ABSTRACT

AEROMICROBIOLOGICAL STUDY OF INDOOR AIR QUALITY IN HOSPITALS – CHARACTERISATION OF BIOAEROSOLS AND THEIR ASSOCIATION WITH NOSOCOMIAL INFECTIONS

Background

Healthcare facilities are a unique environment, where patients including those with chronic illness requiring longer durations of hospital stay or those with altered immune status are admitted. Sometimes patients may acquire a healthcare associated infection after hospitalisation. The environment may occasionally contribute to such infections inadvertently. This study was undertaken to generate baseline data on airborne microorganisms, characterise aerobic bacteria and fungi in different locations of the hospital environment, determine whether temporal variation exists in airborne microbial concentrations of a hospital location, determine the most common isolate across hospitals, study the relationship between environmental and clinical isolates in a select area using molecular typing methods such as Polymerase Chain Reaction (PCR) and Pulsed Field Gel Electrophoresis (PFGE), and determine if endotoxin can be airborne.

Methods

Institutional Ethics Committee approval was sought prior to start of the study. Indoor air samples (in duplicates) were collected simultaneously using exposed plate (for 30 minutes), impingement (BioSampler @ 12.5 L/min for 20 min) and filtration (personal sampling filter cassette loaded with gelatin filter @ 3.5 L/min for 15 min) methods in different locations of different hospitals to
characterise airborne microflora. Sampling was also conducted over different periods of the year in the same location of one hospital to determine temporal variation. A walk-through was conducted to assess the extent of activity prior to sampling. Temperature and relative humidity were recorded during sampling. Bacterial plates were incubated at 37°C and observed for growth after 48 h; fungal plates were incubated at 25°C and 37°C and observed up to 7 days. Microorganisms were identified using standard microbiological procedures. PCR targeting *Pseudomonas* specific 16s rDNA was performed to obtain 618 bp amplicons. Representative strains were sequenced and compared with established sequences of pathogenic *Pseudomonas* strains from NCBI database for evolutionary details. Few strains of phenotypically similar coagulase-negative *Staphylococci* (CNS) of environmental (air) and clinical origin were subjected to genotyping by PCR and PFGE to confirm the similarity. Air samples were collected from a low-cost intensive care unit (ICU) by impingement in sterile normal saline (BioSampler @ 12.5L/min for 16 min) and blood samples were collected from patients (after 48h of admission) and health personnel (with consent) from the same ICU. Pre-treated sera (diluted in sterile pyrogen-free distilled water to 1:10 and heated at 70°C for 10 min) and air samples were tested for presence of endotoxin using LAL gel-clot detection method. Formation of stable clot was considered positive. Questionnaire was circulated among health personnel to determine any adverse health effects.

**Results**

Bacteria were found to be in higher concentrations when compared to fungi, irrespective of the sampling method and the sampled location. Among bacteria, Gram-positive cocci (CNS and *Micrococcini*) were more predominant than
Gram-negative bacilli (*Pseudomonas* sp.). In fungi, moulds (*Aspergillus* sp.) were frequently encountered. Exposed plate method was found to recover a variety of bacteria and fungi than impinger and filter methods. Airborne microbial loads were not influenced significantly by the changing seasons and the size of the hospitals. However, there was significant variation in their loads with the sampling location (OR < ICU < ward). *Pseudomonas* sp. was present in indoor air of different hospitals, including the ICUs and ORs. Phylogenetic analysis showed the similarity between the study isolates of *Pseudomonas* strains that were environmental (air) in origin and the clinical isolates in the NCBI database. Environmental strain of methicillin resistant *Staphylococcus haemolyticus* isolated from air sample was found to be similar to that of the strain recovered from clinical (tissue) sample of a patient admitted subsequently within a week in the same location. Further, endotoxin was present in indoor air of ICU of a low-cost healthcare facility and among health personnel working in the same ICU. Health personnel are under higher risk (10.31 times more than patients) of adverse health effects due to exposure to airborne endotoxins.

**Conclusion**

Exposed plate method is a useful method for collecting air samples in areas of low bioaerosol concentrations such as healthcare facilities, since it is cost effective, reproducible and can be preferred for preliminary assessment of indoor microbial air quality in hospitals. Profiling of bioaerosols in different locations of hospitals under study showed that GPC were in higher concentrations than GNB among bacteria, while fungi were seen in less numbers. Since GPC are primarily shed from skin and hair of occupants, there is a need for guidelines on proper ventilation and permissible limits for number of personnel.
The presence of GNB indicates the need for infection control practices to reduce aerosolisation of these organisms during healthcare delivery. The mere presence of fungi indicates the need for use of proper air handling systems to restrict the entry of fungal spores and prevent fungal growth. Similarity between the study isolates (environmental) and clinical isolates of *Pseudomonas stutzeri* in the NCBI database suggests that a pathway may exist, allowing for the introduction of these organisms from the hospital environment into patients during healthcare delivery. Similarity of coagulase-negative *Staphylococci* of environmental and clinical origin indicates that air may be a possible source of nosocomial infection. The study also documented presence of airborne endotoxin. Health personnel are at a higher risk of adverse health effects due to exposure to airborne endotoxins when compared to patients.

*Key words*

Indoor air; Exposed plate method; Nosocomial infections; Coagulase-negative *Staphylococci; Pseudomonas; Airborne endotoxin*