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# Indoor Airborne Microflora in Jehangir Hospital in Pune City, India

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## Abstract

Hospitals and other healthcare facilities are complex environments that require ventilation for comfort of patients and control of hazardous emissions. This study was carried out the level of airborne microbial load of Jehangir hospital indoor in Pune-India. Microorganisms are the primary sources of indoor air contamination. 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 .Using a microbial air sampler, air samples were taken from a hospital in Pune city. Three factors were investigated to determine how these factors affect the microbial counts, namely the kind of hospital, the type of room and the time of sampling. Some bacterial species were identified in hospital, *Staphylococcus aureus* (15.74%) was found to be the most common organism, followed by *Micrococcus luteus* (13%) and coagulasenegative *Staphylococcus* (13.50%). 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. Microbial rates in the patient room, main entrance and intensive care unit (ICU) were found to be influenced by the time of sampling, while the operating room and neonatal ward were not. These high rates in the Jahangir hospital might be attributed to the age of the building (hospital was built in 1955) 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. 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.

**Key words**: Air sampling, airborne microflora , bacteria, hospital

**Introduction**

Hospital indoor air contains a diverse range of microbial population. The significance of these microbes is debatable in some quarters, whereas elsewhere it may be considered significant. The importance of the estimation of the quantity and types of airborne microorganisms are that these values can be used as an index for the cleanliness of the environment as well as an index they bear in relation to human health and as source of hospital-acquired infections(Jaffal *et al*., 1997 , Splendlore and Fanin, 1983). Microorganisms are the primary sources of indoor air contamination (Lewis, 1994). Hospitals and other healthcare facilities are complex environments that require ventilation for comfort of patients and control of hazardous emissions (Chuaybamroong *et al*., 2008; McCarthy et al., 2000). Moreover, the biological quality of air in hospital environments is of particular concern as patients may serve as a source of pathogenic microorganisms to staff and hospital visitors, in addition to fellow patients (Obbard and Fang, 2003). Although hospitalization and medical procedures are designed to cure diseases, they can sometimes inadvertently introduce pathogenic microorganisms into the body and initiate a nosocomial infection (NI) (Atlas, 1995). The most important source of airborne pathogens inside the hospital is the infected patient (Hambraeus, 1988). Airborne transmission occurs when pathogenic microorganisms are transferred from an infected to a susceptible individual via the air (Atlas, 1995). The predo-minant mechanism that makes the pathogens airborne is the production of aerosol droplets by sneezing or coughing, and their subsequent loss of water which allows them to float in the air over considerable distances and for a long time (Emmerson, 1995). Biological aerosols contain bacteria, viruses, yeasts, molds and fungal spores ( Nevalainen *et al*., 1993). Under special clinical circumstances, skin lesions may also be a source of airborne particles (Hambraeus, 1988) .In spite of 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. Some bacteria, particularly Gram-positive bacteria such as *Streptococcus pneumoniae and Staphylococcus aureus*, can survive for several months in dust particles. 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 (Atlas, 1995; Augustowska and Dutkiewicz, 2006; Matar *et al*., 2005). Controlling airborne pathogens in healthcare facilities is not only important for the safety of the patient, but it is also important for hospital personnel. Various contamination control procedures can limit exposure and risk of infection (Montz and Edward, 2000). There is a demand to reduce airborne microorganisms and their fall out (the bio burden of microorganisms causing infection in healthcare facilities). Furthermore, it is important to identify and accumulate bio burden data of these facilities where the maintenance of a clean environment and the accumulation of data on airborne microorganisms are required (Shintani *et al.*, 2004; Li and Hou, 2003). 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/m3); 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 (Pasquarella *et al*., 2000). There is no single method of choice for sampling airborne loads ( Jaffal *et al*., 1997; Shintany *et al*., 2004). However, impactor air samplers are the most widely used for the quantification of contamination (Nesa *et al*., 2001; Morris *et al*., 2000). 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 (Gangneux *et al.,* 2006; Morris *et al.,* 2000; Prigione *et al*., 2004). Hospital aerosols must be regularly investigated. Gröschel (1980) reported that sampling of air may be performed in hospitals for several purposes, e.g. epidemiologic, surveillance, research, safety or quality control purposes. Other studies have 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 Obbard and Fang, (2003). Li and Hou (2003) have concluded that the significant particle concentration fluctuations in operating rooms may be related to variations in operating personnel numbers and activities. Dust might accumulate in these areas and spores may enter the patient room as contaminants on personnel's clothing.

