

Research Article

Recovery of Airborne Aerobic Bacteria and Fungi from Hospitals of Varying Bed Strength in a Tropical Setting

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How to cite this article:

Sudharsanam S, Ethiraj M, Thangavel G et al. Recovery of Airborne Aerobic Bacteria and Fungi from Hospitals of Varying Bed Strength in a Tropical Setting. *Int J Adv Res MicroBiol Immunol* 2019; 1(1): 12-18.

Date of Submission: 2019-09-18

Date of Acceptance: 2019-10-27

A B S T R A C T

Objectives: Study was undertaken in Chennai, India to 1) determine the impact of hospital bed strength and sampling location on recovery of airborne microorganisms; and 2) characterize microorganisms (aerobic bacteria and fungi) isolated.

Methods: Indoor air samples (duplicates) were collected from intensive care units (ICUs), operating rooms (ORs) and wards of six (two < 10 bed (group I), three ~100 bed (group II) and one > 100 bed (group III)) hospitals over one-year period using exposed-plates for time periods of 30 minutes. Sampling media included 5% Sheep Blood agar and MacConkey agar for bacteria and Rose Bengal agar for fungi.

Result: A total of 2 ORs and 2 wards from group I, 3 ICUs, 3 ORs and 3 wards from group II, and 4 ICUs, 3 ORs and 8 wards from group III hospitals were sampled. Airborne microbial loads ranged between 46-59 CFU/ plate in ICUs, 17 -26 CFU/ plate in ORs and 63-100 CFU/ plate in wards. Airborne microbial loads varied significantly with sampling locations ($p < 0.001$); variation in airborne microbial loads with hospital bed strength was statistically insignificant (Kruskal-Wallis test). Coagulase-negative Staphylococci and Pseudomonas sp., and Aspergillus sp. were the predominant bacteria and fungi recovered respectively from air of all the hospitals under study.

Conclusion: Appropriate location-specific measures such as regulated temperature-humidity levels and air exchanges need to be implemented to contain their spread.

Keywords: Airborne Microbial Loads, Exposed Plate Method, Sampling Locations, Hospital Bed Strength

Introduction

Indoor air of healthcare facilities harbors microorganisms,

including those that are of nosocomial significance.¹⁻⁶ Several factors can contribute to the survival of airborne microorganisms.⁷ Airborne microbial loads are affected by

temperature, relative humidity and number of personnel present in a closed environment.^{8,9} Impact of the changing seasons on airborne microbial loads has also been studied.^{10,11}

A study carried out in three types of residential settings that were grouped based on occupant density and number of rooms showed that total indoor bio aerosol concentrations vary substantially between housing types within the same geographical region.¹² However, little is known whether microbial loads in indoor air of healthcare facilities can be affected by the bed strength (determined by the number of beds) of the hospitals. Hospital design plays a major role, where improper/ faulty design may impact quality of indoor air adversely. In addition, condensates formed following excessive rainfall might also contribute to increased concentrations of airborne microorganisms. Installation of air-conditioning in the existing healthcare facilities without adherence to proper engineering controls to maintain temperature and relative humidity at desired levels may facilitate microbial growth. Lack of appropriate guidelines on hospital design has led to increasing adoption of existing infrastructure for healthcare delivery.

Therefore, a study was undertaken to 1) determine the impact of the hospital bed strength and sampling location on recovery of airborne microorganisms; and 2) characterize

microorganisms (aerobic bacteria and fungi) isolated.

Materials and Method

A cross-sectional study was undertaken to compare airborne microbial loads obtained by exposed-plate method within and between hospitals.

The study was carried out in six different hospitals situated in Chennai, India, for a period of one year from January 2009 to December 2009.

Different hospitals included in the study were located in an urban suburb in South Chennai (Tamil Nadu, India), about 10 km from the centre of the city. Samples were collected after seeking permission and consent from hospital authorities. The hospitals were coded in order to maintain confidentiality.

