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## Assessment of Airborne Pathogens & Non Pathogens and Fungi in Healthcare Settings Pune India

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### ABSTRACT

The indoor air environment can potentially place patients at greater risk than the outside environment because enclosed spaces can confine aerosols and allow them to build up to infectious levels. It becomes imperative to undertake a study of the microbiological air quality of the airborne micro-flora in the environments of two major hospitals, Ruby Hall Clinic and Jehangir in Pune-India. In Jehangir hospital *Staphylococcus aureus* (15.74%) was found to be the most common organism. The correlation between the location of sampling and concentration of *Staphylococcus aureus* showed patient room ( $65 \pm 3$ ) > neonatal ward ( $22 \pm 1.41$ ) > intensive care unit ( $22 \pm 2.82$ ) > operating room ( $16 \pm 1.73$ ) > the main entrance of the hospital ( $0 \pm 0$ ). While *Staphylococcus aureus* (25.2%), followed by coagulase negative *Staphylococcus* (21.78%) and *M. luteus* (16.28%) were found to be the most common in a Ruby Hall hospital. The correlation between the location of sampling and concentration of *Staphylococcus aureus* in Ruby hall hospital, showed patient room ( $45 \pm 4.24$ ) > intensive care unit ( $22 \pm 2.82$ ) > neonatal ward ( $12 \pm 2.80$ ) > operating room ( $8 \pm 1.41$ ) > the main entrance of the hospital ( $3 \pm 2.85$ ). *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp. and *Alternaria* spp. were isolated in both hospitals. Maximum bacterial rates were detected in the patient rooms, while minimum bacterial rates were detected in the operating rooms and neonatal wards. The time of visit showed higher microbial rates in hospital.

**Keywords:** Air sampling, airborne microflora, bacteria, Fungi

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## INTRODUCTION

Hospital indoor air contains a diverse range of microbial population. Hospitals and other healthcare facilities are complex environments that require ventilation for comfort of patients and control of hazardous emissions<sup>1, 2</sup>. Environmental conditions, e.g. dryness, temperatures and ultraviolet radiation, which may prevent microorganisms from growing in unfavorable environments, they still reach new hosts through the air. The incidence of airborne infections has increased in recent years, because many new buildings are sealed and have self-contained circulating air systems for temperature control<sup>3,4</sup>. Fungal spores and viruses can survive for longer periods of time. The incidence of airborne infections has increased in recent years, because many new buildings are sealed and have self-contained circulating air systems for temperature control. The counting and identification of microbes in air is not an easy task. Various methods are used and these can be divided into four groups: counts of colony forming units per cubic meter of air (CFU/m<sup>3</sup>); counts of CFU on settle plates; counts under a microscope; and measurement of a chemical component of the microbial cells per cubic meter of air<sup>5</sup>. There is no single method of choice for sampling airborne loads<sup>6</sup>. However, impact or air samplers are the most widely used for the quantification of contamination<sup>7, 8</sup>. Their advantage lies in the fact that agar plates can be incubated without further treatment, which means that colonies grow directly from collected viable airborne particles<sup>8-10</sup>. Hospital aerosols must be regularly investigated. One researcher reported that sampling of air may be performed in hospitals for several purposes, e.g. epidemiologic, surveillance, research, safety or quality control purposes<sup>11</sup>. Another researcher has reported that occupant density is a key factor affecting concentrations of airborne bacteria, and humidity is also important depending on the particular location within the hospital<sup>12</sup>. A study has concluded that the significant particle concentration fluctuations in operating rooms may be related to variations in operating personnel numbers and activities<sup>13</sup>. Other study has reported on *Aspergillus* infections, primarily invasive pulmonary aspergillosis<sup>14</sup>. Building works carried out in the vicinity of ward areas can generate large aerosols of infective particles. Several authors have concluded that mold spores may enter the hospital through windows or inadequate air filtration systems<sup>2, 5</sup>. Surfaces, such as carpets, potted plants and multiple-hole false ceilings are potential sources of fungal contamination. Dust might accumulate in these areas and spores may enter the patient room as contaminants on personnel's clothing. This study was assessed the levels of airborne bacteria, and fungi in Jehangir hospital and Ruby Hall Clinic, Pune, India, and investigated the environmental and hospital characteristics that affected the airborne microorganism levels. The

data can be used to set standards for levels of acceptable microbial population and can also be used to suggest suitable guidelines in order to decrease the microbial rates in indoor air.

