CHAPTER 2

REVIEW OF LITERATURE

2.1 Indoor air quality

Indoor air quality (IAQ) is a major problem worldwide, both in developed and developing countries. Problems of IAQ are recognized as important risk factors for human health since majority of the population spend a substantial fraction of time within buildings (WHO, 2009).

IAQ is affected due to lack of fresh air, poorly maintained or operated ventilation systems, disrupted circulation of air in occupied space, poorly maintained temperature and / or relative humidity levels, and sources of contamination from indoor and / outdoor air (NJDHSS-PEOSH, 2004). IAQ is affected by the air pollutants that are either generated from indoor sources, or gain entry into the buildings from outdoor air.

Bioaerosols include the particles and gases released by molds and bacteria that grow indoors on damp surfaces. Other common air pollutants include gases and particles produced by tobacco smoking and biomass combustion and the volatile organic compounds (gaseous chemicals containing carbon and hydrogen and often other elements) emitted by some building products, furnishings, and consumer products (IAQ-SFRB, 2009).

Exposure to Bioaerosols unlike exposure to chemicals do not have threshold limits to assess health impact/toxic effects, due to the complexity in their entity, variations in human response to their exposure and difficulties in
recovering microorganisms that can pose hazard during routine sampling (Macher et al., 1999). While their role in various industrial settings has been well studied (Douwes et al., 2003), the role of these airborne microorganisms in healthcare settings is poorly understood, especially in the context of a developing country with resource limitations. Preliminary studies across India have shown the presence of nosocomially significant microorganisms in indoor air of hospitals, including critical care areas such as intensive care units and operating rooms (Kelkar et al., 2002; Ravisankar et al., 2005; Sudharsanam et al., 2008).

Increasing incidences of nosocomial and occupational diseases due to bioaerosol exposure indicate the need for a thorough knowledge in this respect (Schaal, 1991; Ayliffe, 1991; Eickhoff, 1994; Beggs, 2003). Bioaerosol monitoring in hospitals provides information for epidemiological investigation of nosocomial infectious diseases, research into airborne microorganism spread and control, monitoring biohazardous procedures and use as a quality control measure to determine the quality of indoor air (Stetzenbach, 2005).

### 2.2 Bioaerosols – nature, sources and associated health effects

Bioaerosols are aerosols of biological origin or activity. They are airborne particles that are living (bacteria, viruses and fungi) or originate from living organisms. They are ubiquitous, highly variable, complex, natural or man-made in origin (Macher et al., 1999). Bacterial cells and cellular fragments, fungal spores and by-products of microbial metabolism, present as particulate, liquid or volatile organic compounds may be components of bioaerosols (Stetzenbach, 2005).
2.2.1 Sources of bioaerosols in hospital environment

The microbial loads in hospital indoor air are influenced by the number of occupants, their activity and the ventilation (Ayliffe et al., 1999). Occupants are a potential source of microorganisms as they shed microorganisms from the skin squames and the respiratory tract. Ventilation causes dilution thus reducing the microbial load. Sinks, wash-basins and drains, nebulisers, humidifiers, and cooling towers are the potential sources of Gram-negative bacilli, which colonise on moist surfaces. Dressings and beddings also can act as the sources of airborne microorganisms (Ayliffe et al., 1999). Sweeping of floors and changing of bed linens also can cause suspension of bioaerosols in air (Ayliffe et al., 1999). Fungal spores gain entry into the hospital buildings through crevices in the walls and ventilation ducts with inadequate filtration.

2.2.2 Factors influencing the bioaerosols

The transport and the ultimate settling of a bioaerosol are affected by its physical properties, environmental parameters that it encounters and microbial composition (Stetzenbach, 2002). The physical characteristics are size, density, and shape of droplets or particles (Stetzenbach, 2002), while the environmental factors include temperature, relative humidity, water content, oxygen concentration and magnitude of air currents, which determine the capacity to be airborne (Mohr, 2002).
2.2.2.1 Physical Factors

Physical characteristics of a bioaerosol such as size, shape and density determine settling velocity and location of deposition in the respiratory tract and collection characteristics of air samplers (Mohr, 2002).

Size: The particles in a bioaerosol are generally 0.3 to 10 µm in diameter; however, the respirable size fraction of 1 to 10 µm is of primary concern (Stetzenbach, 2005). Bioaerosols ranging in size from 1.0 to 5.0 µm generally remain in the air, whereas larger particles are deposited on surfaces (Stetzenbach, 2005).

Shape: Bioaerosols have diverse shapes ranging from spheres to spheroids to needle-like to irregular. The shape of bioaerosol particles is important in assessing health hazards. For example, angular particles or fibres maybe more detrimental to health than rounded (spherical) ones even though the particle itself is inert (Heber, 1995).

Density: The density and size determine the settling rate of bioaerosols. Heavier airborne particles remain suspended in air for only a short period and settle down subsequently, although resuspension is possible by physical disturbance. Lighter particles remain airborne for longer duration (Morris, 1995).

2.2.2.2 Environmental factors

Environmental factors influence the survival of airborne microorganisms and affect their ability to colonize on surfaces after deposition. Harsh environmental conditions tend to decrease the numbers of viable airborne microorganisms (Stetzenbach, 2002). Increased temperature and decreased
water content inactivate the microorganisms, reducing their viability in air. Temperature and relative humidity also contribute to the influx of airborne microorganisms. Studies have shown that increased concentrations of some fungal spores in outdoor air and increased concentrations of bacteria released from plant surfaces are associated with high temperature and low relative humidity (Stetzenbach, 2002).

Temperature: Temperatures above 24 °C appear to universally decrease airborne bacterial survival (Tang, 2009). Temperature can affect the state of viral proteins (including enzymes) and the virus genome (RNA or DNA). Viruses containing DNA are generally more stable than RNA viruses, but high temperatures also affect DNA integrity (Tang, 2009).