Air samples were collected from Intensive Care Units (ICUs), Operating Rooms (ORs) and wards of six different hospitals over a period of one year. Hospitals were identified based on the number of beds/ bed strength of hospitals and were grouped into three different categories as : < 10 bed hospitals (group I), ~100 bed hospitals (group II) and > 100 bed hospital (group III). Of the six hospitals studied, two were of group I, three were of group II and one was of group III (Table 1).

Table I. Details of the hospitals under study

Grouping of hospitals	Hospital under study	Bed strength of the hospital	Sampling location	Description of the study area (at the time of sampling)
I (< 10 bed hospitals)	H ₅	6	OR	Sterile
			Ward	Multiple-beds with natural ventilation
	H ₆	8	OR	Post-operative
			Ward	Multiple-beds with natural ventilation
II (~100 bed hospitals)	H ₂	75	ICU	Provided with mechanical ventilation
			OR	Post-operative
			Ward	Single-beds; natural ventilation with occasional mechanical ventilation through the use of mechanical fans
	H ₃	50	ICU	Provided with mechanical ventilation
			OR	Clean, fumigated
			Ward	Single-beds; natural ventilation with occasional mechanical ventilation through use of mechanical fans
	H ₄	40	ICU	Provided with mechanical ventilation
			OR	Clean, Not Mopped, UV sterilized
			Ward	Single-beds; natural ventilation with occasional mechanical ventilation through use of mechanical fans
	III (> 100 bed hospitals)	H ₁	> 500	ICU
OR				With varying degrees of activity
Ward				Multiple-beds, with natural ventilation, supported by mechanical fans

Group I hospitals had < 10 beds (6 and 8 beds respectively), and intensive care units were not available. Wards were naturally ventilated with 6 beds or 8 beds. Group II hospitals comprised hospitals with 10-100 beds (75, 50 and 40 beds respectively), where intensive care units were provided with mechanical ventilation. Wards were provided with single-beds and natural ventilation with occasional use of mechanical fans. Group III was a tertiary hospital with > 500 beds. Intensive care units were provided with AC ventilation. Wards had multiple-beds, with natural ventilation, supported by mechanical fans.

Sampling was carried out using exposed plate method;¹³ plates containing media were used in duplicates for collecting the samples for time periods of 30 min.¹⁴ Petri plates were put at a height of 60-70 cm above the ground level during sampling. Media used for sampling included 5% Sheep Blood agar and Mac Conkey agar for bacteria and Rose Bengal agar for fungi (Hi Media Company Limited, India).

The exposed plates were incubated aerobically at 37 °C for 24-48 h for bacteria, and at 25 °C and 37 °C up to 7 days for yeasts and moulds (since yeasts and yeast-like fungi grow best at 37 °C, while filamentous fungi grow at 25 °C, both temperatures were included in the study). Aerobic bacteria and fungi were identified as per standard microbiological procedures.

Identification of bacteria was based on colony morphology, Gram's staining and appropriate biochemical tests.^{15,16} Filamentous fungi were identified by lacto phenol cotton blue preparation and confirmed by Riddel's slide culture;¹⁷ germ-tube test was performed for yeast-like colonies, and growth on tetrazolium reduction medium was used to speciate *Candida*.

SPSS for Windows v. 11.5 packages (SPSS Inc., USA) was used. Airborne microbial loads obtained within and between hospitals were compared using Kruskal-Wallis Test, a non-parametric test.

Result

Indoor air samples were collected from 3 ICUs in group II hospitals and 4 ICUs in group III hospital, 2 ORs in group I hospitals, 3 ORs in group II hospitals and 3 ORs in group III hospital, and 2 wards in group I hospitals, 3 wards in group II hospitals and 8 wards in group III hospital. Airborne microbial loads obtained were analyzed by comparing loads of different hospitals irrespective of the sampling locations and between locations irrespective of hospital bed strength.

Hospital bed strength wise comparison of Airborne Microbial Loads

A total of 28 indoor air samples were collected from six hospitals over a period of one year. Of these, 4, 9 and 15

indoor air samples were collected from group I, group II and group III hospitals respectively. Airborne microbial loads in wards were found to vary with different hospital groups, while the microbial loads in ICUs and operating rooms were found to be similar irrespective of the bed strength of the hospitals (Fig. 1).

