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Review Article

BIO-AEROSOLS IN INDOOR ENVIRONMENT: COMPOSITION, HEALTH EFFECTS AND ANALYSIS

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Abstract

Bio-aerosols are airborne particles that are living (bacteria, viruses and fungi) or originate from living organisms. Their presence in air is the result of dispersal from a site of colonization or growth. The health effects of bio-aerosols including infectious diseases, acute toxic effects, allergies and cancer coupled with the threat of bioterrorism and SARS have led to increased awareness on the importance of bio-aerosols. The evaluation of bio-aerosols includes use of variety of methods for sampling depending on the concentration of microorganisms expected. There have been problems in developing standard sampling methods, in proving a causal relationship and in establishing threshold limit values for exposures due to the complexity of composition of bio-aerosols, variations in human response to their exposure and difficulties in recovering microorganisms. Currently bio-aerosol monitoring in hospitals is carried out for epidemiological investigation of nosocomial infectious diseases, research into airborne microorganism spread and control, monitoring biohazardous procedures and use as a quality control measure. In India there is little awareness regarding the quality of indoor air, mould contamination in indoor environments, potential source for transmission of nosocomial infections in health care facilities. There is an urgent need to undertake study of indoor air, to generate baseline data and explore the link to nosocomial infections. This article is a review on composition, sources, modes of transmission, health effects and sampling methods used for evaluation of bio-aerosols, and also suggests control measures to reduce the loads of bio-aerosols.

Key words: *Bio-aerosols, indoor air, health effects, monitoring nosocomial infections*

Bio-aerosols are airborne particles that are living (bacteria, viruses and fungi) or originate from living organisms. Bio-aerosols are ubiquitous, highly variable, complex, natural or man-made in origin. The sampling and analysis of airborne microorganisms has received attention in recent years due to concerns with mould contamination in indoor environments, the threat of bioterrorism and the occurrence of associated health effects, including infectious diseases, acute toxic effects, allergies and cancer.[1-3] Bio-aerosols contribute to about 5-34% of indoor air pollution.[4,5]

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 bio-aerosols.[6] Air, contains significant number of microorganisms, acting as a medium for their transmission or dispersal. Inhalation, ingestion and dermal contact are the routes of human exposure to airborne microorganisms, inhalation being the predominant. The particles in a bio-aerosol are generally 0.3 to 100 µm in diameter; however, the respirable size fraction of 1 to 10 µm is of primary concern.[7] Bio-aerosols ranging in size from 1.0 to 5.0 µm generally remain in the air, whereas larger particles are deposited on surfaces.[8]

Exposure to bio-aerosols 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.[9] While their role in various industrial settings has been well studied,[1] the role of these airborne microorganisms in healthcare settings is poorly understood. Increasing incidences of nosocomial and occupational diseases due to bio-aerosol exposure[10-13] indicate the need for a thorough knowledge in this respect. Bio-aerosol 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.[6] In this article, an overview of bio-aerosols, their sources and possible health effects, various sampling methods and a characterisation of common airborne agents is presented.

Factors influencing Bio-aerosols

The transport and the ultimate settling of a bio-aerosol are affected by its physical properties and the environmental parameters that it encounters.[14] The physical characteristics are size, density, and shape of droplets or particles, the environmental factors include magnitude of air currents,

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Table 1: Bio-aerosol concentrations in air systems, indoor surfaces and water treatment plants

Category	Activity type	Bacteria (CFU/m^3)*	Fungi (CFU/m^3)*
Air systems	HVAC	$10 - 10^3$	$10 - 10^7$
		$10^2 - 10^4$	$10 - 10^3$
Indoor surfaces	ceilings and walls	$10 - 10^3$	$10 - 10^4$
	carpet	$10^3 - 10^6$	$10^2 - 10^5$
	house plants	$10 - 10^4$	$10^2 - 10^5$
	operating room	$10 - 10^2$	$10 - 10^2$
Water treatment plants	aeration tanks	$10^2 - 10^3$	$10 - 10^2$
	activated sludge	$10^2 - 10^6$	$10 - 10^3$

