CHAPTER 5

DISCUSSION

Bioaerosols are airborne microorganisms and their breakdown products that are capable of causing adverse health effects in human beings on exposure. The health effects may be mild to severe depending on the individual's immune status. Bioaerosols have been characterised in different settings. However, their estimation in healthcare facilities is not well studied, especially in the context of a developing country like India.

Healthcare facilities are a unique environment, where patients admitted are with varied degrees of immunity, and include vulnerable population comprising of children, elderly and immuno-compromised. Nosocomial infections are caused by microbial agents, which are acquired most often through the hands of health personnel and sometimes from the environment (CDC, 2003).

Reports from different parts of India have documented the presence of microorganisms in indoor air of hospitals (Kelkar et al., 2005; Gniadek and Macura, 2007). While it is known that microorganisms can be present in the air of artificially ventilated closed systems including intensive care units and operating rooms (Kaur and Hans, 2007; Sudharsanam et al., 2008), there is however paucity in data on environmental factors influencing the airborne microbial loads. The survival and multiplication of microorganisms are known to be affected by physical parameters such as temperature and relative humidity (Stetzenbach, 2002).
The influence of seasons on the type and concentrations of microorganisms present in indoor air of hospitals has been previously documented. A study conducted in a hospital ward of a pneumono logical department in Poland found seasonal variations in total microbial loads, with greater variation among fungi than bacteria (Augustowska and Dutkiewicz, 2006). Another study carried out in two haematological units of a French hospital documented seasonal variations in fungal loads, with fungal concentrations significantly lower in winter (2.7-3.1 cfu/m$^3$) than in summer (4.2-5.0 cfu/m$^3$) in both haematology units (Sautour et al., 2009).

In addition to the environmental factors, hospital design also plays a major role, where improper / faulty design may impact quality of indoor air adversely. Furthermore, condensation during excessive rainfall might also contribute to increased concentrations of airborne microorganisms. Increasing adoption 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.

It is therefore essential to carry out long-term studies to characterise airborne microorganisms in different locations of healthcare facilities, study the ambient environmental conditions of an artificially ventilated closed system, and determine the fluctuations in airborne microbial loads with changing seasons and with hospital location, especially in the context of a developing country with tropical climate, to develop infection prevention measures.

Studies have indicated that airborne transmission of nosocomial infections is possible (Beggs, 2003). While investigations carried out during outbreaks have established the link between air and transmission of nosocomial
infections, no prospective studies have been carried out so far. Thus, an attempt was made to determine the role of these airborne microorganisms in causing healthcare associated infections.

The occurrence of airborne GNB in higher concentrations in hospital environment (Sudharsanam et al., 2008; Ekhaise et al., 2010), indicates the possibility that their by-products such as endotoxins also get airborne. Endotoxins are lipopolysaccharides present in cell wall of GNB. They are commonly present in occupational settings such as agriculture, animal feed industries and sewage treatment environment (Douwes et al., 2003).

While presence of endotoxin in house dust has been documented in indoor settings such as residential environments (Peterson et al., 1964; Douwes et al., 2000b), healthcare facilities have not been studied in detail for their presence. It was therefore decided to undertake a study to assess the presence of endotoxins in indoor air of a low-cost intensive care unit of a healthcare facility.

5.1 Microbial profile of air

5.1.1 Characterisation of airborne microorganisms in Hospitals

5.1.1.1 Characterisation of bioaerosols by active and passive methods

The present study was undertaken to characterise airborne bacteria and fungi by using active and passive methods of air sampling. Impinger and filter were the active methods used in the study, while exposed plate was the passive method included in the study.
In this study, it was found that the extent of recovery of microorganisms varied with the sampling method used. For instance, airborne bacterial loads in ORs of group II hospitals were found to range between 9 – 28 CFU/plate by exposed plate method, 560 – 10080 CFU/m³ by impinger method, and 7576 – 28409 CFU/m³ by filter method. Comparison of airborne microbials loads obtained by different methods was not feasible since only one-time sampling was carried out.

Among bacteria, CNS and Micrococci were found to be recovered by all the methods. Pseudomonas sp. was found to be isolated using exposed plate and impinger methods, while their isolation was limited when filter method was used. Exposed plate was found to be useful to recover other GNB such as Klebsiella oxytoca and Acinetobacter sp. These were not recovered when sampling was done using active methods. Among fungi, moulds were predominant, of which Aspergillus sp. was the commonest. They were isolated in active and passive samples, though the species varied. Isolation of moulds was slightly limited in impinger method, when compared to the filter method of air sampling. Yeasts such as Candida tropicalis were found to be recovered only by exposed plate method.

Of the two active sampling methods used in the study, impinger method was found to recover GPC and GNB while recovery of fungi was limited. Filter method, on the other hand, was found to recover fungi than bacteria, especially GNB. It was further found that the concentrations of airborne bacteria and fungi also varied between the methods. This may be attributed to the inherent deficiencies in the methods. Impingers are known for their inability to recover fungal spores due to forceful impingement of air (Buttner et al., 2002).
Filters are suitable for recovery of fungal spores; however they are inappropriate for recovery of vegetative cells due to desiccation and subsequent death of microbial cells (Buttner et al., 2002). Exposed plate method was found to capture majority of the aerobic bacteria and fungi. Exposed plates can be preferred due to its simplicity as this does not require expertise and is inexpensive (Pasquarella et al., 2000).