## MATERIALS AND METHOD

For this study Jehangir hospital and Ruby Hall Clinic were selected to examine airborne microorganism. The hospital which was formerly known as Jehangir was constructed on the 6<sup>th</sup> of February 1946. Ruby Hall Clinic was established in 1959 by Dr. K B Grant. In 1966, Ruby Hall Clinic was converted from a private institution owned. Air samples (500 L air/sample) were taken from the following sites of the hospital: intensive care unit (ICU), neonatal ward (NW), the main entrance of the hospital (ME), and patient room (PR), operating room (OR). At each location, two air samples were taken at three different time periods (10:30 - 12:30 am, 14:00 - 16:00 pm and 18:00 - 20:00 pm). In addition, all samples were taken during Jun2011. A microbial air sampler was used for sampling of airborne bacteria and fungi. The microbial air sampler was operated at an air flow-rate of 100 L/min. The sampling time was 5 min to avoid drying of the agar surface and overloading of the collection plate<sup>15</sup>. The total volume of air that was aspirated onto an agar plate was 500 L in each sample from each location (room). The air sampler was set up at a height representative of the normal human breathing zone, that is, 1.5 m above floor level. Between measurements the sampler was cleaned by swabbing with 70% ethanol. Nutrient agar (NA) (HiMedia Laboratories Limited, Mumbai, India) supplemented with 100 mg/L cyclohex-amide was used for the sampling and cultivation of bacteria<sup>12</sup>. For isolation of fungi, Sabouraud dextrose agar (SDA) (HiMedia) supplemented with 10 mg/L chloramphenicol was used<sup>16</sup>. Twice replica plates of each medium were used for the isolation of bacteria and Fungi. After strike Bactria on nutrient agar plates were incubated at 37°C for 48 h to allow the growth of aerobic bacteria while cultivated fungi on Saboraud dextrose agar plates were incubated for 3–5 days at 28 °C. Bacterial colonies were initially characterized by examination microscopic method for morphology and microscopic appearance, and identified bacteria genus and species. These tests included catalase, coagulase, indole, methyl-red and Voges-Proskauer, fermentation of glucose, lactose, and mannitol, citrate utilization, gelatin hydrolysis, and starch hydrolysis. Blood agar, MacConkey agar, mannitol salt agar, eosin-methylene blue agar and Muller Hinton agar were used for differentiation. The biochemical and physiological characteristics of identified bacterial species were performed according to Bergey's Manual of Systematic Bacteriology<sup>17,18</sup>. A wet mount preparation of each fungal colony was prepared by using Lactophenol-cotton-blue solution and examined microscopically. Identification of fungi was based mainly on growth colonial

appearance, microscopic examination of the spore and hyphal characteristics of the stained preparations<sup>19</sup>. The total number of colony forming units (CFU) was enumerated and converted to organisms per cubic meter of air (CFU/m<sup>3</sup>). The mean of the two samples of each bacterium was calculated in all sample (bacteria and fungi) locations at hospital. The data were processed with statistical significant differences were determined by one-way and two-way analysis of variance.