*Bio-aerosol concentrations given are only expected concentrations at various work environments and not the representative threshold limit values, Source: Adapted from germology.com[17]

relative humidity and temperature, which determine the capacity to be airborne.[14] Bio-aerosols generated from liquid suspensions undergo desiccation, whereas those generated as dusts or powders partially rehydrate.[15] The presence of moulds indicates a problem with water penetration or high humidity.[16]

Sources of Bio-aerosols in Indoor and Outdoor Environments

Bio-aerosols originate from any natural or man-made surface and each source gives rise to an entirely unique assemblage of bio-aerosols. Bioaerosols concentrations in air systems, indoor surfaces and water treatment plants are highlighted in Table 1.[17] Deterioration of building materials, offensive odour and adverse human health effects are associated with microbial contamination of indoor environments.

Buildings

The presence of undesirable bio-aerosols is often associated with sick building syndrome (SBS) and building related illnesses (BRI). Sources include furnishings and building materials; fungal contamination within wall, ceiling, and floor cavities by movement of cells, spores, and cell fragments via wall openings and gaps at structural joints.[17] Lack of fresh air due to increased insulation of buildings, poorly maintained or operated ventilation systems, poorly regulated temperature and relative humidity levels contribute to the presence and multiplication of bio-aerosols.[18] In developing countries, inadequacies in the building design and improper ventilation may contribute to poor indoor air quality.

Healthcare Facilities

The microbial load in hospital indoor air is highly influenced by the number of occupants, their activity and the ventilation.[19] Occupants are a potential source of microorganisms as they shed the 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 the moist surfaces. Dressings and bedding also can be the sources of airborne microorganisms.[19] Sweeping of floors and changing of bed linens also can cause suspension of bio-aerosols in air.[19] Fungal spores gain entry into the hospital buildings through ventilation ducts with inadequate filtration. Since exposure levels are high, this may be an issue in the immunocompromised patients.

Modes of Transmission

Bio-aerosols can be transmitted either at long distances beyond the patient room environment, or within short distances. Small particle aerosols (e.g., generated during endotracheal intubation) are transmitted to persons in the immediate area near the patient. Viruses like Severe Acute Respiratory Syndrome (SARS), influenza and norovirus are transmitted from patients primarily by contact and/or droplet routes, while airborne transmission occurs over a limited distance.[20] *Legionella* may be derived from the environment,[21] others include contaminated food, water, medications (e.g., intravenous fluids) or through vectors.[22] *Aspergillus* spp. can be transmitted from patients or the environment.[22,23]

Roy and Milton proposed a new classification for airborne pathogens when evaluating routes of SARS transmission,[24] based on the agent's capacity to be transmitted and to induce disease. Obligate airborne pathogens produce an infection that, under natural conditions, is initiated only through aerosols deposited in the distal lung tissue such as *Mycobacterium tuberculosis*. Preferential airborne pathogen can naturally initiate infection through multiple routes but are predominantly transmitted by aerosols deposited in distal airways, e.g., measles virus and variola (smallpox) virus. Opportunistic airborne pathogens naturally cause disease through other routes (e.g., the gastrointestinal tract) but can also initiate infection through the distal lung and may use fine-particle aerosols as an efficient means of propagating in favourable environments.