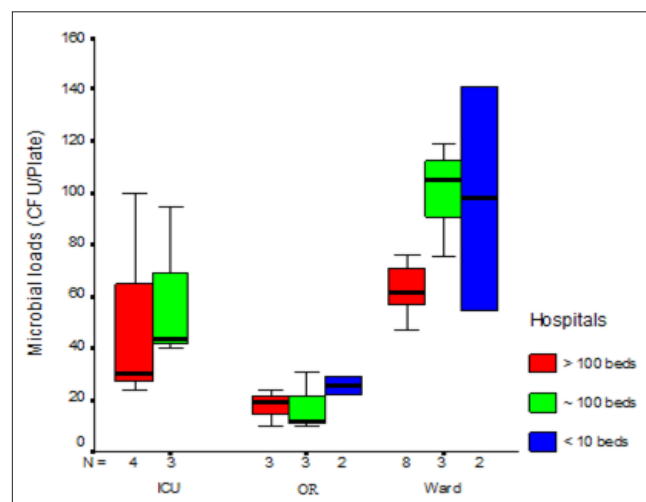


Figure 1. Hospital bed strength wise comparison of Airborne Microbial Loads

Airborne microbial loads were found to be more in ICUs of group II hospital (59 CFU/plate), when compared to that of group III hospital (46 CFU/plate). When the microbial loads in ORs were compared across hospitals, it was found that group I hospitals had higher loads (26 CFU/plate) when compared to group II and III hospitals (~ 17 CFU/plate). Microbial loads in indoor air of wards were found to be less in group III hospital (63 CFU/plate), while they ranged between 98-100 CFU/plate in group I and II hospitals. Mean loads of airborne microorganisms obtained by exposed plate method in different locations are summarized in Table 2.

Airborne microbial loads obtained from different locations of the same hospitals were averaged in order to determine if the bed strength of the hospitals can impact on the loads of airborne microorganisms. Mean loads of airborne microorganisms were 62 CFU/plate, 59 CFU/plate, and 49 CFU/plate respectively in group I, II and III hospitals. The loads were compared and tested for statistical significance by Kruskal-Wallis test. It was found that the variation in the microbial loads with difference in hospital bed strength was statistically insignificant.

Location wise comparison of Airborne Microbial Loads

A total of 7 ICUs, 8 ORs and 13 wards from six different hospitals were sampled over a period of one year. Airborne microbial loads were found to be high in wards, moderate in ICUs and minimum in operating rooms irrespective of the bed strength of the hospitals sampled (Fig. 1).

Table 2. Location-wise and hospital bed strength-wise comparison of airborne microbial loads obtained by exposed plate method

Description	Location	Hospital group	N	Mean	Std. Deviation	p-value
Hospital location wise comparison	Intensive Care Unit	II	3	59	31	p < 0.001
		III	4	46	36	
		Total	7	52	32	
	Operating Room	I	2	26	5	
		II	3	17	12	
		III	3	18	7	
		Total	8	19	9	
	Ward	I	2	98	62	
		II	3	100	22	
		III	8	63	10	
Total		13	77	28		
Hospital bed strength wise comparison	TOTAL	I	4	62	55	p = 0.925
		II	9	59	41	
		III	15	49	26	
		Total	28	54	35	

Airborne microbial loads ranged between 46-59 CFU/plate in ICUs, 17-26 CFU/plate in ORs and 63-100 CFU/plate in wards of the different hospitals sampled (Table 2). In order to determine if airborne microbial loads can vary with locations, loads obtained from the same locations of different hospitals were averaged. Mean loads of airborne microorganisms were found to be 52 CFU/plate, 19 CFU/plate and 77 CFU/plate in ICUs, ORs and wards respectively.

The airborne microbial loads obtained from different locations were compared and tested for statistical significance by Kruskal-Wallis test. The loads were found to vary significantly with locations of the magnitude of ORs < ICUs < wards, irrespective of the bed strength of the hospitals ($p < 0.001$).