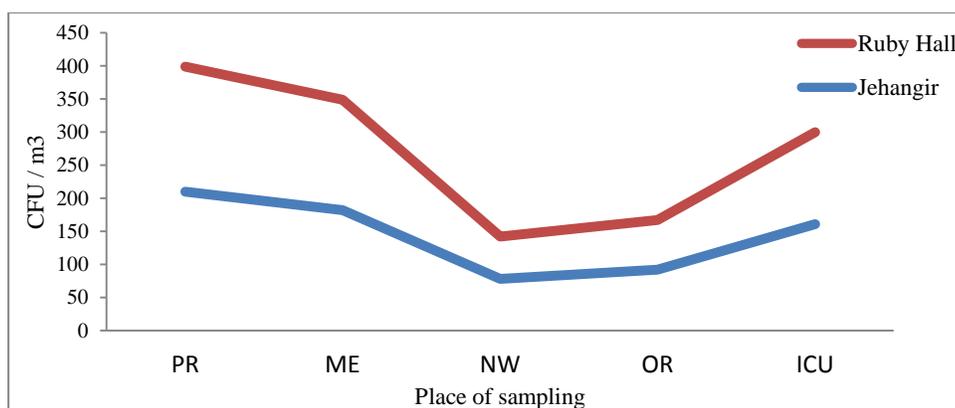
## RESULTS AND DISCUSSION

The bacterial counts on NA (CFU/m<sup>3</sup> air) ranged from 42 CFU/m<sup>3</sup>, which was isolated from the Neonatal Ward, in Rubi Hall Clinic to 230 CFU/m<sup>3</sup> air from the patient room of the Jehangir hospital (Table 1).

**Table 1. Concentration of Airborne bacteria Population (CFU/m<sup>3</sup> air) according to the kind of hospital / room / time of sampling**

Bacterial CFU/m <sup>3</sup> air(n=2)		Morning 10:30-12:30	Afternoon 14-16	Evening 18-20
Jehangir hospital	PR	210	230	189
	ME	170	220	155
	NW	93	85	55
	OR	80	100	95
	ICU	150	190	141
Rubi Hall Clinic	PR	195	200	170
	ME	156	205	140
	NW	80	70	42
	OR	72	85	68
	ICU	130	160	125

Patient room (PR), neonatal ward (NW), intensive care unit (ICU), operating room (OR), the main entrance of the hospital (ME)



patient room (PR), neonatal ward (NW), intensive care unit( ICU), operating room (OR), the main entrance of the hospital(ME)

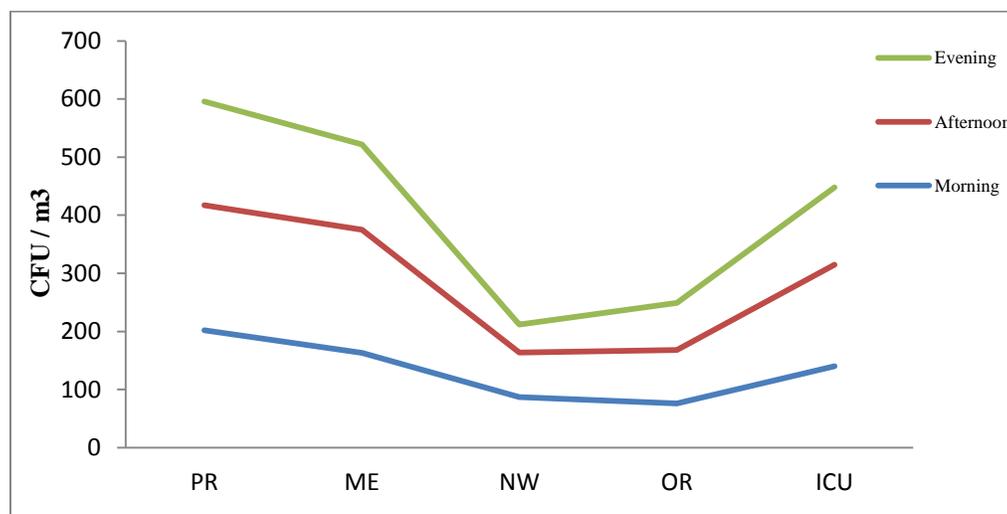
**Figure 1: The effect of the kind of the type of room on CFU/m<sup>3</sup> air in both hospitals**

The bacterial CFU/m<sup>3</sup> air in the Jehangir hospital was significantly higher than in the Rubi Hall Clinic in all five units. In both hospitals, the patient rooms had the maximum bacterial rates, and the minimum rates were detected in the neonatal wards and operation rooms (Figure 1). Depicts table 2 the counts of bacterial swabs taken from the surfaces of the operating rooms and neonatal wards from the both hospitals. In the Jehangir hospital, the counts ranged from 40 to 75 CFU. In the Ruby Hall Clinic, the range was between 32 Operating Room in to 83 Neonatal Ward CFU. Table 2 also shows the bacterial counts from the ventilation grills of intensive care units and patient rooms. The counts ranged from 167 to 260 CFU for the Jehangir hospital, and for the Ruby Hall Clinic the counts ranged from 155 to 240 CFU.