Relative humidity: The effects of relative humidity are complex (Tang, 2009). Studies on airborne Gram-negative bacteria such as *Serratia marcescens*, *Escherichia coli*, *Salmonella pullorum*, *Salmonella derby*, *Pseudomonas aeruginosa* and *Proteus vulgaris* have found increased death rates at intermediate (approx. 50–70 %) to high (approx. 70–90 %) relative humidity environments (Webb 1959; Won and Ross, 1966). For some airborne Gram-positive bacteria like *Staphylococcus albus*, *Streptococcus haemolyticus*, *Bacillus subtilis* and *Streptococcus pneumoniae* (type 1), death rates were highest at intermediate relative humidity levels (Dunklin and Puck 1948; Webb 1959; Won and Ross 1966). In contrast, aerosolized *Klebsiella pneumoniae* (Gram-negative bacillus) demonstrated relative stability at an intermediate relative humidity of 60 % (Bolister *et al.*, 1992).

Fungi and their spores are more resilient and able to withstand greater stresses owing to dehydration and rehydration, as well as UV radiation
(Cox 1989, 1998; Karra and Katsivela, 2007). The presence of moulds indicates a problem with water penetration or high humidity (Pasanen et al., 1993). Studies also indicated that spore concentrations were higher with higher relative humidity levels (Stennett and Beggs, 2004; Rodriguez-Rajo et al., 2005; Erkara et al., 2008); however one study demonstrated opposite findings (Sabariego et al., 2000). The indoor and outdoor concentrations of Aspergillus and Penicillium species may vary considerably in both winter and summer. In addition they may also vary in urban or more suburban environments, with higher temperature and relative humidity, suburban areas being generally more favourable for higher airborne spore concentrations (Li and Kuo, 1994; Pei-Chih et al., 2000; Sakai et al., 2003).

Viruses with lipid envelopes tend to survive longer at lower (20–30%) relative humidity, while non-lipid enveloped viruses tend to survive longer in higher (70–90%) relative humidity (Tang, 2009).

Water content: Water content of aerosolised microorganisms is a principal factor in determining their viability (Mohr, 2002). Loss of water due to dehydration can lead to inactivation of these airborne microorganisms (Mohr, 2002). Bioaerosols generated from liquid suspensions undergo desiccation, whereas those generated as dusts or powders partially rehydrate (Cox, 1995).

Oxygen concentration: Oxygen concentration is another important factor in determining the stability and infectivity of airborne microorganisms (Mohr, 2002). Oxygen in the form of free radicals has been implicated to interact with phospholipids and proteins leading to inactivation of microorganisms (Mohr, 2002). The susceptibility of bioaerosols to oxygen increases with dehydration, oxygen concentration and time of exposure (Mohr, 2002).
Magnitude of air currents: Bioaerosols are greatly influenced by the magnitude of the air currents (Stetzenbach, 2002). Airborne particles remain suspended in air and travel longer distances in the presence of air currents. On the other hand, these particles tend to settle down easily in still air.

2.2.2.3 Microbial composition

The survival of airborne microorganisms is affected by the physiological age and type of the organism (Mohr, 2002). Older cells die off easily due to environmental stress than newly grown populations. Cells in their log phase are metabolically active and tend to be resistant to environmental stresses when compared to cells in stationary phase (Mohr, 2002).

There is variability in survival between groups of microorganisms and within genera. In general, fungal spores, enteric viruses and amoebic cysts are somewhat resistant to the environmental stresses encountered during transport through air. Bacteria and algae are more susceptible, although bacterial endospores are quite resistant (Stetzenbach, 2002). Among bacteria, Gram-negative bacteria are more susceptible to inactivation, injury and/or cell death than Gram-positive bacteria due to higher phospholipid and lower peptidoglycan contents of the cells (Mohr, 2002; Stetzenbach, 2005).

2.2.3 Microbial agents in bioaerosols

2.2.3.1 Bacteria and endotoxins

Airborne bacteria include Gram-positive cocci such as Staphylococcus aureus (including methicillin-resistant strains - MRSA), Mycobacterium tuberculosis and non-tuberculosis mycobacteria, and
Gram-negative bacilli such as *Legionella pneumophila*, *Pseudomonas* spp. and *Acinetobacter* spp. (Fletcher *et al.*, 2004). Airborne *Legionella pneumophila* and *Mycobacterium tuberculosis* are of significant public health concern due to their low infectious dose (Stetzenbach, 2002). Apart from bacterial cells, toxins of bacterial origin such as endotoxins are also seen in domestic, occupational and outdoor environments (Ivens *et al.*, 1999). These are potent pyrogens, capable of causing fever in very low concentrations (Parillo, 1993).

### 2.2.3.2 Fungi and mycotoxins

Common fungal isolates from air are the filamentous fungi such as *Penicillium*, *Aspergillus*, *Acremonium*, *Paecilomyces*, *Mucor* and *Cladosporium* (Lugauskas, 2004). Fungi such as *Aspergillus*, *Fusarium*, *Trichoderma* and *Stachybotrys* act as aeroallergens and also produce mycotoxins. These are lipid-soluble and are readily absorbed by the intestinal lining, airways and skin, and are capable of causing adverse health effects like inflammatory reactions (Etzel *et al.*, 1998). The presence of moulds indicates a problem with water penetration or high humidity (Pasanen *et al.*, 1993). Volatile products of fungal metabolism are also present, which are capable of inducing sensory irritation to eyes and upper respiratory tract (Korpi *et al.*, 1999).

### 2.2.3.3 Viruses

Viruses commonly spread by air include enteric viruses of intestinal origin produced at sewage treatment facilities, Hantavirus, gets aerosolized from rodent faeces (Diglisic *et al.*, 1999), and SARS (Severe Acute Respiratory Syndrome) that spreads airborne quickly (Peiris *et al.*, 2003).
2.2.3.4 Parasites and actinomycetes

Free-living amoeba like *Acanthamoeba* and *Naegleria fowleri* found in soil and water get aerosolized from natural and artificially heated waters such as power plant discharges, and cooling systems such as humidifiers (Lawande et al., 1979). Other airborne microorganisms are actinomycetes such as *Streptomyces* and algae.