Immunopathogenesis

Individuals are exposed to an array of bio-aerosols in a single day that may interact in complex ways to cause

airway inflammation and infection. Smaller cells and spores become trapped within lung tissue and are not easily expelled posing greater health risks.[17]

The clinical expression of airway disease is influenced by a combination of components of bio-aerosols and the dose and duration of exposure (environment), as well as intrinsic differences in the host response to bio-aerosols (genetic polymorphisms).[25] Many of the components of bio-aerosols are pathogen-associated molecular patterns (PAMPs) that bind specific recognition molecules and activate innate immune pathways. The most frequently detected PAMPs in bio-aerosols are endotoxin, peptidoglycan and β-(1→3)-glucans.[26]

Endotoxin signalling is being achieved through TLR4 (Toll-like receptors) pathway, a PAMP recognition molecule. Immune cells first develop tolerance to repeated exposures to endotoxin. Then, there is increased expression of TLR4 on the cell surface that leads to increase in the inflammatory response to lipopolysaccharide (LPS). Respiratory Syncytial Virus (RSV), present in bio-aerosols in domestic and day-care settings, increases TLR4 expression and sensitizes respiratory epithelial cells to endotoxin.[27] Although endotoxin causes inflammation in everyone, people with asthma tend to be more sensitive. Certain proteins found attached to white blood cells and floating free in blood and fluid surrounding lung cells are involved in a person's reaction to endotoxin. A protein called CD14, a mannose receptor specific to LPS and found on the surfaces of mature macrophages,[28] is present in higher levels in people with asthma. EPA (US Environmental Protection Agency) researchers examined healthy controls and asthmatics to investigate the relationship between CD14 and severity of response to endotoxin. They measured CD14 levels in samples of the participants' sputum collected both before and after the exposure and showed a correlation between levels of CD14 and the severity of the inflammatory response; when levels of CD14 were high before exposure to endotoxin, the inflammation was more severe.[29] Estimation of CD14 in serum by enzyme immunoassay (EIA)[30] can be used to predict the severity of a person's response to endotoxin. Exposure to endotoxins is associated with increased severity of asthma and BRI.[31,32]

Peptidoglycan recognition by the innate immune system involves three molecules - TLR2, peptidoglycan recognition proteins (PGRPs), and nucleotide-binding oligomerization domain molecules (NODs).[33] NOD1 and NOD2 are intracellular molecules that recognize peptidoglycans from gram positive and gram negative bacteria. PGRPs are cell surface recognition molecules for peptidoglycan that signal in association with toll receptors. One PGRP, PGRP-S expressed in neutrophils and eosinophils, is bacteriostatic for gram positive bacteria.[34]

β-(1→3)-glucans, polymers of glucose produced in fungi, plants, and some bacteria, are associated with increased respiratory symptoms in a number of occupational settings,[35] and are also potent activators of the innate immune system. A trans-membrane lectin molecule, dectin-1, expressed on macrophages and neutrophils is the β-glucan receptor.[36] Dectin-1 may function as a T-cell co-stimulatory molecule, suggesting that β-glucan stimulation may be a link between innate and adaptive immune responses.[37]

Health Effects

Biological hazards to man arise from exposure to high concentrations or unfamiliar forms of bio-aerosols and three major groups of diseases associated with bio-aerosol exposure are infectious diseases, respiratory diseases and cancer.[1] Current knowledge is unclear regarding risk to cancer whether these excess risks occur from exposures to biological agents or are due to various chemicals used in these industries.[1] Table 2 highlights the microorganisms associated with an airborne route of exposure that result in adverse human health effects.[3]

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.

Bacterial diseases

Various bacterial diseases such as legionellosis and tuberculosis are linked to cause significant public health concern due to their low infectious dose.[14]

Table 2: Microorganisms associated with an airborne route of exposure that result in adverse human health effects

Organism	Source
Aspergillus fumigatus	Mould-contaminated building, compost
Aspergillus versicolor	Mould-contaminated building
Bacillus anthracis	Bioterrorism, animal handlers, veterinarians
Francisella tularensis	Potential WMD*, infected rodents
Legionella pneumophila	Aerosols from water spray
Mycobacterium tuberculosis	Person-to-person
Penicillium species	Mould-contaminated building
Stachybotrys chartarum	Mould-contaminated building
Trichoderma species	Mould-contaminated building
Variola virus	Potential WMD*
Yersinia pestis	Potential WMD*, infected fleas