Airborne Microbial Profile

Bacteria were frequently isolated than fungi. Among bacteria, Gram-positive cocci (GPC) were predominant than Gram-negative bacilli (GNB). Coagulase-negative Staphylococci (CNS) and Micrococci were commonly recovered GPC irrespective of the sampled hospital. Pseudomonas sp. was the GNB isolated from air of all the hospitals under study. Aspergillus sp. was the predominant fungi isolated from all the hospitals sampled. Candida tropicalis was the only yeast in the study. Microbial profile of indoor air of different hospitals obtained by exposed plate method is shown in Table 3.

Group I: CNS, Pseudomonas sp., and A. fumigatus were recovered from wards and ORs of H₅ and H₆ hospitals. In addition, fungi such as A. flavus, A. niger and A. terreus were also isolated from indoor air of wards of the hospitals (Table 3).

Group II: CNS and Pseudomonas sp. were isolated from indoor air of ICUs of all the three hospitals; additionally, S. aureus, K. oxytoca, A. fumigatus, A. terreus and A. flavus and C. tropicalis were also recovered. S. aureus was isolated from indoor air of OR of H₃ hospital; moulds were recovered from indoor air of H₂ and H₄ hospitals, and included A. fumigatus, A. terreus and A. flavus. Airborne microorganisms isolated from wards were S. aureus (H₃ hospital), CNS, Pseudomonas sp., K. oxytoca, Acinetobacter sp., A. fumigatus, A. terreus, A. flavus, A. niger and C. tropicalis (Table 3).

Group III: Bacteria isolated from indoor air of ICUs include CNS, Pseudomonas sp., Klebsiella pneumoniae and Klebsiella oxytoca, A. fumigatus, A. flavus and C. tropicalis were the fungi recovered. CNS and Micrococci were the predominant isolates recovered from ORs; other isolates included Pseudomonas sp., A. fumigatus and A. flavus. A variety of bacteria and fungi were seen in indoor air of wards including E.coli, Pseudomonas sp., Citrobacter diversus, Klebsiella pneumoniae, K. oxytoca, Acinetobacter sp., A. flavus, A. niger, A. fumigatus, A. terreus, and C. tropicalis (Table 3).

Table 3. Microbial profile of indoor air of different hospitals obtained by exposed plate method

Hospital under study	Sampling location code	Indoor Bioaerosols	
		Counts (CFU/plate)	Isolates
H ₁	H ₁ -ICU ₁	100	Micrococci, Pseudomonas sp., A. fumigatus
	H ₁ -ICU ₂	30	CNS, K. oxytoca, Pseudomonas sp., A. fumigatus
	H ₁ -ICU ₃	30	Micrococci, CNS, K. pneumoniae, C. tropicalis
	H ₁ -ICU ₄	24	CNS, Micrococci, A. fumigatus, A. flavus, Hyphae
	H ₁ -OR ₁	19	CNS, Micrococci, Pseudomonas sp.
	H ₁ -OR ₂	10	CNS, Micrococci
	H ₁ -OR ₃	24	CNS, Micrococci, Pseudomonas sp., A. fumigatus, A. flavus
	H ₁ -W ₁	76	CNS, Micrococci, E. coli, Pseudomonas sp., C. diversus, Acinetobacter sp., A. flavus, A. terreus, A. fumigatus, C. tropicalis
	H ₁ -W ₂	57	CNS, Micrococci, K. pneumoniae, A. flavus, A. fumigatus
	H ₁ -W ₃	61	CNS, Micrococci, A. niger, A. fumigatus
	H ₁ -W ₄	47	CNS, Micrococci, Pseudomonas sp., A. flavus, A. terreus, A. fumigatus, Rhizopus
	H ₁ -W ₅	56	Micrococci, CNS, K. oxytoca, C. diversus, A. niger, A. flavus, A. fumigatus, A. terreus, C. tropicalis
	H ₁ -W ₆	76	Micrococci, CNS, Pseudomonas sp., A. niger, A. flavus, A. terreus
	H ₁ -W ₇	62	CNS, Micrococci, Pseudomonas sp., A. niger, A. flavus, A. fumigatus, A. terreus
H ₁ -W ₈	66	Micrococci, CNS, K. oxytoca, C. diversus, Pseudomonas sp., A. niger, A. flavus, A. terreus, C. tropicalis	
H ₂	H ₂ -ICU ₁	41	CNS, Micrococci, Pseudomonas sp., K. oxytoca, A. terreus, A. fumigatus, A. flavus
	H ₂ -OR ₁	31	CNS, Micrococci, Pseudomonas sp., A. terreus, A. fumigatus, A. flavus
	H ₂ -W ₁	107	CNS, Micrococci, Pseudomonas sp., Acinetobacter sp., A. terreus, A. fumigatus, A. flavus, A. niger, C. tropicalis
H ₃	H ₃ -ICU ₁	96	S. aureus, Micrococci, Pseudomonas sp., A. terreus, A. flavus, C. tropicalis
	H ₃ -OR ₁	12	S. aureus, Micrococci, Pseudomonas sp.
	H ₃ -W ₁	77	S. aureus, Micrococci, Pseudomonas sp., A. flavus, C. tropicalis
H ₄	H ₄ -ICU ₁	45	Micrococci, CNS, Pseudomonas sp., A. fumigatus
	H ₄ -OR ₁	10	Micrococci, CNS, A. flavus
	H ₄ -W ₁	120	Micrococci, CNS, Pseudomonas sp., A. terreus, A. fumigatus
H ₅	H ₅ -OR ₁	23	Micrococci, CNS, Pseudomonas sp., A. fumigatus
	H ₅ -W ₁	56	Micrococci, CNS, Pseudomonas sp., A. fumigatus, A. flavus, Hyphae
H ₆	H ₆ -OR ₁	30	Micrococci, CNS, Pseudomonas sp., A. fumigatus, Hyphae
	H ₆ -W ₁	144	Micrococci, CNS, Pseudomonas sp., A. terreus, A. fumigatus, A. niger, Hyphae