**Table2. Enumeration of bacterial colonies from each location in the hospital**

			<b>Bacterial CFU/m<sup>3</sup> air(n=2)</b>		
			<b>Morning 10:30-12:30</b>	<b>Afternoon 14-16</b>	<b>Evening 18-20</b>
Jehangir	Surface	NW	75	63	70
		OR	40	45	43
	Ventilation	PR	212	180	260
		ICU	230	167	170
Ruby Hall	Surface	NW	66	58	83
		OR	32	49	33
	Ventilation	PR	197	240	174
		ICU	155	220	167

patient room (PR), neonatal ward (NW), intensive care unit( ICU), operating room (OR), the main entrance of the hospital(ME) Figure 2. shows that bacterial CFU/m<sup>3</sup> air in the main entrance and the patient rooms were more sensitive to the change in the sampling time, while the other units were not sensitive<sup>1</sup>.



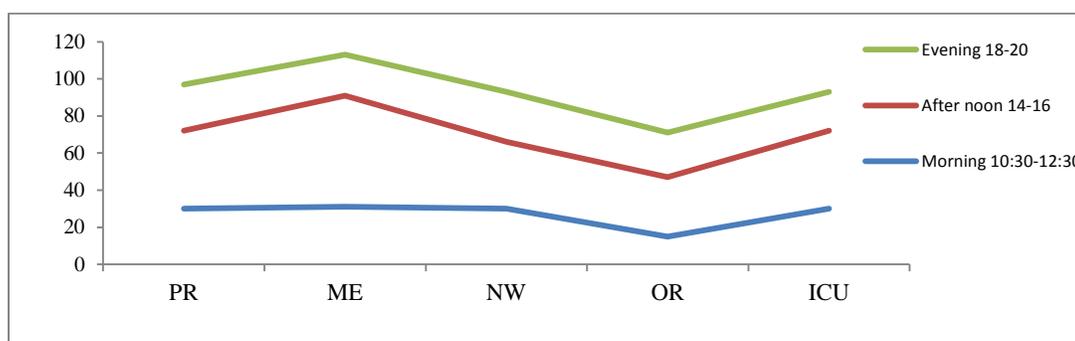
**Figure 2: The effect of type of room and time of sampling on bacteria CFU/m<sup>3</sup> air in both hospitals**

As indicated in Figure 3, fungal CFU/m<sup>3</sup> in three locations (ICU, main entrance and patient room) were more sensitive to the change in sampling time, while the rest of locations were not sensitive. The fungal counts (CFU/m<sup>3</sup> air) on SDA ranged from 18 CFU/m<sup>3</sup> air, which was isolated from the operating room of hospital, to 75 CFU/m<sup>3</sup> air from the main entrance of the Jehangir hospital while in Ruby Hall Clinic ranged from 16 CFU/m<sup>3</sup> air in operating room, to 65 CFU/m<sup>3</sup> air from the main entrance of the hospital. (table 3). P-value from comparison hospitals according to type of room were (ICU p=0.00001, NW p=0.0543, ME p=0.0017, OR p<0.00001, PR p=0.315). The fungal CFU/m<sup>3</sup> air of the Main Entrance was higher than that of the other units in both hospitals. So in the hospital, there were no significant differences between the different units. On the other hand, the operating room had the lowest rates in the Jehangir hospital which in Ruby Hall Neonatal ward is lowest fungi (Figure 4).

**Table 3. Concentration of Airborne fungi population (CFU/m<sup>3</sup> air) according to the kind of hospital / room / time of sampling**