2.2.3.5 Bio-allergens

Allergens are macromolecular structures ranging from low (chemicals such as di-isocyanates) to high molecular weight sensitizers, which are mostly proteins of biological origin. Most potent occupational IgE inducing allergens include enzymes derived from fungi and bacteria produced by biotechnological companies (Sandiford et al., 1994; Cullinan et al., 2000; Cullinan et al., 2001). Other IgE inducing allergens are plant pollens (Miesen et al., 2003).

2.2.4 Aerobiological pathway

An aerobiological pathway describes the launching of airborne particles into the air, transport via diffusion and dispersion, and the deposition of bioaerosols. Aerobiological pathway comprises of five components namely source of microorganism, release, dispersion, deposition and impact (Lacey and West, 2006).

Aerobiological particles originate from sources that may be instantaneous (single event) or continuous (extended periods). Once originated, the numbers and distribution of microorganisms released are largely affected by the niche microenvironments. The microorganisms can get airborne
opportunistically from animal, fungal and plant sources or mechanical activity (Lacey and West, 2006).

Once the particles are released into the air, they disperse, and their concentration per unit volume of air decreases with increasing distance from the point of liberation. Eventually, airborne particles descend due to gravity, by sedimentation (passively settling onto a surface), or by impaction (the sticking of airborne particles onto a surface following an active collision) on an object’s surface (Lacey and West, 2006).

Following deposition, airborne particles can have many health effects including diseases and allergies or colonisation of new habitats. To have an impact, particles need to survive the airborne phase and remain viable for growth or causing an infection. In cases of allergy, however, the particle need not be viable to cause a reaction (Lacey and West, 2006).

**2.2.5 Health effects due to bioaerosols**

Health effects from exposure to bioaerosols are determined by the microbial agents present within the aerosol and the immune status of the exposed individuals (Stetzenbach, 2005). These health effects may arise due to exposure to high concentrations or unfamiliar forms of bioaerosols. Three major groups of diseases have been associated with bioaerosol exposure – infectious diseases, respiratory diseases and cancer (Douwes et al., 2003).
2.2.5.1 Infectious diseases

Infectious diseases arise from viruses, bacteria, fungi, protozoa and helminths and involve the transmission of an infectious agent from a reservoir to a susceptible host through airborne transmission (Douwes et al., 2003).

Bacterial diseases: Various bacterial diseases such as legionellosis and tuberculosis are linked to cause significant public health concern due to their low infectious dose (Stetzenbach, 2002).

Legionellosis: *Legionella pneumophila* causes human legionellosis and community-acquired and nosocomial pneumonia in adults following either occupational or non-occupational exposures. Legionellae get airborne as a result of active aerosolizing processes such as aeration of contaminated water. They are found in water environments that include man-made water systems, biofilms in cooling towers, air conditioning systems, etc. (Douwes et al., 2003). Nosocomial infections and hospital outbreaks have been linked to contaminated hot water supply of temperature 45 °C (Darelid et al., 1994). Use of monochloramine for residual drinking water disinfection may help prevent Legionnaires’ disease (Kool et al., 1999). Monochloramine is better at reaching distant points in a water system and penetrates better into biofilm, but requires a higher pH than free chlorine for optimal disinfection (Kirmeyer et al., 1993).

Tuberculosis: The transmission of tubercle bacilli occurs through the inhalation of aerosolized bacilli in droplet nuclei of expectorated sputum-positive tuberculosis patients during coughing, sneezing and talking. Several outbreaks of multi-drug resistant tuberculosis in UK have highlighted the potential for transmission within the hospital environment (Breathnach et al., 1998).
Anthrax: The transmission occurs due to inhalation of the spores of \textit{Bacillus anthracis} and outbreaks are often linked to bioterrorism that are spread through intentionally contaminated mail, apart from occupational exposures (Traeger \textit{et al.}, 2002).

\textit{Illness due to endotoxins}: Endotoxins are the lipopolysaccharides (toxins) of Gram-negative bacterial cell wall. These are potent pyrogens, capable of causing fever in very low concentrations (Parillo, 1993). High exposure to endotoxins is often associated with nausea and diarrhoea (Ivens \textit{et al.}, 1999).

\textit{Fungal diseases}: Airborne fungi causing respiratory infections and allergic reactions include \textit{Penicillium}, \textit{Aspergillus}, \textit{Acremonium}, \textit{Paecilomyces}, \textit{Mucor} and \textit{Cladosporium} (Lugauskas, 2004). Most of the fungal infections can occur in immuno-compromised hosts or as a secondary infection, following inhalation of fungal spores or the toxins produced by them. Symptoms include persistent cold, watering eyes, prolonged muscle cramps and joint pain (Verma \textit{et al.}, 2003). \textit{Coccidioides, Histoplasma} and \textit{Blastomyces} grow in soil or may be carried by bats and birds and are linked to exposure to wind-borne or animal borne contamination. Volatile products of fungal metabolism are capable of inducing sensory irritation to eyes and upper respiratory tract (Korpi \textit{et al.}, 1999).

\textit{Aspergillosis}: Aspergillosis is the commonest fungal infection. \textit{Aspergillus} species that can grow indoors include \textit{Aspergillus fumigatus} and \textit{Aspergillus flavus} and can cause nosocomial infections (Verma \textit{et al.}, 2003), allergic broncho-pulmonary aspergillosis (ABPA) and sinusitis (Diwakar \textit{et al.}, 2008). Chronic asthmatics may progress to have their bronchial passages colonised by \textit{Aspergillus fumigatus, Bipolaris hawaiensis}, or \textit{Wangiella dermatitidis} (Hodgson, 2003). Constant allergic response maintains the fungal colonization, and first-line
therapy with steroids, brings down the level of inflammation and may result in elimination of the colonising organism (Hodgson, 2003).

**Illness due to mycotoxins:** Mycotoxins are absorbed by the intestinal lining, airways and skin; toxic effects follow exposure to toxins on the surface of the mould spores. *Aspergillus, Fusarium* and *Stachybotrys* act as aeroallergens and also produce mycotoxins (Etzel *et al.*, 1998). A case report from the US described upper respiratory tract irritation and rash in a family living in a Chicago home with a heavy growth of *Stachybotrys atra* producing trichothecene mycotoxins. The symptoms disappeared when the amount of mould was substantially reduced (Croft *et al.*, 1986). Other adverse health effects include pre-term births or late abortions in farm women exposed to mycotoxins with immunotoxic and hormone-like effects (Kristensen *et al.*, 2000).