*Weapons of mass destruction, Source: Modified from Stetzenbach *et al*.[3]

Legionellosis: *Legionella pneumophila* causes human legionellosis and community-acquired and nosocomial pneumonia in adults following either occupational or non-occupational exposures. Legionellae become airborne often as a result of active aerosolising processes (aeration of contaminated water) and may inhabit various water environments including man-made water systems, often in biofilms in cooling towers, air conditioning systems, etc. Nosocomial infections and hospital outbreaks have been linked to contaminated hot water supply of temperature 45°C.[38] The use of monochloramine for residual drinking water disinfection may help prevent Legionnaires' disease.[39] In comparison to free chlorine, 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.[40]

Tuberculosis: The transmission of tubercle bacilli occurs through the inhalation of aerosolised 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.[41]

Anthrax: The transmission occurs due to inhalation of the spores of *Bacillus anthracis* and outbreaks are often linked to bioterrorism that are spread through intentionally contaminated mail, apart from occupational exposures.[42]

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.[43] High exposure to endotoxins is often associated with nausea and diarrhoea.[44]

Fungal diseases

Airborne fungi causing respiratory infections and allergic reactions include *Penicillium, Aspergillus, Acremonium, Paecilomyces, Mucor* and *Cladosporium*.[45]

Most infections, commonest being *Aspergillosis*, can occur in immunocompromised 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, etc.[46] *Coccidioides, Histoplasma* and *Blastomyces* grow in soil or may be carried by bats and birds and is 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.[47] *Aspergillus* species that can grow indoors include *Aspergillus fumigatus* and *Aspergillus flavus* and can cause nosocomial infections[48], allergic broncho-pulmonary aspergillosis (ABPA) and sinusitis. Chronic asthmatics may progress to have their bronchial passages colonized by either *Aspergillus fumigatus*, *Bipolaris hawaiiensis*, or *Wangiella dermatitidis*.[46] Constant allergic response maintains the fungal colonisation, and first-line therapy with steroids, brings down the level of inflammation and may result in elimination of the colonising organism.[46]

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.[49] 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.[50] Other adverse health effects include pre-term births or late abortions in farm women exposed to mycotoxins with immunotoxic and hormone-like effects.[51]

Viral diseases

Viruses are readily transmitted by airborne route, and include SARS virus[52], enteric viruses of intestinal origin produced at sewage treatment facilities, RSV, Hantavirus from rodent faeces,[53] 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.[54] SARS, caused by novel corona virus, is a highly contagious respiratory illness of significant morbidity and mortality, and causes very severe atypical pneumonia.[55,56] The use of aerosol-generating procedures (such as endotracheal intubation, bronchoscopy, and treatment with aerosolised medication) in hospitals may amplify the transmission of SARS.[52]

Diseases caused by parasites and Actinomycetes

Free-living amoebae like *Acanthamoeba* and *Naegleria fowleri* get aerosolised from natural and artificially heated waters,[57] and cause respiratory illness and meningoencephalitis. Actinomycetes such as *Streptomyces* and algae cause allergy, inflammatory reactions and hypersensitivity pneumonitis.

Respiratory Diseases

Many of the BRI are respiratory diseases and include asthma, hypersensitivity pneumonitis and multiple chemical sensitivity.[18] Asthma and allergic rhinitis are the most extensively studied respiratory diseases associated with bio-aerosol exposure. Both innate and adaptive immune mechanisms are implicated in the pathogenesis of disease.