Most of the infections arising from indoor air could potentially be prevented through adequate application of infection control practices (Bomo. *et al*.;(2004)). For instance, measuring the degree of bacterial contamination of indoor air and the susceptibility pattern of the isolates to commonly used antibiotics in the area will help to select appropriate antibiotics for empirical therapy. This also helps to revise and, if necessary, design appropriate hospital infection prevention protocols in an effort to minimize the incidence of costly SSI (Surgical site infection). Moreover, it provides the tools needed to localize the source and control the spread of SSI ( Dharan, &Pittet, 2002).

This study was conducted to gain knowledge regarding the air quality and the quantity of airborne pathogens in the indoor air Jehangir hospital in Pune city.

## MATERIALS AND METHODS

### Sampling sites

For this study, Jehangir hospital was selected from Pune -India. The hospital was the governmental hospital (built in 1955, 170 beds) .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.

Air sampling

A microbial air sampler was used for sampling of airborne bacteria. 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 (Stetzenbach *et al*., 2004). 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 (Obbard and Fang, 2003). Between measurements the sampler was cleaned by swabbing with 70% Culture media and microbial identification Nutrient agar (NA) (HiMedia Laboratories Limited, Mumbai, India) supplemented with 100 mg/L cyclohex-amide was used for the sampling and cultivation of bacteria (Obbard and Fang, 2003). Twice replica plates of each medium were used for the isolation of bacteria. Nutrient agar plates were incubated at 37°C for 48 h to allow the growth of aerobic bacteria. Bacterial colonies were initially characterized by morphology and microscopic appearance, and identified further by biochemical tests. 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 (Krieg and Holt, 1984; Sneath *et al.*, 1986).

**Statistical analysis**

The total number of colony forming units (CFU) was enumerated and converted to organisms per cubic meter of air (CFU/m3). The mean of the two samples of each bacterium was calculated in all sample locations at hospital. The data were processed with statistical significant differences were determined by one-way and two-way analysis of variance.

**RESULTS**

Air samples from each sampled unit were taken and used for enumeration and isolation of airborne bacteria on NA plates.

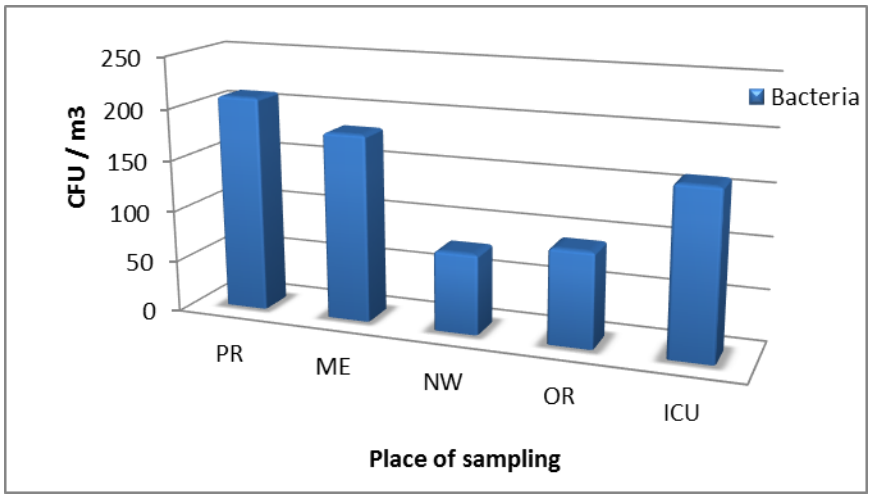
Table 1. Enumeration of bacteria (CFU/m3 air) according to the type of room and the time of sampling

| | **Bacterial CFU/m3 air(n=2)** | | |
|---|---|---|---|
| | Morning 10:30-12:30 | Afternoon 14-16 | Evening 18-20 |
| PR | 210 | 230 | 189 |
| ME | 170 | 220 | 155 |
| NW | 93 | 85 | 55 |
| OR | 80 | 100 | 95 |
| ICU | 150 | 190 | 141 |

ICU: Intensive Care Unit, OR: Operating Room, NW: Neonatal Ward, ME: Main Entrance, PR: Patient Room.