**Viral diseases:** Viruses are readily transmitted by airborne route, and include SARS virus (Yu *et al.*, 2004), enteric viruses of intestinal origin produced at sewage treatment facilities, RSV, *Hantavirus* from rodent faeces (Diglisic *et al.*, 1999), varicella - zoster virus, measles, mumps and rubella viruses. Airborne transmission of rabies virus is uncommon; spread of the infection due to aerosolisation of laboratory strains has been reported, resulting in revised safety recommendations for laboratory personnel working with rabies virus (Aitken and Jeffries, 2001).

**SARS:** SARS caused by novel corona virus, is a highly contagious respiratory illness of significant morbidity and mortality, and causes very severe atypical pneumonia (Peiris *et al.*, 2003; Lee *et al.*, 2003). The use of aerosol-generating procedures such as endotracheal intubation, bronchoscopy and treatment with
aerosolized medication in hospitals may amplify the transmission of SARS (Yu et al., 2004).

*Diseases caused by parasites and actinomycetes:* Free-living amoeba like *Acanthamoeba* and *Naegleria fowleri* get aerosolized from natural and artificially heated waters (Lawande et al., 1979), and cause respiratory illness and meningo-encephalitis. Actinomycetes such as *Streptomyces* and algae cause allergy, inflammatory reactions and hypersensitivity pneumonitis.

### 2.2.5.2 Respiratory diseases

Several airway diseases or syndromes have been associated with exposure to bioaerosols, including asthma (atopic and non-atopic), rhinitis/allergic rhinitis, chronic bronchitis, hypersensitivity pneumonitis and organic dust toxic syndrome (Douwes et al., 2003). Asthma and allergic rhinitis are the most extensively studied respiratory diseases associated with bioaerosol exposure.

Hypersensitivity pneumonitis or Extrinsic Allergic Alveolitis (EAA) is an inflammatory airway disease caused by an unusual immune response to antigens like fungi (Farmer's lung), bird excreta (pigeon breeder's disease), and microbial contaminants in grain dust (Bourke et al., 2001).

Organic dust toxic syndrome (ODTS) occurs within hours of a high dose inhalation of endotoxin, fungal spores and mycotoxins (Von Essen et al., 1990), which may lead to chronic obstructive pulmonary disease (COPD) (Vogelzang et al., 1998).

Bioallergens are potent allergens and include enzymes derived from fungi and bacteria produced by biotechnological companies (Sandiford et al.,...
1994; Cullinan et al., 2000; Cullinan et al., 2001), and plant pollens (Miesen et al., 2003).

2.2.5.3 Cancer

Established biological occupational carcinogens are the mycotoxins. Aflatoxin from *Aspergillus flavus* is capable of causing liver cancer (Hayes et al., 1984; Sorenson et al., 1984), while Ochratoxin A is a possible human carcinogen. Exposure to aflatoxin and ochratoxin occurs by ingestion, but can also occur by inhalation in industries such as peanut processing, livestock feed processing or when exposed to grain dust (Sorenson et al., 1984; Autrup et al., 1993). Studies have found associations between exposure to wood dust and various specific cancers, in particular, sinonasal cancer in furniture making, and in other wood-related jobs including sawmills (Demers et al., 1998).

2.3 Bioaerosol assessments

Healthcare settings represent a unique assemblage of indoor microflora as bioaerosols in indoor air, which may be a source of nosocomial infection. In order to evaluate the quality of indoor air in hospitals, passive and active sampling methods can be used. Wherever higher concentrations of bacteria and fungi are found, active sampling techniques like filter and impinger methods can be used in addition to passive sampling to determine the concentrations and composition of bioaerosols. The choice of the sampling method, in terms of air flow rate and the duration of sampling, is made based on the extent of the loads of bioaerosols.

In areas like high-containment laboratory and hospitals, where low bioaerosol concentrations are expected, air samplers with flow-rates $\geq 25$L/min is
essential for monitoring. Air samplers with flow-rates <5L/min are not suitable and practical when the bioaerosol concentration is <10^2 CFU/m³ (Macher et al., 1995).

### 2.3.1 Principles of bioaerosol collection

Most bioaerosol sampling devices involve techniques that separate particles from the air stream and collect them in or on a pre-selected medium. Sampling methods are of three types - inertial, non-inertial and gravitational.

Inertial sampling methods used for bioaerosol collection include impaction and impingement. These samplers allow particle collection by size-selective sampling. Non-inertial sampling methods do not depend on inertia and therefore particles collected are less dependent on size. Filtration is a commonly used non-inertial sampling method. Gravitational sampling is due to settling of particles onto a collection surface due to gravity, and gravity settling is the method of choice for sampling by this method.

#### 2.3.1.1 Gravitation or settling

An adhesive substrate (such as a coated microscope slide or a Petri-dish containing agar medium) is exposed, face upwards to the atmosphere to collect particles settling by gravity. This method is simple and has been used frequently, sometimes in preference to other aerobiological samplers (Crook, 1995a). However, it is a passive (non-volumetric) method that does not give information on the volume of air from which the particles have been collected; also it over-represents larger particles sampled during the exposure period because of their faster sedimentation rate (Crook, 1995a). Use of settle plates can however provide a hint on the extent of contamination of an environment with airborne microorganisms.
2.3.1.2 Impaction

Impaction is used to separate a particle from a gas stream based on the inertia of the particle. The particles impact onto growth medium with one or more bacterial or fungal colonies forming at some impaction sites (Jensen and Schafer, 1998). The samplers differ by the number and the shape of the nozzles (slit samplers and sieve samplers) and the number of stages (cascade impactors) (Buttner et al., 2002). Cascade impactors comprise of a stack of impaction stages consisting of one or more nozzles of decreasing diameter so that successive stages collect progressively small particles, and a target or substrate (Buttner et al., 2002). The nozzles may take the form of holes or slots. The target may consist of a greased plate, filter material, or growth media (agar) contained in Petri dishes. A filter may be used as the final stage so that particles not impacted on the previous stages are collected.