Different types of active and passive methods are available for air sampling (Macher et al., 1995). Active methods are volumetric, and are often preferred, since the volume of air from which particles are recovered is known unlike the passive method. However, the choice of the sampling method should be made based on the type of setting under study.

Active methods often find their use in settings like agriculture and poultry farming, where bioaerosol concentrations are high. Healthcare facilities, on the other hand, are settings where indoor bioaerosol concentrations are less. Use of active methods in such settings may not be appropriate as the recovery of airborne microorganisms is hampered due to their inherent deficiencies. Exposed plate method, thus finds its use particularly in the context of a healthcare setting where the recovery is primarily due to particle fall-out. Though airborne microbial concentrations are less in these settings, it is essential to determine the different types of microorganisms that are present in the hospital environment for improved patient care.

Given the advantages, use of exposed plate method has certain limitations. This method may not be appropriate to determine the relationship between inhalable microbial exposure levels and nosocomial infections.
Moreover, since only a small fraction of microbial load is collected due to gravitational pull, many species could be missing and remain unidentified.

5.1.1.2 Comparison of indoor and outdoor bioaerosol concentrations

Estimation of bioaerosols is best conducted when it is assessed in both indoor and outdoor air (Macher et al., 1995). Air sampling was therefore undertaken both inside and outside the hospitals. Walk-through was conducted at the time of sampling to determine potential sources of bioaerosols, colonisation of microorganisms or any evidences for dampness through visual inspection.

When indoor and outdoor microbial loads obtained by exposed plate method in five different hospitals were compared, it was found that indoor microbial loads were less than that of outdoor air in all the sampled locations of the hospitals under study except in ward of H4 (Indoor/Outdoor ratio = 2.18) and H6 (Indoor/Outdoor ratio = 2.18) hospitals.

Indoor bacterial loads obtained by exposed plate method were higher in wards of H4 and H6 hospitals, while fungal loads in indoor air were found to be higher in ICU of H2 hospital, OR of H2 and H5 hospitals and wards of H2, H4, H5 and H6 hospitals. Walk-through conducted at the time of sampling did not reveal dampness or any possible sources indoor.

Indoor bioaerosol concentrations obtained by impinger and filter methods were found to vary with hospitals and was found to be inconsistent. Indoor concentrations in operating rooms obtained by impinger method were found to be less than that of outdoor air, except in H4 and H6 hospitals. Indoor bioaerosol concentrations obtained by filter method was found to be less in operating rooms of H4, H5 and H6 hospitals, and in wards of H4 and H6 hospitals.
Increased bacterial loads in indoor air of the studied wards may be attributed to the lack of supply of fresh outside air (Macher et al., 1995). Increased levels of indoor fungi with respect to that of outdoor air also suggest the need for proper ventilation (Macher et al., 1995). Locations such as wards are generally mechanically ventilated, where dampness may support the growth of fungi within walls and ceilings (WHO, 2009). Increased fungal loads in controlled settings such as OR and ICU was observed. Initial walk-through of these areas did not reveal any evidence of fungal growth indoors. However, it is possible that these fungal spores might have gained entry through cracks in the walls, or through A/C units and ventilator ducts (Kelkar et al., 2005).

Since indoor air of different hospitals were found to harbour a variety of microorganisms including nosocomial pathogens and fungi, it is possible that there are no appropriate guidelines for hospital design. This indicates an urgent need for proper hospital design with adequate ventilation.

The aim of outdoor air sampling was to compare with that of indoor air; therefore characterisation of outdoor microflora was done depending on the type of microorganisms recovered from indoor air.

5.1.1.3 Microbial profile of indoor air in different hospitals

Bacteria were present in higher concentrations than fungi in all the hospitals, irrespective of the method used to collect air samples. Among bacteria, GPC were recovered in higher concentrations 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.
In this study, bacteria such as *Staphylococcus aureus*, coagulase-negative *Staphylococci*, *Micrococcus*, *Pseudomonas*, *Acinetobacter* and fungi such as *Aspergillus* were recovered from hospital air which is similar to previous reports (Jaffal *et al.*, 1997; Sarca *et al.*, 2002; Obbard and Fang 2003; Ekhaise *et al.*, 2008; Qudiesat *et al.*, 2009). *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.

Possible sources of GPC are the occupants who constantly shed the normal flora of skin and hair such as *Staphylococci* in the form of skin squames (Ayliffe *et al.*, 1999). Increased concentrations of GPC may be attributed to the lower susceptibility of these organisms to unfavourable environmental conditions due to the presence of pigments and photo-reactivation mechanisms that provide protection from sunlight, and higher peptidoglycan contents in their cell walls, which provides protection from drying and heat stress (Stetzenbach, 2005).

GNB normally colonise moist surfaces (Kiska and Gilligan, 2003). Their recovery from air may be due to aerosol generating procedures that may cause liberation of these GNB into air (WHO, 2002). Previous studies have shown that GNB get generated and dispersed in air in the form of aerosols from contaminated fluids from humidifiers, nebulizers, and wet cleaning equipment (Ayliffe and Lowbury, 1982). GNB were however recovered in less numbers in this study. This may be attributed to their susceptibility to environmental stress due to lower peptidoglycan contents, leading to their injury and/or cell death (Stetzenbach, 2005).
While *Staphylococcus aureus* are known nosocomial pathogens, CNS are gaining importance due to their increasing ability to cause a range of infections, especially in immuno-compromised (Jarvis and Martone, 1992; Piette and Verschraegen, 2009). Their presence in indoor air, especially in high concentrations, may facilitate colonisation of the anterior nares of health personnel, leading to transmission of nosocomial infections. Further, they may cause infections due to particle fall-out directly on wounds, or indirectly through inanimate objects. In addition, *Micrococc*, which are common hospital contaminants, have been implicated to cause localised cutaneous infections in immunocompromised (Smith *et al*., 1999).