**Enumeration of bacterial colonies from air samples**

The bacterial counts on NA (CFU/m3 air) ranged from 55 CFU/m3 air, which was isolated from the Neonatal Ward, to 230 CFU/m3 air from the patient room of the hospital (Table 1). In hospital, the patient rooms had the maximum bacterial rates, and the minimum rates were detected in the neonatal wards and operation rooms (Figure 1).




**ICU:** Intensive Care Unit, **OR:** Operating Room, **NW:** Neonatal ward, **ME:** Main Entrance, **PR:** Patient Room.
Figure 1. The effect of the kind of the type of room on CFU/m3 air in Jehangir hospital

| | | **Bacterial CFU/m3 air(n=2)** | | |
|---|---|---|---|---|
| | | Morning 10:30-12:30 | Afternoon 14-16 | Evening 18-20 |
| Surface | NW | 75 | 63 | 70 |
| | OR | 40 | 45 | 43 |
| Ventilation | PR | 212 | 260 | 180 |
| | ICU | 170 | 231 | 167 |

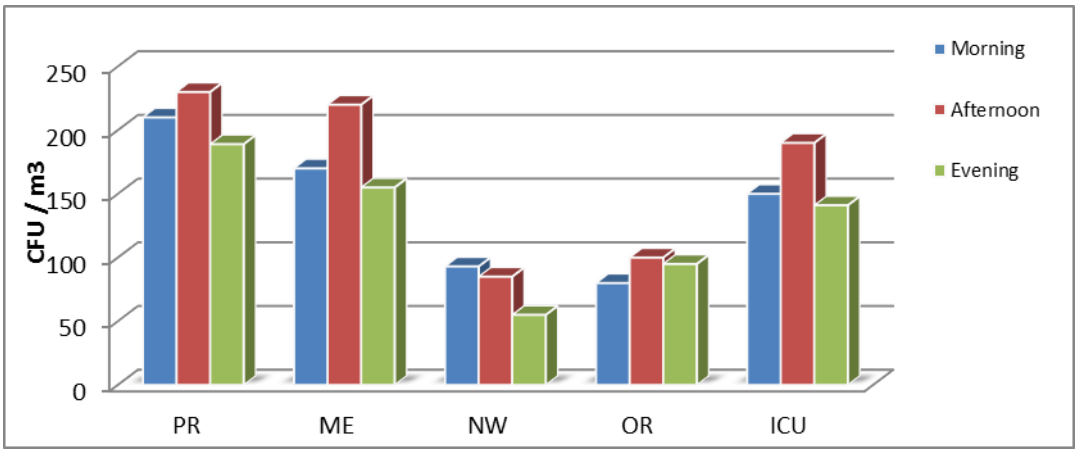
**Enumeration of microbial colonies from swab samples**

Depicts table2 the counts of bacterial swabs taken from the surfaces of the operating rooms and neonatal wards from the hospital. The counts ranged from 40 to 75 CFU. In 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 hospital.

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

ICU: Intensive Care Unit, OR: Operating Room, NW: Neonatal Ward, ME: Main Entrance, PR: Patient Room.

Figure 2 shows that bacterial CFU/m3 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.




**ICU:** Intensive Care Unit, **OR:** Operating Room, **NW:** Neonatal ward, **ME:** Main Entrance, **PR:** Patient Room.

Figure 2. The effect of type of room and time of sampling on CFU/$m^3$ air in hospital

The types of microorganisms isolated from the air of the five different locations are shown in Tables 3 . The largest quantities of isolated bacteria in the hospital was S. aureus (125 CFU/m3),followed by Micrococcus luteus (104 CFU/$m^3$) and coagulase negative Staphylococci (106 CFU/$m^3$).Others Isolated bacteria are shown in table3.

