial load.

7. Source of Funding

ICMR funded project.

8. Conflict of Interest

None.

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