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.[58]

Organic Dust Toxic Syndrome (ODTS) occurs within hours of a high dose inhalation of endotoxin, fungal spores and mycotoxins,[59] which may lead to chronic obstructive pulmonary disease (COPD).[60]

Bioallergens are potent allergens and include enzymes derived from fungi and bacteria produced by biotechnological companies,[61-63] and plant pollens.[64]

Cancer

Established biological occupational carcinogens are the mycotoxins. Aflatoxin from *Aspergillus flavus* is capable of causing liver cancer.[65,66] 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 grain dust exposure occurs.[66,67] 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.[68]

Role of Bio-aerosols in Healthcare Settings

Operating rooms are a high risk area for both patients and staffs; air-quality management is important so that such environments are ensured to be free of airborne infectious agents. Adequate air changes and installation of filtration equipment are a necessity; proper air-conditioning systems can significantly reduce airborne concentrations of fungi.[69] Airborne bacteria have a considerable impact on infection during surgery. When the levels of airborne bacteria are reduced in operating rooms (OR), contamination of wounds is substantially reduced.[70]

Role of Airborne Infectious Agents in Nosocomial Infections

Airborne nosocomial infections are transmitted directly or indirectly through air and may cause respiratory (primarily pneumonia) and surgical-site infections.[71] Earlier studies have shown increasing evidences of airborne transmission in nosocomial outbreaks of methicillin resistant *Staphylococcus aureus* (MRSA)[72,73], *Acinetobacter* spp.[73,74] and *Pseudomonas* spp.[75]

A variety of bacteria such as *Acinetobacter, Bacillus, Corynebacterium, Escherichia, Listeria, Micrococcus, Staphylococcus* and *Streptococcus*, and fungi such as *Alternaria, Aspergillus, Cladosporium, Penicillium* and *Scopulariopsis* were isolated from operating theatre, birthing-room, emergency department, service area for infectious diseases, intensive care unit (ICUs) and canteen in Trakya University Hospital (Edirne, Turkey).[76]

A recent report evaluated the characteristics of total particles and viable bacterial and fungal species in clean rooms of different classes in hospital and found that the significant particle concentration fluctuations might be related to variations in operating personnel numbers and activities for operating rooms, and suggested that further evaluation of bio-aerosols characteristics in relation to nosocomial infection and the efficiency of particulate control in clean rooms are needed.[77]

In another study, the frequency of nosocomial infection related to air-colonisation was higher in patients of anaesthesia intensive care unit (16.4%) than in general surgery intensive care unit (4.9%), the most frequent being bacteraemia and surgical wound infections respectively. The most frequently isolated microorganisms were MRSA and *Acinetobacter baumannii,* suggesting that airborne viable particles in operating theatres and intensive care units can be a significant risk factor for the development of nosocomial infections.[78] A study from Pune, India assessed environmental bacteria carrying particle (BCP) load and found potentially pathogenic fungi in the filters of the air-conditioning units, highlighting the need for standardising the microbiological evaluation protocols for operating rooms.[79]

The Central Pollution Control Board (CPCB), New Delhi, India[80] studied the bacterial, fungal and total pathogenic populations in different months in various settings and found seasonal variation in fungal and bacterial concentrations. The CPCB report raised concerns regarding quality of indoor air in various sectors, including medical devices manufacture, operation theatres and hospitals, but did not include the details of identification of individual bacterial and fungal species.[80]

A pilot study conducted in a healthcare facility in Chennai, India characterised Bio-aerosols and found *Staphylococcus aureus* in microbiology laboratory, female ward and animal house, *Shigella* in BWD (biomedical waste disposal), *Pseudomonas* and *Acinetobacter* species in wards, animal house and BWD, and *Aspergillus fumigatus* in laboratory and animal house; indicating that bio-aerosols in healthcare facilities may be a significant occupational safety and health concern.[81]

Bio-aerosol Evaluation

In general, indoor microflora concentrations of a healthy work environment are lower than outdoor concentrations at the same location.[82] The purpose of bio-aerosol sampling is to verify and quantify their presence in air and in most cases no single sampling method can collect, identify and quantify all of the bio-aerosol components existing in a particular environment. When sampling is indicated, it is advisable to sample before, during, and after the sampling area is occupied, including times when the heating, ventilating, and air conditioning system is activated and inactivated.[82]

In comparison to settings like agricultural and poultry farming, where bio-aerosol concentrations are high, microbial loads are less in laboratories and wards. Healthcare settings represent a unique assemblage of indoor microflora as bio-aerosols 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 bio-aerosols. Areas such as ICUs, ORs, labour rooms and orthopaedic wards, where indoor air quality are of concern can be targeted.