These samplers are most commonly chosen to sample viable bacteria and fungi. They are desirable to examine respirable and non-respirable fractions separately. These are, however, restricted to areas of bioaerosol concentrations less than $10^3$ CFU/m$^3$. Closely growing or overlapping or multiple colonies cannot be counted or identified accurately (Jensen and Schafer, 1998).

2.3.1.3 Impingement

Liquid impingers are a special type of impactor. Impingers are useful for the collection of culturable aerosols (Jensen and Schafer, 1998). Impingers use a liquid (e.g., a simple salt solution such as 0.3 mM phosphate-buffered dilution water) as collection medium. Additives to the collection medium such as proteins, antifoam, or antifreeze aid in resuscitation of bacterial cells, prevent
foaming and loss of the collection fluid, and minimize injury to the cells (Jensen and Schafer, 1998). The jet is positioned, a set distance above the impinger base and consists of a short piece of capillary tube designed to reduce cell injury when the air is dispersed through the liquid and the particles are entrapped.

The samplers operate by drawing aerosols through an inlet tube. In some samplers, the inlet tube is curved to simulate particle collection in the nasal passage that makes it useful for separating respirable (collection fluid) and non-respirable (inlet tube) microorganisms. When such a sampler is used, the curved inlet tube is washed with a known amount of collecting fluid after sampling for recovery of total airborne organisms from the environment. After sampling for the appropriate amount of time, the liquid sample can be analysed by dilution (through liquid addition) or concentration (by filtration) to maximize accuracy in quantitation (Buttner et al., 2002). A liquid sample can also be used with a variety of analytical methods, including culture, microscopy, immunoassay, flow cytometry and molecular methods (Buttner et al., 2002).

These samplers are suitable for areas with bioaerosol concentrations equal to or greater than $10^3$ CFU/m$^3$, as collected air particles can be diluted and characterised. However, these samplers have a disadvantage due to decreased recovery of fungal spores (Jensen and Schafer, 1998).

2.3.1.4 Filtration

Collection of particles from a non-biological aerosol sample is most commonly achieved by filtration (Jensen and Schafer, 1998). Filter media are available in both fibrous (typically glass) and membranous forms. Particles that are smaller than the pore size of the filter may be efficiently collected. Sampling
filter media may have pore sizes of 0.01 – 10 μm. The collection of an airborne particle on a filter depends on the aerodynamic diameter of the particle, the filter pore size, and the airflow rate. The overall efficiency of membrane filters is approximately 100 % for particles larger than the pore size (Jensen and Schafer, 1998).

Membrane filters are manufactured in a variety of pore sizes from polymers such as cellulose ester, polyvinyl chloride, and polycarbonate. For analysis by microscopy, cellulose ester or polycarbonate membranes are the usual choices. Filters are held in disposable plastic filter cassettes during bioaerosol sampling. A three-piece cassette may be used either in open- or closed-face modes (Jensen and Schafer, 1998).

Filtration techniques are used for the collection of certain fungi and endospores-forming bacteria that are desiccation-resistant. The sampled organisms are washed from the surface of smooth-surface polycarbonate filters. The organisms in the wash solution are either cultured or re-filtered to distribute the organisms uniformly on the membrane filter.

These samplers are suitable for areas with bioaerosol concentrations less than 10 CFU/m³. In areas of high concentration, the organisms may have to be eluted, diluted, and then re-filtered for microscopic analysis. In case of re-filtration, the microorganisms are stained and examined microscopically (Jensen and Schafer, 1998). To culture the organisms, the membrane filter from each sampling cassette is washed with a 0.02 % Tween™ 20 in aqueous solution (three 2 mL washes) with agitation (Jensen and Schafer, 1998).
Though filter methods are known for their simplicity, low cost and versatility, loss of viability of vegetative cells may occur due to desiccation stress during sampling (Buttner et al., 2002). Removal of filter deposits from the sample may be necessary in subsequent analysis (Crook, 1995b).

2.3.2 Methods for detection of microorganisms

Viable microorganisms are metabolically active (living) organisms with the potential to reproduce. These may be divided into two subgroups: culturable and non-culturable. Viable bioaerosol sampling involves collecting a bioaerosol and culturing the collected particulate. Only culturable microorganisms are enumerated and identified, thus leading to an underestimation of bioaerosol concentration. In order to determine the total bioaerosol concentration, estimation of both culturable and non-culturable organisms is essential.

2.3.2.1 Culturable organisms

After sample collection, colonies of bacteria and fungi are grown on culture media at a defined temperature over a 3 – 7 day period. Culturable microorganisms may be identified or classified by using microscopy, classical microbiology, or molecular biology techniques such as restriction fragment length polymorphic (RFLP) analysis (Hensel and Petzoldt, 1995; Madelin and Madelin, 1995). Microscopy includes the various staining and wet-mount techniques used to identify the bacteria and fungi and their staining characteristics. Staining techniques commonly used for bacteria are Gram staining and acid-fast staining, while lactophenol cotton blue and calcofluor-white are the common fungal staining techniques. Apart from these staining, the microorganisms can also be observed directly in the microscope without the addition of stains to obtain the
total cell counts. Classical microbiology techniques include observation of growth characteristics; cellular or spore morphology; simple and differential staining; and biochemical, physiological, and nutritional testing for culturable bacteria. The type of growth medium or cell culture used and the incubation conditions (e.g., temperature, humidity, atmosphere and duration) influence the type of microorganisms (viz. bacteria, fungi, viruses or amoebae) isolated.

Counting of culturable microorganisms is potentially a very sensitive technique and many different species can be identified. Traditionally used culture methods have proven to be of limited use for quantitative exposure assessment. Culture-based techniques thus usually provide qualitative rather than quantitative data that can, however, be important in risk assessment, since not all fungal and bacterial species pose the same hazard.