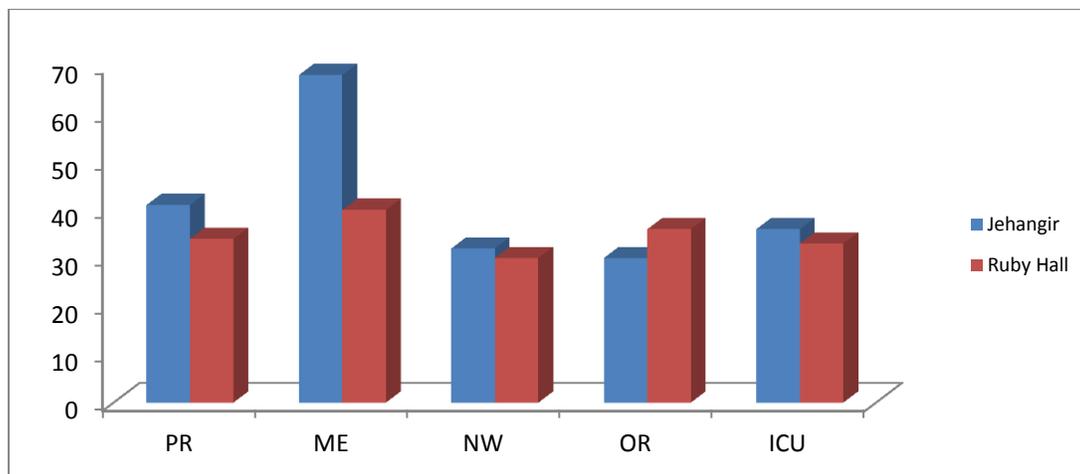
		Fungal CFU/m <sup>3</sup> air(n=2)		
		Morning 10:30-12:30	Afternoon 14-16	Evening 18-20
Jehangir Hospital	PR	42	52	30
	ME	36	75	25
	NW	30	35	25
	OR	18	40	30
	ICU	40	45	22
Ruby Hall	PR	35	41	26
	ME	32	65	21
	NW	28	35	25
	OR	16	31	26
	ICU	32	40	25

Patient room (PR), neonatal ward (NW), intensive care unit( ICU), operating room (OR), the main entrance of the hospital(ME).The types of microorganisms isolated from the air of the five different locations are shown in Tables4, 5.



**Figure 3: The effect of type of room and time of sampling on Fungi CFU/m<sup>3</sup> air in both hospitals**

In Jehangir hospital the largest quantities of isolated bacteria in the hospital was *S. aureus* (125 CFU/m<sup>3</sup>), followed by *Micrococcus luteus* (104 CFU/m<sup>3</sup>) and coagulase negative *Staphylococci* (106 CFU/m<sup>3</sup>). Others Isolated bacteria and fungi are shown in table4. Among fungi, *Aspergillus* spp. was the highest (66 CFU/m<sup>3</sup>) followed by *Penicillium* spp. (40 CFU/m<sup>3</sup>) while *Alternaria* spp were zero in all units except ICU unit that was 8 CFU/m<sup>3</sup>.



**Figure 4: The effect of the kind of the type of room on Fungi CFU/m<sup>3</sup> air in both hospitals**

In Ruby Hall hospital the largest quantities of isolated bacteria in the hospital was *S. aureus* (90 CFU/m<sup>3</sup>), followed by *Micrococcus luteus* (58 CFU/m<sup>3</sup>) and coagulase negative *Staphylococci* (78 CFU/m<sup>3</sup>).

**Table 4. Airborne microorganisms isolated from five locations in Jehangir hospital**

Types of organisms	CFU / m <sup>3</sup> air (%)					
	ICU	OR	NW	ME	PR	Total
<i>Staphylococcus aureus</i>	22±2.82 (17.6%)	16±1.73 (12.8%)	22±1.41 (17.6%)	0±0 (0.0%)	65±3 (52%)	125 (100%)
<i>Enterococcus faecalis</i>	20±1.45 (48.78%)	3±1.40 (7.31%)	10±2.82 (24.39%)	35±2.82 (7.3%)	41±5.65 (12.19%)	41 (100%)
<i>Micrococcus luteus</i>	21±1.43 (20.19%)	22±2.82 (21.15%)	14±2.82 (13.46%)	14±5.60 (13.46%)	33±4.24 (31.74%)	104 (100%)
<i>Bacillus subtilis</i>	20±1.38 (32.25%)	8±2.80 (12.90%)	8±1.41 (13%)	10±5.65 (16.12%)	16±4.24 (25.80%)	62 (100%)
<i>Bacillus cereus</i>	14±1.40 (40%)	12±4.24 (34.28%)	6±1.41 (17.17)	3±1.41 (8.55 %)	0±0 (0%)	35 (100%)
Coagulase-negative <i>Staphylococci</i>	14±2.82 (13.20%)	14±1.41 (13.20%)	6±1.41 (5.66)	35±4.24 (33.04%)	37±2.81 (34.90%)	106 (100%)
Unidentified Gram-positive rods	10±4.74 (23.25%)	8±2.80 (18.60%)	0±0 (0%)	9±4.24 (20.93%)	16±2.81 (37.22%)	43 (100%)
<i>Pseudomonas aeruginosa</i>	5±1.41 (18.51%)	0±0 (0%)	0±0 (0%)	22±4.24 (81.49%)	0± 0 (0%)	27 (100%)
<i>Klebsiella</i> spp.	12±1.41	5± 1.40	3±1.73	21± 2.82	12±4.24	53