Among fungi, *Aspergillus* sp. was the predominant isolate irrespective of the hospitals. This may be attributed to the fact that *Aspergillus* sp. have relatively low moisture requirements and their ability to tolerate dry conditions enable them to colonize and/or survive in areas where only minimal or intermittent moisture is available that cannot support other fungi (Klich, 2002). Increasing incidences of nosocomial Aspergillosis and their association with the building hygiene, design and indoor air (Anderson *et al*., 1996) indicate the need for further investigations.

### 5.1.2 Characterisation of airborne fungi in various locations of hospital

Preliminary study findings have shown that the concentrations of bacteria are higher than those of fungi. It was further observed that CNS and *Micrococc* were the predominant bacterial isolates while *Aspergillus* sp. was found to be the predominant fungal isolate recovered from indoor air of different locations, and in different hospitals.
Since the presence of fungi in indoor air of hospitals was documented, a further study was undertaken in one hospital over a period of three years to determine if any other fungi are present in indoor air of the hospital environment. Air samples were collected from ICUs, ORs, wards, laboratories and other places such as laundry (where soiled bed linen and other clothes from the hospital were washed and disinfected).

When the pattern of fungi isolated over three years was analysed, moulds were found in higher frequency when compared to that of yeasts. Among yeasts, *Candida non-albicans* were the only isolate. Among moulds, it was found that *Aspergillus flavus* was the commonest fungi recovered in 2006 (29 %; n=13), while *Aspergillus niger* predominated in the years 2007 (43 %; n=6) and 2008 (39 %; n=16).

When the recovery of fungi by different methods was analysed, it was found that recovery was better when exposed plate (65 %; n=65) and filter (22 %; n=22) methods were used, while that of impinger method (13 %; n=13) was found to be less. This may be attributed to the inherent deficiencies in the sampling method used as previously described in section 5.1.1.1.

When the data was analysed based on the sampling locations, it was found that *Aspergillus* sp. was the predominant fungi recovered irrespective of the location. Other fungi recovered included *Absidia* sp. and *Fusarium oxysporum*. The study findings corroborate with previous studies carried out in Indian hospitals, where *Aspergillus* sp. were recovered from indoor air of ICUs and ORs (Kelkar et al., 2005; Gniadek and Macura, 2007). Presence of fungal spores in sterile areas such as ORs may facilitate their entry during invasive procedures at the time of surgery.
Fungi may be present and remain unnoticed indicating the need for air sampling. The mere presence of fungi indicates the need for measures to ensure that no fungi are seen in indoor air of hospitals. This may be accomplished through proper air handling units. The study findings were immediately reported to concerned hospital authorities and corrective measures were taken.

Inhalation of *Aspergillus* spores has been shown to cause invasive aspergillosis in both immuno-compromised (Mukhopadyay *et al*., 2008) and immuno-competent (Gupta *et al*., 2004; Agarwal *et al*., 2005) hosts. They are also known to cause allergic broncho-pulmonary aspergillosis, rhinitis and sinusitis (Diwakar *et al*., 2008), and adverse effects due to exposure to mycotoxins such as aflatoxin (*A. flavus*), patulin (*A. terreus*) and fumitremorgin (*A. fumigatus*) (Frisvad and Thrane, 2000). This indicates the need for engineering control measures to minimize the entry of these species into the hospital environment.

*Absidia* sp. and *Fusarium oxysporum* were also recovered in the study. These are saprophytic fungi that are capable of causing opportunistic infections. *Fusarium* infections in immuno-competent hosts usually result from direct inoculation of skin and soft tissue or ingestion or inhalation of conidia (Nelson *et al*., 1994; Nucci and Anaissie, 2002) that can result in onychomycosis, keratitis, endophthalmitis, and mycetoma. *Fusarium* infections in immuno-compromised patients may include cellulitis, pneumonia, and fungemia with disseminated skin lesions (Nucci and Anaissie, 2002); these can be acquired via direct inoculation or through the sinopulmonary tract. Exposure to *Absidia* sp. has been associated with acute sinusitis and rhinocerebral mucormycosis in immuno-
compromised individuals (CDC, 2006). They have also been implicated to cause allergies, dermatitis and keratitis.

Recovrey of *Candida non-albicans* from indoor air of hospital locations including ICUs and ORs is worrisome, since these yeasts are increasingly documented as a cause for invasive candidiasis (Xess *et al*., 2007; Kothari and Sagar, 2008).

The main aim of the study was to characterize the airborne fungi in different locations by active and passive methods of air sampling. The preliminary findings suggest that filter and exposed plate methods are better options for recovery of airborne fungi. Further studies can be aimed at targeting simultaneous sampling by active and passive methods to corroborate the findings. The study was designed to characterise and determine the frequency of occurrence of different fungi; their concentrations and associated health effects were therefore not studied.