2.3.2.2 Non-culturable organisms

Non-culturable organisms are not living organisms, or do not reproduce in the laboratory because of intracellular stress or because the conditions (e.g., culture medium or incubation temperature) are not conducive to growth. The bioaerosol is collected on a "greased" surface or a membrane filter. The organisms are then enumerated and identified using microscopy, classical microbiology, molecular biological, or immunochemical techniques (Hensel and Petzoldt, 1995). Many fungal spores and pollen grains as well as dust mites and some amoebic cysts can be identified by direct microscopic examination because of the organism’s distinctive sizes, shapes and surface features (Madelin and Madelin, 1995).
Analytical techniques which may be applied to both nonviable and viable microorganisms, but not distinguish between them, include polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). Such methods may be used to identify specific microorganisms and to locate areas of contamination. Though these latter methods are generally qualitative, current research efforts involve modifying the methods to obtain semi-quantitative or quantitative results. In case of PCR, two main methods are available that provide reproducible, accurate measurements of initial target DNA concentrations in samples: real-time QPCR using fluorogenic probes and competitive PCR. The former measures product accumulation utilizing a fluorogenic probe, while the latter relies on the presence of a known amount of internal control DNA competitor in each PCR reaction. Real-time QPCR has a high degree of sensitivity and accuracy, is less labour intensive than competitive PCR, and is amenable to high sample throughput (Stetzenbach et al., 2004).

2.3.3 Detection of microbial constituents

2.3.3.1 Endotoxin assay

Studies have been carried out in outdoor environment to determine the levels of airborne endotoxins and the associated health effects due to occupational exposure (Liu et al., 2011). However, little is known whether these endotoxins can be airborne in critical areas like healthcare facilities. Reports have documented the presence of endotoxins in systemic circulation in patients with sepsis, and also in many clinical settings that are non-infectious in nature, such as following cardiopulmonary bypass, in patients with congestive heart failure, in chronic renal failure, in cirrhosis and in patients with a ruptured abdominal aortic aneurysm (Marshall, 2010). Studies have shown that endotoxins are normally not
present in systemic circulation in healthy individuals since they are constantly removed by the immune system (Turunen et al., 1981). Exposure to airborne endotoxins are known to cause adverse health effects including fever, cough, headache and respiratory impairment, and can exacerbate asthma on inhalation, nausea and gastrointestinal symptoms (Duchaine et al., 2001).

Several assays are available for the detection of endotoxins, of which three have been widely used for the detection of endotoxin in clinical specimens: the rabbit pyrogen assay, the LAL bioassay, and immunoassays (Hurley, 1995). LAL (Limulus Amebocyte Lysate) assay is the method of choice as the advantages of this assay are increased sensitivity, potential for quantitation, reactivity with the biologically active component lipid A, and relative convenience of operation. LAL test is a comparative and not an analytical bioassay method as measured endotoxin activity levels change with changes in factors other than lipopolysaccharide (LPS) concentrations (Finney, 1978). It is based on the clotting-reaction of the blood of the horseshoe crab (Limulus polyphemus) induced by the presence of endotoxins. In the presence of calcium amebocytes (blood cells), lysates (LAL) from Limulus polyphemus gets activated to an enzyme, converting soluble protein to an insoluble complex (clot).

The rabbit pyrogen assay is performed by in-vivo application of the samples in rabbits. Immunoassays do not quantitate biological activity and are relatively insensitive, typically of the order of 10 ng of endotoxin per ml in plasma (Kimura, 1976). Since reactivity is with the polysaccharide component of the LPS molecule, these assays are restricted by endotoxin immunotype. Changes in the physical state of LPS as a consequence of disaggregation (Shnyra et al., 1993) result in changes in the biological activity of LPS, which is not proportionately
reflected by changes in antibody binding in an immunoassay. Immuno-*Limulus* assay is an assay in which a monoclonal antibody that is either type specific or cross-reactive is used to capture endotoxin onto a solid phase in microtiter plates and then the endotoxin is detected with the chromogenic LAL (Mertsola *et al*., 1989; Saxen *et al*., 1993).

Duchaine *et al*.

(2001) carried out environmental assessment in two prior occupational hygiene studies of swine barns and sawmills to compare concurrent, triplicate, side-by-side endotoxin measurements using air sampling filters and bioaerosol impingers. Endotoxin concentrations in impinger solutions and filter eluates were assayed using the LAL assay. The study demonstrated that impinger sampling is an acceptable method for quantitation of area endotoxin concentrations. Further, when sampling is performed with impingers for airborne microorganism quantitation, these same impinger solutions can yield valid endotoxin exposure estimates, negating the need for additional filter sampling (Duchaine *et al*., 2001).

Pearson *et al*.

(1985) studied the endotoxin levels in plasma of healthy individuals and patients with Gram-negative sepsis using LAL assay. The assay was performed using plasma diluted 1:10 and treated at 70 °C for 10 min. It was found that nephelometric and chromogenic LAL methodologies were equally effective (Pearson *et al*., 1985).

Novel assays are available that are based on the endotoxin activity. Marshall *et al*.

(2002) performed an observational cohort study in critically ill patients in the medical/surgical intensive care unit (ICU) of a tertiary care hospital. Whole blood endotoxin levels on the day of ICU admission were measured using a novel chemiluminescent assay – the endotoxin activity assay
(EAA) – and the chromogenic modification of the LAL assay. It was shown that the correlations of endotoxaemia associated with Gram-negative infection from any source, and with a diagnosis of sepsis and leukocytosis were not apparent using the LAL method and hence, the EAA may be a useful diagnostic tool for the investigation of invasive Gram-negative infection and incipient sepsis.

Another study was carried out by Marshall et al (2004) using a novel assay for endotoxin, based on the ability of antigen-antibody complexes to prime neutrophils for an augmented respiratory burst response. It was carried out as a cohort study in 857 patients admitted to an ICU. It was found that on the day of ICU admission, 57.2% of patients had either intermediate (≥0.40 endotoxin activity [EA] units) or high (≥0.60 units) EA levels. Stepwise logistic regression analysis has shown that elevated Acute Physiology and Chronic Health Evaluation II score, Gram-negative infection, and emergency admission status were independent predictors of EA.