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 bio-aerosols; there is however no internationally accepted recommendations on sampling flow-rate and the media used for sampling. Reports suggest that high-containment laboratory and hospitals require air samplers with flow-rates ≥ 25 L/minute for monitoring, and those with flow-rates < 5 L/minute are not suitable and practical when the bio-aerosol concentration is $<10^2$ CFU/m^3,[83] and recommends less sampling time for bioimpactor samplers with an airflow rate of 100 L/minute, and an air impact speed of less than 20 m/second (to avoid the risk of impact stress and dehydration of the agar surface).[84] As the environment is polluted in India, it is necessary to carry out repeated sampling to develop a standard protocol in the context of Indian settings.

Environmental Sampling for Bio-aerosols

Most bio-aerosol sampling devices involve techniques that separate particles from the air stream and collect them in or on a pre-selected medium. In addition, surface sampling is used to locate areas of contamination due to re-aerosolisation from surfaces and in identifying the source(s) of bio-contamination. The following methods may be used to monitor ambient indoor air quality in hospitals.

Inertial sampling methods used for bio-aerosol collection include impingement whereas filtration is a commonly used non-inertial sampling method. Gravity settling is the widely used gravitational sampling method.

Gravitation or settling

An adhesive substrate such as a coated microscope slide or a petri-plate containing agar medium is exposed face upwards to the atmosphere to collect particles settling by gravity. This method is simple, frequently used, sometimes in preference to other aerobiological samplers.[85] It is, however, a passive (non-volumetric) method that does not give information on the volume of air from which the particles have been collected. It also over-represents larger particles sampled during the exposure period because of their faster sedimentation rate.[85] Use of settle plates can provide a hint whether an environment is more or less contaminated with airborne microorganisms.

Impingement

Liquid impingers are a special type of impactor. Impingers are useful for the collection of culturable aerosols.[82] 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.[82] In this method, air samples are impinged into 20 mL of inert liquid medium at the rate of 12.5 Litres/minute for 20 minutes (Figure). The samplers operate by drawing aerosols through an inlet tube. Curved inlet tube helps to simulate particle collection