### 5.2 Factors influencing the bioaerosols in indoor air of a healthcare facility

Having recovered bioaerosols from indoor air of different locations of healthcare facilities, what could be the factors that determine the presence and/or survival of these airborne microorganisms? A study was therefore undertaken to determine the microbial loads in indoor air of an artificially ventilated closed system and characterize the microorganisms isolated, and study the influence of ambient physical parameters (temperature and relative humidity) and extent of activity on airborne microbial loads. A naturally ventilated open system was also
studied, in addition, to generate baseline data on the factors influencing the airborne microbial loads.

Airborne microbial loads were found to be limited when temperature was maintained at optimum levels, in both closed (20 – 24 CFU/plate at an indoor temperature of 22 – 26 °C) and open (62 – 99 CFU/plate where indoor temperature ranged between 31.2 – 32.5 °C) systems. It was further observed that the recovery of airborne microbial loads was maximum (63 CFU/plate in closed system and 140 CFU/plate in open system) at high levels of relative humidity (80 % and 72 % in closed and open systems respectively), with temperature being recorded below desired levels (20.5 °C and 26.3 °C respectively in closed and open systems). Number of personnel did not have an appreciable impact on the airborne microbial loads; however higher loads were recovered from indoor air of open system when there were more than 15 occupants.

GNB such as Pseudomonas sp. were isolated when there was an increase in relative humidity (70 – 80 %) in the closed system. Yeasts such as Candida krusei were recovered from indoor air of open system at increased relative humidity levels (72 – 73 %).

The preliminary findings of the study indicate that environmental parameters such as temperature and relative humidity have impact on the airborne microbial loads irrespective of the nature of the ventilation. In order to determine the impact of the extent of activity on the airborne microbial loads, closed system was chosen since the environmental conditions are controlled, unlike an open system where it is difficult to monitor due to periodic dilution by outdoor air.
When the data was analysed regarding the time of sampling, environmental factors, i.e., temperature and relative humidity, and the correlation with the extent of activity, it was found that the microbial loads were high at times of activity at ambient temperature-humidity conditions. Analysis further showed microbial loads to be the maximum at start of the day. This may be attributed to the suspension of the particles settled overnight due to air currents created by restart of the air-conditioning system following overnight shut down. Loads were found to be subsequently reduced with continued functioning of the air-conditioning systems. A study from Germany documented that shutting down of air-conditioning system of operating rooms at nights to conserve energy did not result in microbial contamination of the ORs, and suggested that normal ventilation should be established at least 30 minutes before surgical activity (Dettenkofer et al., 2003). Since the energy demands are high in a healthcare facility, it is important to consider measures that can save energy without compromising infection control practices. The microbial loads were found to remain high during the on-going activities and decreased to minimum at the end of the day. This may be attributed to several factors, such as the level of activity and physical parameters like temperature and relative humidity.

Microbial loads increased with raised humidity at constant temperature. This finding corroborates with earlier findings, indicating a need to maintain a temperature of 20 – 24.4 °C and a relative humidity of 50 – 60 % to inhibit microbial growth (Gruendemann and Mangum, 2001). The maintenance of appropriate humidity levels may be accomplished by the use of refrigerated dehumidifiers or chemical dehumidifiers.
When the data was analysed on the impact of the number of personnel on microbial loads, it was found that airborne microbial loads were not directly affected by the number of personnel present.

When loads of GPC and GNB were analysed, it was found that loads of GPC were higher than those of GNB. Coagulase-negative \textit{Staphylococci} and \textit{Micrococci} were isolated from all samples. \textit{Pseudomonas} sp. was the predominant GNB, and was found to be isolated in times of increased levels of humidity. \textit{Aspergillus niger}, \textit{Aspergillus fumigatus}, \textit{Aspergillus terreus} and fungal hyphae were isolated during different times of the day. The pattern of microorganisms isolated was found to be similar to the earlier studies (Kaur and Hans, 2007; Sudharsanam \textit{et al.}, 2008); however, fungal species were isolated in addition, \textit{Aspergillus} sp. being the predominant species. Though fungi were isolated, they were present in less numbers, and were not recovered throughout the study period. Their presence may be attributed to high humidity levels.

Earlier reports have documented GPC to be more prevalent in indoor than outdoor air, due to greater shedding from exposed skin surfaces of the occupants (Tang, 2009). Studies have further shown the survival of aerosolized Gram-negative bacteria such as \textit{Pseudomonas} species to be greatest in high RH low temperature conditions (Tang, 2009).

The study has certain limitations. The purpose of the study was to determine the environmental factors in artificially ventilated closed systems, due to ease in conduct of the study as the environmental conditions were expected to be constant. Further studies can be undertaken in open systems to determine the combined effects of environmental factors and activity.
5.3 Temporal variation of airborne microorganisms

Having studied that environmental factors such as temperature and relative humidity affect the bioaerosols in indoor air, it is essential to determine if there is any variation in the airborne microbial loads obtained over a period of time. A prospective study was therefore undertaken in a naturally ventilated ward over a period of one year, where sampling months were randomly chosen and samples were collected between 15th and 20th days of the month.

5.3.1 Choice of air sampling methods

So far, to our knowledge, no long-term studies have been carried out in the context of a developing country with resource limitations. Although different active and passive methods of air sampling are available, no single method is considered efficient for recovery of microorganisms, or is ideal in every sampling situation (Macher et al., 1995).