OR-Operating room; ICU-Intensive Care Unit; W-Ward.

CNS-Coagulase-negative Staphylococci.

Discussion

Several reports have documented presence of microorganisms in indoor air of hospitals including critical

care areas.¹⁻⁶ The transport and the ultimate settling of these airborne microorganisms are affected by several factors including their physical properties and environmental parameters that they encounter.⁷

Factors such as temperature, relative humidity and number of personnel can affect the airborne microbial loads in a closed environment.^{8,9} Airborne microbial loads were found to increase when there was a drop in temperature and raise in relative humidity,⁹ thus indicating the need to maintain a temperature of 20-24.4 °C and a relative humidity of 50-60 % to inhibit microbial growth.^{8,9} Impact of changing seasons on airborne microbial loads has been found to vary with geographical region.

A study conducted in a hospital ward of a pneumonological department in Poland found seasonal variations in total microbial loads, with greater variation among fungi than bacteria.¹⁰ On the other hand, they do not have an impact on airborne microbial loads in a tropical setting.¹¹

Given these factors, whether bed strength of the hospitals can influence the airborne microbial loads is not known. So far, to our knowledge, no studies have been carried out to determine if the bed strength of the hospital can impact the bioaerosol concentrations. A study in residential settings has shown that total indoor bioaerosol concentrations vary substantially between housing types (based on occupant density and number of rooms) within the same geographical region.¹² An attempt was therefore made to carry out a study to compare airborne microbial loads within and between hospitals and determine the variations with the hospital bed strength and sampling locations.

A report has documented that exposed-plate method can be used to capture microorganisms efficiently with little variation in duplicate samples, especially in hospitals for preliminary assessment of indoor air quality and determine pathogenic microorganisms due to particle fall-out.¹¹ Thus, exposed plate method was the method of choice to carry out the study.

A total of six hospitals were chosen for the study, two < 10 bed hospitals (group I), three ~ 100 bed hospitals (group II) and one > 100 bed hospital (group III). Sampling locations included wards and operating rooms in group I hospitals, and intensive care units (ICUs), wards and operating rooms in group II and group III hospitals. Kruskal-Wallis test, a non-parametric test was used to determine the variations in airborne microbial loads with respect to sampling location and bed strength of the hospitals, since the data collected was discrete and three variables (ICU, OR and ward in case of location-wise comparison, and hospital groups I, II and III in case of hospital bed strength-wise comparison) were involved.