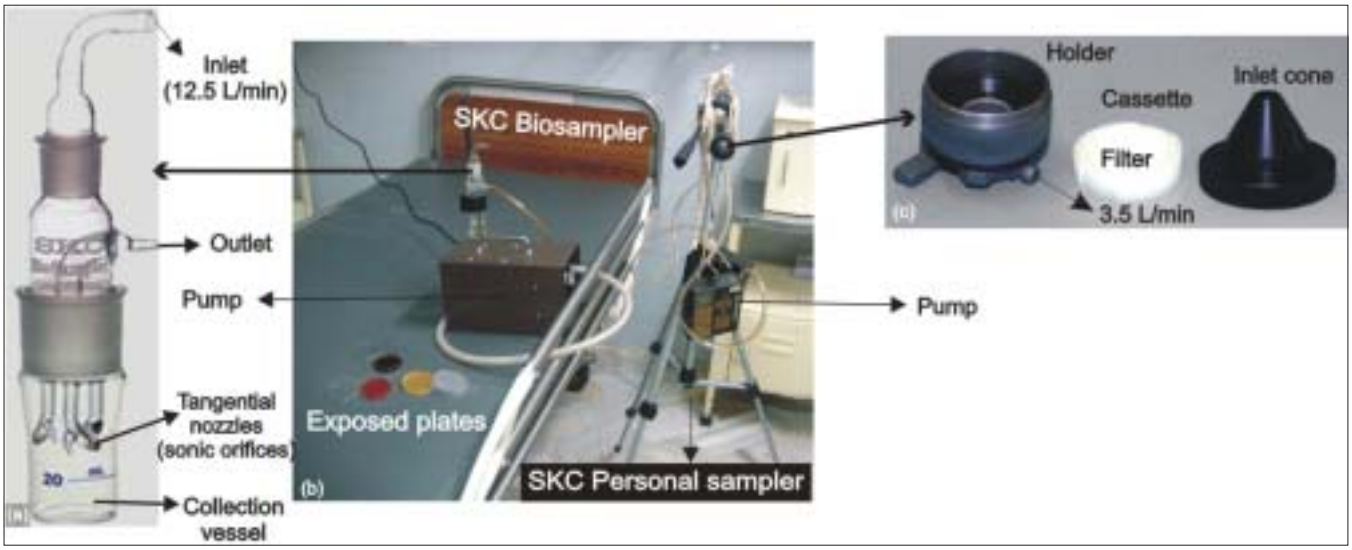

Figure: (a) Impinger sampling method (SKC Biosampler) (b) Active (Filter and Impinger) and passive samplers in operation for simultaneous sampling of ambient air (c) Sampling method using filter (SKC Personal sampler)

in the nasal passage for separating respirable (collection fluid) and non-respirable (inlet tube) microorganisms. 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.[84] A liquid sample can also be used with a variety of analytical methods, including culture, microscopy, immunoassay, flow cytometry and molecular methods.[86]

Filtration

Collection of particles from a non-biological aerosol sample is most commonly achieved by filtration.[82] Filter media are available in both fibrous (typically glass) and membranous forms. Particles smaller than the pore size may be efficiently collected. Sampling filter media may have pore sizes of 0.01 - 10 µm. Membrane filters are manufactured in a variety of pore sizes from polymers such as cellulose ester, polyvinyl chloride, and polycarbonate. Filters are often held in disposable plastic filter cassettes during bio-aerosol sampling.[82]

Sampling is done by allowing the air to pass through the filter (preferably gelatine or polycarbonate) at the rate of 3.5 Litres/minute for 15 minutes (Figure). The sampled organisms are washed from the surface of the filters and the wash solution cultured or refiltered to distribute the organisms uniformly on the membrane filter. In areas of high concentration, the organisms have to be eluted, diluted and refiltered for microscopic analysis.[82] Filtration techniques are used for the collection of certain fungi and endospores-forming bacteria that are desiccation-resistant. 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.[86,87]

Surface Sampling

Since air sampling alone does not provide assurance that an area is free of biological contamination[88], due to re-aerosolisation of the organisms from surfaces during routine activity, surface sampling is essential to identify the areas and sources of contamination in determining the effectiveness of remediation and clean-up of contaminated indoor environments.[88-90]

Analysis for Detection of Microorganisms and Microbial Constituents

Detection of Microorganisms

Viable microorganisms include culturable and non-culturable. During sampling, only culturable microorganisms are enumerated and identified, leading to an underestimation of bio-aerosol concentration. Therefore estimation of both culturable and non-culturable organisms using appropriate microscopy to identify bacteria and fungi (using gram staining for bacteria, and lactophenol cotton blue and calcofluor white for fungi) and classical microbiology techniques such as observation of growth characteristics, cellular or spore morphology, and biochemical tests for identification is essential.[91] After sample collection, colonies of bacteria and fungi are grown on culture media at a defined temperature over a 3 - 7 day period and then identified. Molecular biology techniques such as restriction fragment length polymorphic (RFLP) analysis are further used for better identification.[92,93]

Analytical techniques applied for nonviable and viable microorganisms include polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA); these are usually qualitative, while semi-quantitative and quantitative methods are evolving.