GPC are usually recovered by active methods of sampling. GNB, however, may get destroyed due to forceful impingement of air samples or desiccation at the time of filtration of air samples. Previous studies have documented isolation of GNB using exposed plate method (Sudharsanam et al., 2008; Ekhaise et al., 2008), thereby, making it essential to include exposed plate method for better recovery. Choice of the media used in the study was made based on the availability of the resources. In the context of a healthcare facility, media such as blood agar, MacConkey agar and Sabouraud's Dextrose agar are readily available in the laboratory facilitating easier sampling at times of an investigation without delay.
5.3.2 Temporal variation in airborne microbial loads

A repeated measure sampling was done as it allows for an analysis to take into account the unique microbial and meteorological conditions within the sampling location. This allows for seasonal comparisons that are not influenced by how high or low the baseline is for the location (Mota et al., 2008). A temporal variation in the airborne microbial loads was documented; however there was no statistical significance in the variation captured by active and passive sampling methods. The lack of appreciable variation in the airborne microbial loads may be attributed to the inherent deficiencies in the method used for sampling. The variation within duplicate samples obtained by exposed plate method was minimal and had a strong correlation of 89.7 % with a statistical significance of p=0.01; active methods, on the other hand, had huge variations within the duplicate samples with no statistical significance.

This study indicates that exposed plate is more suitable and appropriate for areas like healthcare facilities as compared to outdoor settings like agriculture and poultry farming, where microbial loads are comparatively high and require active sampling to determine the loads. Exposed plate method is well-known for its reproducibility and reliability (Pasquarella et al., 2000). Additionally, this method is cost-effective and finds its use in surveillance of air in healthcare facilities for isolation of GNB (Sudharsanam et al., 2008; Ekhaise et al., 2008). Volumetric samplers often measure the total number of microorganisms in the air, and therefore an indirect measurement of the likely microbial contamination of a surface at risk through fallout (Pasquarella et al., 2000).
Sampling by active methods (filter and impinger) showed that the recovery of the microorganisms was not appreciable, and variation was found to be statistically not significant. The variation within the samples collected in duplicates was found to be huge and inconsistent, irrespective of the sampling method used. The fluctuation in the recovery of microorganisms may be attributed to the inherent deficiencies of these methods such as forceful impingement / desiccation, which may cause microbial cell damage thereby affecting the recovery of the Gram-positive / Gram-negative organisms.

5.3.3 Comparison between active methods of air sampling

The comparison of airborne microbial concentrations obtained by impinger and filter methods showed that there was no significant correlation between the concentrations. This may be attributed to the inconsistent and differential recovery of microorganisms by the methods due to inherent deficiencies, as previously described.

5.3.4 Comparison between indoor and outdoor microbial loads

Microbial loads in indoor air were less than that of the outdoor air irrespective of the method used for isolation of the culturable bioaerosols. Pattern of indoor and outdoor isolates was similar. Whenever outdoor microbial loads increased, there was a corresponding increase in microbial loads of indoor air. This indicates that indoor microflora is influenced by outdoor environmental conditions. Isolation pattern of microbial loads from indoor and outdoor air was similar in both active and passive methods thereby validating the methodology used.
5.3.5 Microbial profile of indoor air

Microorganisms were isolated throughout the year with fluctuations in their loads with the sampling months or the changing seasons. Concentrations of GPC were very high than GNB. Coagulase-negative Staphylococci (CNS) and Micrococci were predominant in all seasons irrespective of the sampling method. Enterobacter and Pseudomonas were the predominant GNB. Exposed method was efficient in isolation of GNB; impinger and filter methods did not facilitate recovery of GNB during the study period except one sampling month (January). It was further observed that Pseudomonas sp. was isolated in summer and monsoon months but not in winter. Aspergillus niger fungi was isolated throughout the year irrespective of the changing season. Candida krusei was the only yeast isolated at the onset of summer (April).

High concentrations of GPC may be attributed to their ability to survive under stressful environmental conditions (Stetzenbach, 2005). Other factors that may contribute are improper ventilation and presence of increased number of occupants beyond room capacities (Ayliffe et al., 1999), as GPC are constantly shed from skin, clothing and hair. Though CNS were the predominant isolates, increasing incidences of nosocomial infections by CNS make it a cause for concern (von Eiff et al., 2001).

Isolation of GNB was occasional and in lower concentrations. One of the reasons may probably be their vulnerability to harsh environmental conditions (Stetzenbach, 2005). Pseudomonas sp. and Enterobacter sp. were the commonly isolated GNB. These organisms are often found to grow on moist surfaces. Their presence may be attributed to the presence of wash-room in the vicinity of the sampling area, which may provide moisture required for survival and growth.
Since both *Pseudomonas* sp. and *Enterobacter* sp. are known nosocomial agents, understanding of the hospital milieu is essential to identify the environmental conditions that favour the growth of these microorganisms. This will provide insights into aspects for proper designing and construction of hospitals and help to target preventive measures.

Though fungi were seen in fewer numbers, their mere existence in the hospital air is of concern. Species of *Aspergillus* have been associated with incidences of nosocomial infections in immuno-compromised patients and children either as primary or secondary infections. Allergic reactions have also been reported following inhalation of fungal spores, making it essential to pay attention to their presence in hospital air (Anderson *et al.*, 1996).

### 5.4 Variations in airborne microbial loads within and between hospitals

So far, to our knowledge, no studies have been carried out to determine if the size of the hospital can impact the bioaerosol concentrations. An attempt was therefore made to carry out a study to determine if variations exist in airborne microbial loads with size of the hospitals and with the sampling locations.