Chieko (2005) evaluated the accuracy of C-reactive protein (CRP), procalcitonin (PCT), neopterin and endotoxin in the differential diagnosis of sepsis and non-infectious systemic inflammatory response syndrome (SIRS). Studies have shown that CRP, PCT and neopterin are released both in sepsis and in non-infectious inflammatory disease. Endotoxaemia was detected in no more than half of the patients with Gram-negative bacteraemia. In addition, Gram-negative bacteraemia was also detected in half of the patients with endotoxaemia. Thus, endotoxin assay in combination with CRP, PCT or neopterin may help as a diagnostic marker for Gram-negative bacterial infection.
2.3.3.2 Fungal biomass assay

Some markers for the assessment of fungal biomass include ergosterol measured by gas chromatography-mass spectrometry (Pasanen et al., 1999) or fungal extracellular polysaccharides measured with specific enzyme immunoassays (Douwes et al., 1999).

2.3.4 Threshold Limit Values for bioaerosols

Threshold Limit Values (TLVs) for bioaerosols are referred to the air concentrations of substances under conditions to which people are repeatedly exposed day after day without adverse health effects. There are no established guidelines specifying the threshold limit values for interpreting environmental measurements of total culturable or countable bioaerosols such as total bacteria or fungi, specific culturable or countable bioaerosols such as Aspergillus fumigatus, infectious agents such as Legionella pneumophila and assayable biological contaminants such as endotoxins, mycotoxins, allergens, or microbial volatile compounds (Macher et al., 1999).

The reasons for absence of established guidelines specifying the threshold limit values are:

(a) Bioaerosols do not comprise of a single entity as they are generally complex mixtures of many microorganisms as well as non-living particles released from fungi, bacteria, animals and plants, due to the diverse and interactive nature of the indoor microenvironments.

(b) Human responses to bioaerosols range from innocuous effects to serious, even fatal diseases depending on the specific material involved and the susceptibility of human beings to it. Some are
harmful and their confirmed presence is a cause of concern while
others are normal components that typically cause little, if any, harm,
eliciting responses in sensitive persons, even at ambient
concentrations or in all persons, when in sufficient quantity.

(c) For a majority of microorganisms that are likely to be recovered during
routine air and source sampling, very little is known about the
minimum dose needed to pose a hazard.

2.4 Bioaerosols in healthcare facilities

2.4.1 Characterisation of bioaerosols in hospitals

A number of studies have been carried out in different parts of the
world to determine the airborne microbial profile of hospitals. From these studies,
it was found that a variety of microorganisms are present in indoor air of
hospitals, including bacteria such as Staphylococcus aureus, coagulase-negative
Staphylococci, Micrococcus, Streptococcus, Acinetobacter, Bacillus,
Corynebacterium, Escherichia, Pseudomonas, Proteus mirabilis, Listeria,
Moraxella, and fungi such as Alternaria, Aspergillus, Cladosporium, Penicillium,
Rhizopus, Fusarium and Scopulariopsis. (Jaffal et al., 1997; Sarca et al., 2002;
Obbard and Fang, 2003; Ekhaise et al., 2008; Quidesat et al., 2009). Details of
the sampling locations, method of air sampling, and media used are summarised
in Table 2.1.

Few studies have been carried out in India to determine the microbial
profile of hospital air. A study carried out at the National Institute of
Ophthalmology, Pune, India on Environmental Bacteria Carrying Particle (BCP)
load by sedimentation method has shown that the operating rooms had a
significant risk of airborne infections. Study has further shown the incidence of various pathogenic fungi being isolated from the filters used in air-conditioning systems (Kelkar et al., 2002).

Another study by Kaur and Hans (2007) documented the presence of bacterial isolates such as *Staphylococcus aureus*, Coagulase-negative *Staphylococcus*, *Acinetobacter* spp. and *Klebsiella* spp. in air of seven different operating rooms over a period of one year. Shivakumar et al (2007) studied the level of atmospheric microbial contamination before, during, and after dental treatment procedures in the dental operatory of a mobile dental unit, and found atmospheric microbial contamination (CFUs/plate) to be minimum at the start of the working session, increase with activity during the working sessions, and decrease at the end of the working day. However details of the organisms were not mentioned in the study.

A three month pilot study (February-April 2006) conducted in three hospitals in the Tamil Nadu region of India found that overall counts of Gram-positive organisms were higher than Gram-negative organisms, with *Staphylococci* and *Micrococci* being the predominant Gram-positive bacteria. *Klebsiella* sp. and *Pseudomonas* sp. were the predominant potentially pathogenic Gram-negative bacteria isolated. Among moulds, *Aspergillus niger* and *A. flavus* were commonly isolated (Sudharsanam et al., 2008).
Table 2.1: Summary of sampling locations, methods and media used for air sampling from existing literature to characterise bioaerosols in hospitals