Airborne microbial loads did not vary significantly with the bed strength of the hospitals, though the least was documented in group III hospital and the highest in group I hospitals. This preliminary finding suggests that the control measures that are followed to maintain different hospital

locations influence the recovery of airborne microorganisms irrespective of the bed strength of the hospitals.

Recovery of airborne microorganisms from ORs was the least followed by ICUs and then wards, which is to be expected. Variations in airborne microbial loads were found to be significant with respect to sampling locations irrespective of the bed strength of the hospitals. The location-wise variations in airborne microbial loads may be attributed to the procedures followed that are effective resulting in distinct differences in the recovery of airborne microorganisms and their loads by location.

Operating rooms are generally clean and disinfected periodically leading to minimal survival and/ or least recovery of microorganisms from air. Intensive care units are critical care areas with restricted entry. The recovery of microorganisms was therefore found to be moderate, and more than that of ultra-clean areas like ORs. Wards are locations with no specific requirements for maintenance of sterility or restricted entry; therefore recovery of airborne microbial loads was maximum.

Bacteria were present in higher numbers than fungi in all the hospitals. Among bacteria, GPC were recovered in more numbers when compared to GNB. Micrococci and CNS were the commonest GPC, while *Pseudomonas* sp. was the predominant GNB. Among fungi, *Aspergillus* sp. were commonly seen in all the hospitals. Bacteria such as *Staphylococcus aureus*, coagulase-negative *Staphylococci*, *Micrococcus*, *Pseudomonas*, *Acinetobacter* and fungi such as *Aspergillus* were recovered from hospital air. Our study findings are similar to previous reports.¹⁻⁶ *Staphylococcus aureus* was recovered from indoor air of H₃ hospital while *Acinetobacter* sp. was isolated from that of H₂ hospital. Additionally, *Klebsiella oxytoca* from H₂ hospital and *Candida tropicalis* from H₂ and H₃ hospitals were also documented.

While it is not possible to maintain the hospital environment free of these nosocomial pathogens, there is a need to adopt location specific measures in order to contain their spread. These include appropriate temperature relative humidity levels and air exchanges, as recommended by Centre for Disease Control and Prevention (CDC).¹⁸ ORs are ultra-clean areas where temperature of 20-23 °C, relative humidity < 68% and adequate air changes per hour (15 ACH) need to be ensured. Critical areas such as ICUs require clean environments; these can be provided with air handling units with a minimum of 6 ACH to minimize the entry of fungal spores. Wards do not require maintenance of clean/ ultra-clean conditions. However, wards such as post-operative wards need adequate ventilation to minimize the risk of nosocomial infections due to particle fall-out.

Corrective measures including adequate ventilation need to be ensured to prevent contamination at the time of

procedures and not just disinfection alone. Excessive exposure to disinfectants to avoid contamination can promote the growth and survival of bacteria such as *Pseudomonas aeruginosa*.

The study was not designed to carry out simultaneous sampling in all the hospitals included in the study. Data obtained from six different hospitals over a period of one year were collated to compare the airborne microbial loads based on hospital bed strength and location. Further studies may be carried out targeting hospitals accredited by National Accreditation Board for Hospitals & Healthcare Providers (NABH), and between NABH accredited and non-accredited hospitals to compare airborne microbial loads.

Conclusion

There was significant variation in the airborne microbial loads with the sampling location. The loads were highest in wards, followed by ICUs and ORs. Number of beds did not impact airborne microbial loads. Since it is not possible to rid the hospital environment of these nosocomial pathogens, appropriate location-specific measures such as regulated temperature-humidity levels and air exchanges need to be implemented to contain their spread.

Acknowledgement

The authors thank the Council of Scientific and Industrial Research (CSIR), India for providing partial financial assistance through the Senior Research Fellowship [09/949/(0002)/2009/EMR-I] to carry out the research work. The authors thank Dr Anupma Jyoti Kindo, Professor of Microbiology, Sri Ramachandra University for assistance in identifying the moulds.

Conflict of Interest: None

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