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. Exposed plate method was only used for sampling; active methods were not included in the study due to inherent
inconsistencies existing in the active methods, as described earlier. Kruskal-Wallis test, a non-parametric test was used to determine the variations in airborne microbial loads with respect to sampling location and size 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 size-wise comparison) were involved.

Recovery of airborne microorganisms from ORs was the least followed by ICUs and then wards. Variations in airborne microbial loads were found to be significant with respect to sampling locations. The location-wise variations in airborne microbial loads may be attributed to the procedures that are followed. Operating rooms are generally sterile areas and aseptic conditions are maintained 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 is more than that of sterile areas like ORs. Wards are locations where there are no requirements for maintenance of aseptic conditions and restrictions; therefore recovery of airborne microbial loads was maximum.

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 CDC (2003). ORs are sterile 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 minimise the entry of fungal spores. Wards do
not require maintenance of aseptic conditions. These areas, however, need adequate ventilation, especially in wards such as post-operative wards, to minimise the risk of nosocomial infections due to particle fall-out.

Airborne microbial loads were found to vary with hospitals; the least being documented in group III hospital and the highest in group I hospitals. The variation was however not significant. Though there was no significant variation in the airborne microbial loads with the size of the hospitals, from the study findings described earlier, it was found that indoor microbial loads are higher than that of outdoor air, indicating the lack of dilution by outdoor air due to improper ventilation. The study emphasizes the need to develop corrective measures for adequate ventilation.

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 size and location.

Lot of time and money are spent in delivering quality healthcare. Heavy investments are made on high-end instruments to assist doctors for improved diagnosis and treatment. These are considered as primary areas of concern for optimum and quality healthcare delivery, and efforts are taken. However, nature of construction, design of building and ventilation are often overlooked and not given importance. This study has looked at the impact of environmental factors on the recovery of airborne microorganisms.

It is known that only certain areas of the hospital need to have sterile environment such as operating rooms. It is therefore not unusual that
microorganisms may be recovered in other areas. There are no threshold limits for exposure to bioaerosols unlike chemicals. Lack of such standards is indicative of the fact that it is difficult to rid the environment of microorganisms. It is therefore essential to maintain the airborne microbial loads at their minimum, through proper hospital design with adequate ventilation.

5.5 Correlation of environmental and clinical strains

5.5.1 Correlation of environmental strains of *Pseudomonas* with pathogenic species

*Pseudomonas* species are among the commonest etiologic agents of HAI s in India. Prevalence of *Pseudomonas* infections has been documented as 13.6% in neonatal ICU and 12.7% in general ICU (Kamath et al., 2010; Mohanasoundaram, 2010). They are known to survive in a variety of environmental niches. Their ability to survive in adverse conditions has enabled them to successfully cause HAI s. These organisms are known to survive on inanimate objects and dry surfaces. For example, *Pseudomonas aeruginosa* can persist on inanimate objects for 6 h – 16 weeks, and upto 5 weeks on dry surfaces (Kramer et al., 2006). Principal reservoirs for *Pseudomonas* species are the hands of the personnel, sinks or other moist surfaces, and solutions used to rinse catheters (Brown and Baublis, 1977), where transmission is known to occur primarily via hands. However, airborne dissemination has been implicated to play a significant role in patient-to-patient spread (Panagea et al., 2005). This study was therefore carried out to determine whether air can be a reservoir of *Pseudomonas* species.
*Pseudomonas* sp. was found to be recovered from indoor air of all the hospital sampled, including the ICUs and ORs, irrespective of the size of the hospital. When one-time sampling was conducted in duplicates in 28 different locations of six hospitals, *Pseudomonas* sp. was present in indoor air of 20 (71%) locations. *Pseudomonas stutzeri* was the predominant species isolated. Of the four isolates that were sequenced, three isolates were *Pseudomonas stutzeri* that were obtained from indoor air of three different hospitals. They were found to be similar and clustered in the same group in the phylogenetic tree along with the clinical strain of *Pseudomonas stutzeri* (CCUG 11256; GenBank Accession number U26262) available in the existing database.

*Pseudomonas stutzeri* are capable of causing opportunistic infections in humans (Gilardi, 1972) and have been associated with a wide range of infections such as bacteraemia/septicaemia, bone infections, endocarditis, eye infections (endophthalmitis and panophthalmitis), meningitis, pneumonia and/or empyema, skin infection, urinary tract infection and ventriculitis, resulting in increased morbidity (Noble and Overman, 1994; Lalucat et al., 2006) and to cause pseudobacteremia (Keys et al., 1983).

*Pseudomonas stutzeri* are commonly found in the hospital environment. *Pseudomonas stutzeri* has been isolated from deionised water used for hemo-dialysis (Goetz et al., 1983). Procedures or activities that cause aerosolisation may be the source of these organisms especially from moist surfaces (Keys et al., 1983; Felts et al., 1972).

The study strains were susceptible to majority of the commonly used antibiotics. However, a longitudinal follow-up study was not undertaken to determine if these strains acquired resistance to antibiotics. Susceptible strains of
*Pseudomonas stutzeri* have been recovered from hospital environment (Lalucat *et al.*, 2006). These strains were found to acquire resistance subsequently following exposure to antibiotics in the hospital environment resulting in survival of resistant mutant strains (Carvalho-Assef *et al.*, 2010).