PCR was first demonstrated as a means to detect bacteria[94] and viruses[95] in air samples in 1994, and was later used to detect airborne *Pneumocystis jiroveci*[96] and *Aspergillus* spp.[97] It has also been used for the enhanced detection of bacterial WMD surrogates and actual bioterrorism agents. Quantitative PCR is being evaluated in bio-aerosol monitoring.

Detection of microbial constituents

Endotoxin assay: Samples are collected from air by filter method, using polycarbonate capillary pore membrane filters. After sampling, it is extracted by sonication and then analysed for the presence of endotoxins by limulus amebocyte lysate (LAL) test, or chromogenic kinetical limulus test. LAL test is a comparative and not an analytical bioassay method as measured endotoxin activity levels change with changes in factors other than lipopolysaccharide concentrations.[98] The measurement of optical density is by means of a modified spectrophotometer.[99]

Fungal biomass assay: Some markers for the assessment of fungal biomass include ergosterol measured by gas chromatography-mass spectrometry[100] or fungal extracellular polysaccharides measured with specific enzyme immunoassays.[101]

Data Interpretation for Bio-aerosols

Threshold limit values (TLV) for bio-aerosols are referred to air concentrations of substances under conditions to which people are repeatedly exposed day after day without adverse health effects.[9] There are no established guidelines specifying the threshold limit values for interpreting environmental measurements of bio-aerosols because bio-aerosols do not comprise of a single entity. Human responses to bio-aerosols range from innocuous effects to serious diseases depending on the exposure and the susceptibility of human beings to it (e.g., genetic factors, age, personal habits, medication). Also, little is known about the minimum dose needed to pose a hazard.

While there are no internationally accepted guidelines,

recommendations have been made by World Health Organization (Indoor air quality: Biological contaminants),[102] Federal-Provincial Advisory Committee on Environmental and Occupational Health, Canada (Indoor Air Quality in Office Buildings: A Technical Guide)[103] and NASA standard NhB5340.2.[104]

Though ACGIH (American Conference of Governmental Industrial Hygienists) had published numerical guidelines earlier, it currently does not support any existing numerical criteria for interpreting data on biological agents from source or air samples in non-manufacturing environments.

Control Measures for Reducing Bio-aerosols

In order to reduce bio-aerosol loads in indoor environments, certain control measures can be followed.[4] These include, proper identification and elimination of the microbial source in occupational and house-hold settings, maintenance of equipment, humidity control, natural ventilation, use of filters in ventilation, and air cleaning by the use of disinfectants and biocides. Periodical use of disinfectants and biocides is one of the methods to ensure controlled bio-aerosol concentrations. Air in the operating rooms and other critical areas like isolation rooms can be disinfected by fumigation using various microbicidal agents. Bacillocid[105] is the most commonly used commercially available surface and environmental disinfectant that has very good cleansing property along with bactericidal, viricidal, sporicidal and fungicidal activity. It is either sprayed or mopped liberally allowing a contact time of 30 minutes and provides complete asepsis within 30 - 60 minutes. It does not require cleaning with detergent or carbolic acid or formalin fumigation. It does not require shutdown of the disinfected areas such as operating rooms for 24 hours.

Conclusions

In the context of healthcare settings, bio-aerosols can cause occupational hazards and nosocomial infections. Modern built environment can be a potential source of bio-aerosols. Bio-aerosol monitoring in hospitals can be used for tracking of nosocomial infections, identify the source and spread of airborne microorganisms to control hospital associated infections (HAI). This will also serve as a tool to measure biosafety while handling biohazardous materials. The complexity of these bio-aerosols requires a multidisciplinary approach. There is heightened awareness regarding the study of bio-aerosols and its impact on human health and quality of indoor air and environment in the West. In the context of a developing country, there is a need for increased awareness for targeted surveillance for infection control.

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