<i>Escherichia coli</i>	(22.76%) 21±2.80	(9.43%) 10±1.81	(5.66%) 8±1.41	(39.62%) 16± 4.24	(22.53%) 18± 2.82	(100%) 73
<i>Enterobacter</i> spp.	(28.76%) 1±1 (5%)	(13.69%) 5± 1.41	(10%) 0± 0 (0%)	(21.91%) 14± 4.24	(24.65%) 0± 0 (0%)	(100%) 20
Unidentified Gram-negative coccus	0±0 (0%)	0±0 (0%)	2±2 (17%)	6± 2.82 (50%)	4± 4.24 (33.33%)	12 (100%)
Unidentified Gram-negative rods	12± 1.41 (12.90%)	5±4.24 (5.37%)	8± 4.24 (8.5%)	32± 2.80 (34.40%)	36± 1.41 (38.70%)	93 (100%)
<b>Fungi</b>						
<i>Aspergillus</i> spp.	18 ± 2.82 (27%)	4±1.41 (6%)	6 ± 1.41 (9.0%)	21±5.65 (31.81%)	17± 2.82 (25.75%)	66 (100%)
<i>Penicillium</i> spp.	25± 2.82 (62.5%)	3±1.41 (7.5%)	2±2.00 (5%)	10±2.82 (25%)	0±0 (0.0%)	40 (100%)
<i>Rhizopus</i> spp	4±1.41 (21%)	0±0 (0.0%)	3±1.41 (15.78%)	0±0 (0.0%)	12±2.82 (63.15%)	19 (100%)
<i>Alternaria</i> spp	8± 1.41 (100%)	0±0 (0.0%)	0±0 (0.0%)	0±0 (0.0%)	0±0 (0.0%)	8 (100%)

patient room (PR), neonatal ward (NW), intensive care unit( ICU), operating room (OR), the main entrance of the hospital(ME)

Also Isolated *Enterococcus faecalis* bacteria (10CFU/m<sup>3</sup>) and fungi are shown in table5. Among fungi, *Aspergillus* spp. was the highest (45CFU/m<sup>3</sup>) followed by *Penicillium* spp. (35 CFU/m<sup>3</sup>). While *Rhizopus* spp. and *Rhizopus* spp were not find in Ruby Hall hospital.

**Table 5. Airborne microorganisms isolated from five locations in Ruby Hall hospital**

Types of organisms	CFU / m <sup>3</sup> air (%)					
	ICU	OR	NW	ME	PR	Total
<i>Staphylococcus aureus</i>	22±2.82 (24%)	8±1.41 (8.90%)	12± 2.80 (13.33%)	3± 2.85 (3.33%)	45±4.24 (50.44%)	90 (100%)
<i>Enterococcus faecalis</i>	6±1.40 (60%)	0±0 (0.0%)	0±0 (0.0%)	0±0 (0.0%)	4±2.82 (40%)	10 (100%)
<i>Micrococcus luteus</i>	14±1.40 (24.13%)	5± 1.41 (8.62%)	6±2.82 (10.25%)	16±4.24 (27%)	17±2.82 (30%)	58 (100%)
<i>Bacillus subtilis</i>	16±1.35 (47%)	5±2.80 (14.70%)	1±1 (2.94%)	7±4.24 (20.58%)	5±4.24 (14.70%)	34 (100%)
<i>Bacillus cereus</i>	8±1.41 (33.33%)	0±0 (0.0%)	8± 2.82 (33.33%)	3±3 (12.5%)	5±4.24 (20.83%)	24 (100%)
Coagulase-negative <i>Staphylococci</i>	26±2.82 (33.33%)	6±1.40 (7.7%)	0±0 (0.0%)	16±5.25 (20.51%)	30±4.24 (38.46%)	78 (100%)
Unidentified Gram-positive rods	7±2.82 (39%)	0±0 (0.0%)	0±0 (0.0%)	6±4.24 (33.30%)	5±2.82 (28%)	18 (100%)
<i>Pseudomonas aeruginosa</i>	2±1.41 (4.90%)	0±0 (0.0%)	0±0 (0.0%)	25±5.65 (61%)	14±2.82 (34.10%)	41 (100%)
<i>Klebsiella</i> spp.	8± 2.80	3±1.41	4± 1.40	1±1	5±2.82	21