The similarity documented between the study isolates of *Pseudomonas stutzeri* strains that are environmental in origin and the clinical isolate of *Pseudomonas stutzeri* in the NCBI database suggests that a pathway may exist, allowing for the introduction of these organisms from the hospital environment into patients during healthcare delivery. Further studies may be undertaken to perform simultaneous sampling of environmental and clinical samples to obtain a correlation of the isolates.

The repeated isolation of *Pseudomonas stutzeri* from different hospitals which showed a homology with the clinical isolate of *Pseudomonas stutzeri* in the NCBI database suggests a possible new reservoir for *Pseudomonas stutzeri* in the hospital environment. Airborne transmission is a possible route for acquisition of surgical site infection and wound infection. Since *Pseudomonas aeruginosa* and other *Pseudomonas* species have predilection for the hospital environment, it is not possible to rid the hospital environment of these nosocomial pathogens. There is a need to heighten awareness on possible sources of such nosocomial agents in order to plan for appropriate engineering control measures to contain the spread of these nosocomial agents, especially in areas where immuno-compromised patients are admitted.
5.5.2 Correlation between clinical and environmental coagulate-negative *Staphylococci*

Nosocomial infections by coagulate-negative *Staphylococci* are on the rise. About 30% of the nosocomial blood-stream infections are caused by CNS, of which bacteraemia related to indwelling devices are predominant. Other infections by CNS include central nervous system shunt infections, endophthalmitis, surgical site infections, peritonitis in patients with continuous ambulatory peritoneal dialysis and foreign body infections. Approximately 55 – 75% of the nosocomial CNS are methicillin resistant. In addition, they also cause endocarditis and urinary tract infections in immuno-competent hosts. (Piette and Verschraegen, 2009).

A study was conducted by Khadri and Alzohairy (2010) to investigate the prevalence of MRSA and MRCoNS, and found the rate of multidrug resistance to be 69% for MRSA and 72.5% for MRCoNS strains. Another study by Deepa *et al* (2010) recorded an increasing trend of MR-CoNS in neonatal septicaemia, with the prevalence of MRCoNS during 2008, 2009 and 2010 being 41.57 %, 47 % and 57.36 % respectively.

Chylak *et al* (1999) carried out a study, where methicillin resistant strains of *S. haemolyticus* isolated from patients and from the hospital ward environment were typed. The study findings suggested that a nurse may have been the source of infection because the same genotype of *S. haemolyticus* was isolated from her nasal anterior as from the majority of patients.

Airborne nosocomial staphylococcal infections have been documented. Shiomori *et al* (2001) quantitatively investigated the existence of
airborne methicillin-resistant *Staphylococcus aureus* (MRSA) in a hospital environment, and found the isolates from the air and inanimate environments to be identical to the MRSA strains that caused infections or colonisation in in-patients. The study thus indicated that MRSA gets re-circulated among the patients, the air and the inanimate environments, especially when there is movement in the rooms (Shiomori *et al.*, 2001). However, so far, no studies have reported the possibility of airborne CNS in causing nosocomial infections.

In this study, airborne microorganisms in different hospitals were characterised, and it was found that CNS was the predominant GPC, accounting for 47% of the total GPC isolated. An attempt was made to look for the existence of a correlation between airborne (environmental) and clinical strains of CNS recovered during the study period.

Based on the retrospective analysis of the laboratory reports, it was found that there was occurrence of staphylococcal wound infections in an orthopaedic ward that were nosocomial in origin. Air samples were collected from the orthopaedic ward by exposed plate method. Daily visits were made to the laboratory, and samples were screened for the occurrence of any nosocomial staphylococcal wound infections. Strains recovered from air and clinical samples were stocked.

Thus, a total of 15 clinical and six environmental strains of coagulase-negative *Staphylococci* were isolated over a period of two months. When these strains were subjected to screening by phenotypic and genotypic methods for similarity, only two strains (one clinical and one environmental) isolated within a week’s time were found to have similar phenotypic characteristics with identical antibiotic susceptibility pattern. Both the strains were found to be methicillin
resistant *Staphylococcus haemolyticus*. They were found to be similar (F=0.51) when subjected to typing by PFGE.

Airborne transmission of *S. aureus* has not been linked to areas other than operating rooms, burn units, and neonatal nurseries (CDC, 2003). To the best of our knowledge, this is the first study documenting the similarity of clinical and environmental strains of methicillin resistant CNS in an orthopaedic ward. This study indicates that air can be a possible source of nosocomial infection. The patient may have acquired the infection from the air due to particle fall-out.

Although airborne transmission of nosocomial infection by CNS was observed, incidence of only one such case over a period of two months is noteworthy. This suggests that air may not be a predominant source for these infections. Their presence in air is inevitable as they are normal skin flora and are constantly shed by the occupants. It is possible that they settle on surfaces including high touch areas like door knobs due to particle fall-out. Frequent contact with such surfaces may lead to colonisation of hands of health personnel, indicating the need for proper hand hygiene practices.

The study was carried out when there was no ongoing outbreak of nosocomial infections by CNS. The occurrence of only one episode of airborne transmission of nosocomial infection suggests that there is no need to carry out frequent air sampling as a part of routine surveillance. Active surveillance may be conducted during outbreaks of nosocomial infections.
5.6 Assessment of endotoxin in indoor air and personnel in an intensive care unit of a hospital

In this study, we characterized airborne microorganisms in different hospitals, looked at the factors that impact airborne microbial loads, determined if there is temporal variation in the bioaerosol concentrations, and used tools such as PFGE to determine the significance of microorganisms such as CNS in healthcare associated infections, characterized *Pseudomonas* sp. from indoor air of different hospitals to identify its ecological niche, and constructed a dendrogram to determine their similarity with isolates of *Pseudomonas* sp. recovered from other ecological niches. In view of these, it is essential to study and determine if the breakdown products of microorganisms are also present in indoor air of hospitals.