<table>
<thead>
<tr>
<th>Sampling location</th>
<th>Sampling method</th>
<th>Media used</th>
<th>Study period</th>
<th>Sample size</th>
<th>Country</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Male surgical and medical wards, female surgical and medical wards, paediatric ward, operating room and ICU of Al-Ain hospital (14 year old, with 511 beds and 23 wards)</td>
<td>Mechanical air sampler – one stage culture plate impactor MK2 (Casella London): 0.15 m$^3$ of air per sample for 5 min</td>
<td>Jaffal et al., 1997</td>
<td>Abu-Dhabi, United Arab Emirates</td>
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<td>Operating theatre, birthing-room, emergency department, service area for infectious diseases, intensive care unit and canteen</td>
<td>Exposed plate method: exposure to room air for 10-min periods</td>
<td>Rose-Bengal streptomycin agar and 5% sheep blood agar</td>
<td>September 2000 to February 2001</td>
<td>One-month intervals</td>
<td>Edirne, Turkey</td>
<td>Sarca et al., 2002</td>
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<tr>
<td>Main lobby, a visitor restricted ward and a pharmacy</td>
<td>Andersen Particle Impactors: operating at an air-sampling rate of 28.3 L/min for 5 min</td>
<td>Trypticase soy agar</td>
<td>Five duplicate air samples at each location in a single day</td>
<td>Singapore</td>
<td>Obbard and Fang, 2003</td>
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<tr>
<td>Operating rooms</td>
<td>Sedimentation method: exposure for 30 min</td>
<td>Blood agar</td>
<td>21 months</td>
<td>276 occasions</td>
<td>Pune, India</td>
<td>Kelkar et al, 2002</td>
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<tr>
<td>Operation theatres of a tertiary care teaching hospital (1000 beds)</td>
<td>Settle plate method: exposure for 1 h</td>
<td>Blood agar and MacConkey agar</td>
<td>One year</td>
<td>344 samples from seven operation theatres</td>
<td>India</td>
<td>Kaur and Hans, 2007</td>
</tr>
<tr>
<td>Sampling location</td>
<td>Sampling method</td>
<td>Media used</td>
<td>Study period</td>
<td>Sample size</td>
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<td>Reference</td>
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<td>Dental operatory of a mobile dental unit (MDU). Before activity: in the middle of fumigated MDU; during activity: patient’s chest, approx. 40 cm away from patient’s mouth, and on the operator’s mouth mask; after treatment: in the middle of MDU</td>
<td>Gravitometric settling: exposure for 30 min prior to activity, and 20 min at times of activity</td>
<td>Brain Heart Infusion agar with 5% sheep blood</td>
<td>Before, during and after dental treatment procedures, on different days with one-month interval</td>
<td>Davangere, India</td>
<td>Shivakumar et al 2007</td>
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<tr>
<td>Male ward, female ward, children ward, theatre and bacteriological laboratory of Faith Medical Centre (private) and Central (government) Hospital</td>
<td>Settled plate technique: exposure for 30 min</td>
<td>Potato dextrose agar and nutrient agar</td>
<td>Air samples taken at three different time periods each day</td>
<td>Benin City, Nigeria</td>
<td>Ekhaise et al., 2008</td>
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<tr>
<td>Intensive care unit, operating room, and three different wards of three hospitals</td>
<td>Petri-plate gravitational settling method: exposure for 20 min</td>
<td>5% Sheep Blood agar, MacConkey agar and Sabouraud Dextrose agar</td>
<td>February to April 2006</td>
<td>Chennai, India</td>
<td>Sudharsanam et al., 2008</td>
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<tr>
<td>Intensive care unit, operating room, neonatal ward, patient room and main entrance of both governmental hospital (built in 1960, with 294 beds) and a private hospital (built in 1986, with 80 beds)</td>
<td>Microbial air sampler: operated at an air flow-rate of 100 L/min for 5 min</td>
<td>Bacteria: Nutrient agar supplemented with 100mg/L cyclohexamide Fungi: Sabouraud dextrose agar supplemented with 10mg/L chloramphenicol</td>
<td>December 2005 Three air samples taken at three different time periods</td>
<td>Zarqa city, Jordan</td>
<td>Qudiesat et al., 2009</td>
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</table>
2.4.2 Variations in indoor bioaerosol concentrations

A study conducted in a hospital ward of a pneumonological department in Poland found seasonal variations in total microbial loads, with greater variation among fungi than bacteria; the study further documented that even though microbial loads were less in indoor air of hospital, there is possibility of asthma exacerbations in some patients because of the presence of *Aspergillus fumigatus* and other potentially pathogenic species (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).

2.4.3 Airborne bacteria in healthcare facilities and association with nosocomial infections

A healthcare-associated infection (HAI) is “an infection occurring in a patient during the process of care in a hospital or other health-care facility, which was not present or incubating at the time of admission. This includes infections acquired in the hospital but appearing after discharge and also occupational infections among staff” (WHO, 2011).

HAI are endogenous or exogenous in origin. Endogenous sources include the patients’ own flora, while the exogenous sources include the hospital environment, including the people, inanimate objects, food, water and air in the hospital.

Airborne nosocomial infections are transmitted directly or indirectly through air and may cause respiratory (primarily pneumonia) and surgical-site
infections (Kowalski, 2007). Earlier studies have shown increasing evidences of airborne transmission in nosocomial outbreaks of methicillin resistant *Staphylococcus aureus* (MRSA) (Farrington *et al.*, 1990; Bernards *et al.*, 1998), *Acinetobacter* spp. (Allen and Green, 1987; Bernards *et al.*, 1998) and *Pseudomonas* spp. (Jones *et al.*, 2003).

Shiomori *et al* (2001) quantitatively investigated the existence of airborne methicillin-resistant *Staphylococcus aureus* (MRSA) in a hospital environment. Phenotyping and genotyping of the MRSA isolates were also performed to study MRSA epidemiology. Study revealed presence of MRSA in air samples collected during a period of rest as well as when bed-sheets were being changed. MRSA isolates were detected in all stages, of which 20 % were within a respirable range of < 4 µm. MRSA were also isolated from inanimate environments, such as bed-sheets, sinks and floors in the rooms of the patients with MRSA infections, as well as from the patients’ hands. Epidemiological study demonstrated that clinical isolates of MRSA were of one origin and that the isolates from the air and inanimate environments were identical to the MRSA strains that caused infections or colonisation in in-patients, indicating 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).

Another study was carried out by Jones *et al* (2003) to look for the presence of epidemic strains of *P. aeruginosa* in the environment of a Cystic Fibrosis (CF) centre during a cross infection outbreak and examine their potential modes of spread between patients. Microbiological sampling of the environment of the CF facility was performed, including room air sampling. Individual *P. aeruginosa* strains were identified by bacterial fingerprinting. The typing
patterns were compared with those of epidemic strains responsible for cross infection among the patients. Epidemic *P. aeruginosa* strains were isolated from room air when patients underwent spirometric tests, nebulisation, and airway clearance, but were not present in other areas of the inanimate environment of the CF centre. This study indicates that aerosol dissemination may be the most important factor in patient-to-patient spread of epidemic strains of *P. aeruginosa* during the cross infection outbreaks at adult CF centres (Jones *et al.*, 2003).