	(38%)	(14%)	(19%)	(4.76%)	(23.80%)	(100%)
<i>Escherichia coli</i>	10±2.82 (30.30%)	3±1.41 (9.09%)	41±1.41 (12.12%)	4±4.24 (12.11%)	12±2.82 (36.38%)	33 (100%)
<i>Enterobacter spp.</i>	0±0 (0.0%)	1±1 (25%)	0±0 (0.0%)	0±0 (0.0%)	3±1.41 (75%)	4 (100%)
Unidentified Gram-negative coccus	1±1 (20%)	0±0 (0.0%)	0±0 (0.0%)	3±1.40 (60%)	1±1 (20%)	5 (100%)
Unidentified Gram-negative rods	26±1.41 (51%)	0±0 (0.0%)	3±3 (6%)	14±4.24 (28%)	8±5.65 (15%)	51 (100%)
<b>Fungi</b>						
<i>Aspergillus spp.</i>	7±2.82 (15.55%)	10± 1.41 (22.26%)	10±2.82 (22.20%)	6± 4.24 (13.33%)	12± 4.24 (26.66%)	45 (100%)
<i>Penicillium spp.</i>	3±1.41 (8.57%)	14±2.82 (40%)	18± 2.80 (51.24%)	0±0 (0.0%)	0±0 (0.0%)	35 (100%)
<i>Rhizopus spp</i>	0±0 (0.0%)	0±0 (0.0%)	0±0 (0.0%)	0±0 (0.0%)	0±0 (0.0%)	0±0 (0.0%)
<i>Alternaria spp</i>	0±0 (0.0%)	0±0 (0.0%)	0±0 (0.0%)	0±0 (0.0%)	0±0 (0.0%)	0±0 (0.0%)

patient room (PR), neonatal ward (NW), intensive care unit( ICU), operating room (OR), the main entrance of the hospital(ME)

In this study, the three investigated factors, the type of room and the time of sampling, individually or combined, type of hospital were found to influence the microbial rate in indoor air of hospital. The results from this study showed that the Jehangir hospital had a higher degree of contamination than Ruby hall clinic with airborne bacteria and in indoor air. These high rates in the hospital might be attributed to the age of the building (hospital was built in 1946) but the age of the Ruby Hall 1966, poor and deficient hygienic conditions, low degree of cleanness and minimal disinfection procedures against airborne bacteria might raise the airborne bio-contaminants. Another factor which might be involved in the latter finding is the number of beds in hospital; the Jehangir hospital houses 170 beds), this high bed number in hospital means a high number of patients, personnel, and visitors occupying the hospital building, and consequently high number in each ward of the hospital (high occupant density). And the multiple patients per room (more than one patient in each room) might raise the number of people in rooms and in the corridors. Hospitals consist of different units with different levels of healthcare services, among these units, there must be a number of highly clean or disinfected units which have to deal with severely ill patients or critical cases such as intensive care units, the operation rooms or neonatal wards. Considering the type of room (location of sampling) as a factor affecting the indoor rate of airborne microorganisms, there was a significant effect of different levels of the degree of cleanness and disinfection strategies, which might lead to increased bacterial rates in the patient room .The high number of visitors that