A study was undertaken to assess the presence of endotoxins in indoor air of an ICU. Air samples were collected by impinger method, and samples were qualitatively tested for the presence of endotoxin by LAL gel clot method. It was found that 75% (n=6) of the air samples tested positive indicating the presence of endotoxin in indoor air.

Endotoxin has been recognized as an important factor in the aetiology of occupational lung diseases including (non-allergic) asthma and organic dust toxic syndrome (ODTS) (Douwes and Heederik, 1997; Douwes *et al.*, 2003). Inhalation of endotoxin has been associated with clinical effects such as fever, shivering, arthralgia, malaise, blood leukocytosis, neutrophilic airway inflammation, asthma symptoms, as well as dose-dependent lung function impairment and decreased lung diffusion capacity (Douwes and Heederik, 1997;
In this study, six samples of air tested positive for endotoxins. Where are these endotoxins coming from? It is possible that several aerosol generating procedures and related activities may cause repeated low dose of endotoxin to be periodically released, which results in the endotoxin levels to be detectable.

Health personnel may constantly be exposed through such procedures which may lead to prior sensitization without any health effects (Liu, 2002; Radon, 2006). However, frequent / repeated exposures may lead to adverse health effects. Since the possibility of risk due to exposure to airborne endotoxin is unknown, all the health personnel working in different times of the day and night were included in the study. It was found that 52 % tested positive for the presence of endotoxin in blood, with a risk of 10.31 times more than that of the patients admitted to the same ICU.

A positive association between endotoxin exposure and health effects including reversible (asthma) and chronic airway obstruction, respiratory symptoms (symptoms of asthma, bronchitis and byssinosis) and increased airway responsiveness has been documented (Douwes and Heederik, 1997; Douwes et al., 2003; Pernis et al., 1961; Michel et al., 1996; Rylander, 2006; Bakirci et al., 2007). Subjects with increased bronchial hyper-responsiveness and/or asthma are more sensitive to develop symptoms (Michel et al., 1989), although large differences in airway responsiveness to inhaled endotoxin exist in healthy (non-allergic) subjects suggesting that potentially only susceptible individuals are at risk (Kline et al., 1999). A causal association between endotoxin and asthma exacerbation in children and adults has been established (Michel et
Few studies have also described beneficial effects due to endotoxin exposure, especially with respect to development of allergies leading to a state of tolerance (von Mutius et al., 2000; Braun-Fahrländer et al., 2002; Eduard et al., 2004).

The presence of endotoxins were documented in blood samples collected from health personnel with work experience ranging from few months to five years. It was found that the maximum positivity to endotoxins was among samples from health personnel with work experience of 3 – 5 years. It was also found that 44 % of health personnel with less than 3 years of experience tested positive to endotoxin. However, samples from health personnel with 5 – 9 years of work experience tested negative to endotoxin. When symptoms were reviewed and correlated with endotoxin, it was found that 23 % had upper respiratory tract ailments, 19 % had lower respiratory disorders and 16 % had gastro-intestinal disturbances.

From the study findings, it is unclear as to why people with 5 – 9 years of work experience did not test positive for endotoxin. Perhaps, prolonged / repeated exposures of health personnel to endotoxins might have resulted in immunity against airborne endotoxin over time. However, long-term studies need to be studied to determine the associated health effects.

Of the three patients (enrolled after 48 h of admission) tested positive to endotoxin, one patient’s sample was found to be positive for bacteraemia due to GNB. The source of endotoxin may be from inherent bacteraemia. Two other patients’ samples were endotoxin positive while cultures tested negative. It is not very clear whether they acquired from hospitalisation through ventilator or aerosol
generating procedures, or due to administration of antibiotics that might have led to destruction of bacteria releasing endotoxin into circulation.

Can endotoxin exposure pose a risk for development of HAI is unknown. Can inhaled endotoxin lead to endotoxaemia in patients? Though the possibility is less, further studies need to be carried out.

The limitation of the study is that only the presence of endotoxins was documented, and their levels were not quantified due to resource limitations. Secondly, a questionnaire based one-time assessment was carried out among health personnel to identify the associated health effects due to endotoxin exposure, and the results reported the existence of health effects. It is unclear as to whether the symptoms reported are due to the presence of endotoxins in the samples. Only longitudinal studies with endotoxin quantification can determine whether there is a definite role and impact of endotoxins in causing health effects, so that appropriate intervention can be suggested.

The study on endotoxin is first of its kind in India, documenting the presence of endotoxins in indoor air of low-cost intensive care unit of a healthcare facility and among health personnel working in the same ICU. Endotoxins are known to be present in cell wall of GNB and are liberated into the environment when the bacterial cells disintegrate. While the mechanism of the release is not very clear, it is known that GNB are known for their susceptibility to environmental stress due to lower peptidoglycan contents that may lead to the injury and/or death of cells and release the endotoxins into the environment (Stetzenbach, 2005). Procedures that generate aerosols may result in GNB becoming airborne and be a source for transmission.