**Table 3.** Airborne microorganisms isolated from five locations in Jehangir hospital

| **Types of organisms** | **CFU / $m^3$ air (%)** | | | | | |
|---|---|---|---|---|---|---|
| **Bacteria** | **ICU** | **OR** | **NW** | **ME** | **PR** | **Total** |
| *Staphylococcus aureus* | 22(17.6%) | 16(12.8%) | 22(17.6%) | 0(0.0%) | 65(52%) | 125(100%) |
| *Enterococcus faecalis* | 20(48.78%) | 3(7.31%) | 10(24.39%) | 35(7.3%) | 41(12.19%) | 41(100%) |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Micrococcus luteus* | 21(20.19%) | 22(21.15%) | 14(13.46%) | 14(13.46%) | 33(31.73%) | 104(100%) |
| *Bacillus subtilis* | 20(32.25%) | 8(12.90%) | 8(13%) | 10(16.12%) | 16(25.80%) | 62(100%) |
| *Bacillus cereus* | 14(40%) | 12(34.28%) | 6(12.9) | 3(8.57\|%) | 0(0%) | 35(100%) |
| Coagulase-negative *Staphylococci* | 14(13.20%) | 14(13.20%) | 6(5.66) | 35(33.01%) | 37(34.90%) | 106(100%) |
| Unidentified Gram-positive rods | 10(23.25%) | 8(18.60%) | 0(0%) | 9(20.93%) | 16(37.20%) | 43(100%) |
| *Pseudomonas aeruginosa* | 5(18.51%) | 0(0%) | 0(0%) | 22(81.48%) | 0(0%) | 27(100%) |
| *Klebsiella* spp. | 12(22.64%) | 5(9.43%) | 3(5.66%) | 21(39.62%) | 12(22.64%) | 53(100%) |
| *Escherichia coli* | 21(28.76%) | 10(13.69%) | 8(10%) | 16(21.91%) | 18(24.65%) | 73(100%) |
| *Enterobacter* spp. | 1(5%) | 5(25%) | 0(0%) | 14(70%) | 0(0%) | 20(100%) |
| Unidentified Gram-negative coccus | 0(0%) | 0(0%) | 2(16%) | 6(50%) | 4(33.33%) | 12(100%) |
| Unidentified Gram-negative rods | 12(12.90%) | 5(5.37%) | 8(8.5%) | 32(34.40%) | 36(38.70%) | 93(100%) |

**ICU:** Intensive Care Unit, **OR:** Operating Room, **NW:** Neonatal ward, **ME:** Main Entrance, **PR:** Patient Room.

## DISCUSSION

In this study, the two investigated factors, the type of room and the time of sampling, individually or combined, were found to influence the microbial rate in indoor air of hospital. The results from this study showed that the Jehangir hospital had a high degree of contamination 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 1955), 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. These are recognized source of hospital contamination (Jaffal *et al.*, 1997). 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. Kumari *et al*. (1998) have reported the role of ventilation grills as a potential source of methicillin-resistant S. aureus.

In the main entrance, which is the passageway between the hospital and its environment, the large numbers of patients, visitors and personnel raise the microbial rates especially at the afternoon because of the maximum activity of people there. 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 (Jaffal *et al*., 1997; Rainer *et al.*, 2000).

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. Li and Hou (2003) have reported that in the hospital operating rooms in Taipei, Taiwan, the concentration of airborne bacteria also varied from 10 to 102 CFU/m3, but in the bone marrow transplantation rooms the concentration was much lower, changing to 0 to 2 CFU/m3. Similar data have been published by other workers (Augustowska and Dutkiewicz, 2006 and Pastuszka *et al*., 2005; Krogulski, 2008, and 2006).

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 Jehangir hospital. This might be correlated to the fact that hospital allows visitors to enter the ICU without any precautions.

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.

In the present study, the bacterial rates were more sensitive to the number of people and it also agrees with the results obtained by Talon (1999), Emori and Gaynes (1993), and Sudharsanam *et al*. (2008). The results obtained by these researcher showed that there is mounting evidence that the environment of high number of patients colonized with bacteria serves as a potential reservoir for dispersal and hence, possible infection in the hospital environment. Obbard and Fang (2003) showed that occupant density is a key factor affecting concentrations of airborne bacteria; their results showed that occupant density was dependent upon the time and these support our findings.

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 (Schaal, 1991; O'Connell and Humphreys, 2000; Vincent et al., 1995; Warris et al., 2001; VandenBergh et al.,1999 Rainer et al., 2000; McCarthy et al., 2000). The number of potentially pathogenic organisms in the hospital air was high. Pathogenic organisms represented more than 37% of the total count of bacteria isolated. S. aureus was found to be the most common organism isolated presenting 15.74%. A similar observation was observed in the study of Jaffal, *et al*. (1997) and showed that S. aureus was more common in the pediatric and female surgical wards.

Conclusion

Nine bacterial genera were isolated and identified from indoor air of Jehangir hospital in Pune city. The kind of 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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