commonly enter the patient rooms, and the amount of materials brought from outside by the visitors, such as food, fruits, and flowers, were more common in patients rooms. The results from this study seem to support the statement made by most of the workers that patient room had the highest total count of microorganisms. Furthermore, old and poor ventilation systems might serve as another potential source of airborne micro-organisms in intensive care units as well as patient rooms, these microorganisms might be introduced into the indoor air of hospital units. The exchange between indoor and outdoor air raise the microbial rate brought from outside the hospital into the main entrance, and this coincides with many studies which have reported the role of outdoor microbial concentrations through opened windows and doors in raising the microbial rates and homogenization of indoor air of buildings <sup>16</sup>. The number of microorganisms in the operation room and neonatal ward was low. This was anticipated due to the high sanitary standards in this area, compared to other hospital areas. It is worth noting that microbial rates in the operation room were dependent on the hospital. The location of the operation room is very important in order to reduce the microbial exchange with the other units through the air. Intensive disinfection procedures are performed along the day to reduce the microbial rates as much as possible, but the efficiency of these procedures is dependent upon the different factors. Furthermore, the bacterial swabs from surfaces in operation room and neonatal ward indicated that the resident microorganisms have a significant role in raising the bacterial rates in hospital. Room settings and surfaces are potential sources of microorganisms, which are always exchanged with the indoor air, higher surface microorganisms coincide with higher microbial rates in indoor air and vice a versa. Regarding intensive care unit, this unit has to deal with critical cases and there must be sufficient strategies to reduce the microbial rates as much as possible. The microbial rates in this study showed high rates in both hospitals (with higher rates in governmental hospital as mentioned before). This might be correlated to the fact that both hospitals allow visitors to enter the ICU without any precautions (Figure 4.). Moreover, the hospitals in Pune usually have specific times for visiting patients (14:00 - 16:00 pm). In these times, the hospitals are crowded with the visitors in addition to the hospital employees and patients. The time of sampling found to be more effective on the rate of indoor air microorganisms in the Jehangir hospital rather than the Ruby Hall Clinic hospital. These findings agree with the findings of other studies of earlier authors who examined microflora of air in hospitals <sup>2, 3, 5, 11</sup>. Moreover, researcher has specified that bacterial levels were found to be higher and more sensitive to the activities of personnel than fungal concentrations<sup>13</sup>. In the present study, the bacterial rates were more sensitive to the number of people and it also agrees with the results obtained by <sup>20</sup>. The airborne bacterial species which were indicated in Tables 3 were

found to be suspended in indoor air of hospital and might be a potential source of NI in hospital. These species had been reported in several studies that used different isolation and identification procedures<sup>2, 16, 21, 22</sup>. In general, fungal rates were less sensitive to the type of room. This result is in line with the finding of a study that reported that no remarkable differences could be observed in the concentration of airborne fungal propagules inside the hospital units, and in the corridors in front of it<sup>16</sup>. Other studies have indicated that the determination of the concentrations of airborne viable fungi is affected by activities, sources, accuracy of the sampler, growth medium used, and viability of spores<sup>1,11,20,23</sup>.

Moreover, one study have reported that the key to the growth and spreading of fungi in building units is a moisture supply<sup>24</sup>. The result revealed the isolation of ten fungal isolates and six bacterial isolates<sup>25</sup>. The fungal species which were indicated in Tables 5 were found to be suspended in indoor air .The common general of fungi frequently isolated from the hospital air, *Aspergillus*, *Alternaria* and *Penicillium*. In the present study, *Aspergillus* spp. was found to be the most common fungus isolated in the hospital. The study of airborne fungal spores is important to understand the dissemination, spread, and movement of the microbes, particularly the pathogenic ones in the hospital atmosphere<sup>3, 11,19,26,27</sup>.

## CONCLUSION

Nine bacterial genera were isolated and identified from indoor air of Jehangir and Ruby Hall hospital in Pune city. The kind of hospital is a significant factor that influences the rate of indoor air microorganisms because of differences in the age of the hospital buildings, and disinfection strategies. Bacterial rates were more sensitive to the type of unit inside the hospital than fungal rates. The limitations on of the time of visits in the hospitals leads to increase the number of people in hospital building in short period of time and consequently raise the airborne microbial rates at this period of time. Well-constructed ventilation systems and air-conditioning systems are needed to decrease the concentrations of microorganisms that may be introduced into the indoor air of hospitals. The age of hospital, the type of room and the time of sampling are three factors that affect the indoor airborne microbial rates.

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