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Microbial quality assessment of indoor air in a large hospital building during winter and spring seasons

Ayesha Asif[a], Muhammad Zeeshan[a,*], Imran Hashmi[a], Uzma Zahid[b], Muhammad Faraz Bhatti[c]

[a] Institute of Environmental Sciences and Engineering (IESE), School of Civil and Environmental Engineering (SCEE), National University of Sciences and Technology (NUST) H-12 Campus, Islamabad 44000, Pakistan

[b] Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan

[c] Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST) H-12 Campus, Islamabad 44000, Pakistan

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ABSTRACT

Sensitivity of hospital building environment due to presence of potential sources of wide range of airborne microbes, make it a complex environment. Present study aimed to investigate seasonal (winter & spring) variation in airborne microbial levels as well as species on various locations (i.e. operation theatres (OT1 & OT2), wards (GMW & SW), out-patient department (OPD) and emergency services (ES)) of a large hospital building. Air samples were collected during peak hours, twice a week, covering one month of each season. Statistically significant variation ($p > 0.05$) in bacterial concentrations over two seasons was found only for OPD. However, fungal concentrations significantly varied ($p < 0.05$) over two seasons for all sites except for OT1 and OT2. Concentrations among most of sites were significantly different. Highest bacterial level was found in OPD (mean: 1649.7 CFU/m^3) while lowest in the two OTs (mean: 221 CFU/m3 for OT1 and 236 CFU/m^3 for OT2). Highest fungal level was found in GMW (mean: 193.4 CFU/m^3) while lowest in the two OTs (mean: 41.1 CFU/m^3 for OT2 and 58 CFU/m^3 for OT1). Bacterial identification showed dominancy of gram positive cocci (89.8%) followed by gram positive rods (7.2%) and gram negative rods (3%). Identified bacterial strains belonged to genera staphylococcus, micrococcus, kocuria, aerococcus, kytococcus, bacillus and pseudomonas. The most abundant fungal genera included cladosporium (47%), aspergillus (17.1%), penicillium (7.1%), alterneria (6.2%), geotrichium (3.68%) and ulocladium (3.2%). Cleaning frequencies appeared to be important factor in maintaining low microbial load in air.

1. Introduction

Tightening of buildings in the modern architectural trends for achieving higher energy efficiency has affected the indoor air quality (IAQ) [1] for all buildings in general and hospital buildings in specific. Airborne micro-organisms, which are matter of great concern for public health, show variation in concentration with time, indoor as well as outdoor conditions and geographic location [2]. Various natural and anthropogenic sources and factors contribute towards high concentration buildup of micro-organisms in indoor air [3,4] e.g. biological (e.g. indoor plants), physical (e.g. temperature and humidity) and chemical factors (e.g. presence of airborne organic particulate matter) [5], outdoor sources, number of occupants [4–6]. Lack of the availability of an efficient mechanical Heating, ventilation and Air Conditioning (HVAC) control system, the indoor environment of the building is strongly influenced by the fluctuations in its surroundings thus making the scheduled monitoring of the indoor environment of sensitive buildings (e.g. hospital, schools, food courts etc.) essential [7]. In hospital environment, airborne microbial population is present in diverse range [8], where concentrations depend primarily on number and types of patients [9]. In addition, medical activity, cleaning frequency and cleaning procedures of hospitals [10], weather and ventilation rate [11] and building design [12] are also the decisive factors for airborne microbial concentration levels which combined to make the situation challenging to maintain satisfactory IAQ [10].

Studies reported an increasing trend of infections caused by airborne micro-organisms due to the recent concept of air-tight buildings [9]. Various health issues linked with the exposure of airborne micro-organisms are infectious diseases, toxic reactions [6], pneumonia, hypersensitivity, bronchitis [13], tiredness, headache [14], asthma, allergies [15], alveolitis [4], rhinitis [16] and hay fever etc. where severity of the symptoms being function of pathogenicity of micro-organisms, immune system of persons and environmental conditions [17]. In normal conditions, species of fungi are not supposed to cause

* Corresponding author.
E-mail address: mzalikhan@gmail.com (M. Zeeshan).

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any infection, but they are found to spread diseases in immunosuppressed patients of hospitals [11,12,18]. Although World health organization (WHO) showed concern towards indoor biological agents and building moisture [19], majority of countries have no clear regulations or proposed guidelines for acceptable concentrations of micro-organisms in indoor environments particularly [14].

Keeping in view the sensitivity of hospital buildings, due to the existence of airborne micro-organisms and patients with immune deficiencies, many researchers worked on airborne microbial indoor air quality of hospitals. The main focus of these studies were operation theatres [8,20], orthopedic ward [12], hematological units [18], intensive care unit (ICU), patient rooms, and neonatal wards [9]. Some of the studies also focused on seasonal variation of airborne microbes [11,18]. There are studies on seasonal variation of airborne micro-organisms which showed the dominancy of gram-positive bacteria in indoor air [3,21,22]. A few studies also reported the seasonal variation in fungal levels with no significant variation in bacterial levels [11,18].

Sensitivity and complexity of hospitals vary from one place to another. OTs are supposed to be the most sensitive places and require strict maintenance of acceptable levels of airborne micro-organisms. Similarly, other wards require control measures according to the sensitivity of patients present there. Most of the previous research work on microbial indoor air quality of hospitals has been focused on specific locations like OTs, ICUs and wards. However, very few studies have assessed the seasonal variation of airborne microbial load of different sections of a hospital. Thus, the aim of present study was to investigate airborne bacterial and fungal levels of different sites in a hospital along with their seasonal variations. For this purpose, six sites of a large publicly managed hospital of Islamabad, Pakistan was selected. The most dominantly observed bacterial and fungal colonies were identified up-to species level following the standard methods. Important factors contributing towards buildup of higher airborne microbial levels were identified so that the air quality may be managed more efficiently.

2. Methodology

2.1. Selected hospital information

An 1100-bed publicly managed hospital of Islamabad, Pakistan, founded in 1985, covering area of 5.1 ha, was selected for airborne microbial investigation during spring and winter seasons. Hospital was selected for the study as it consists of 32 specialized clinics with 12 critical units, catering for approximately 5000 patients per day with a variety of medical histories, making the environment extremely complex. Six sites were selected for the purpose of study which include (i) emergency operation theatre (OT1) (ii) general surgery operation theatre (OT2), (iii) surgical ward (SW), (iv) general medicine ward (GMW), (v) emergency services (ES) and (vi) out-patient department (OPD).

OT1 remains operational 24 h a day having 15–20 patients operated per day whereas in OT2, patients were operated from 8 h to 14 h, having average 8 patients operated per day. Both OTs were washed with disinfectants in morning and mopped with water before and after each surgery. Besides, periodic deep cleaning, including walls and ceiling was performed on need basis. Selected wards for study purpose had a capacity for 8 patients each with 2–3 attendants. ES remains operational 24 h while working hours for OPD were from 8 h to 14 h. Both locations had high occupation levels. Floors of both monitored wards, OPD and ES were mopped with disinfectants twice a day. Table 1 shows the complete description of monitored sites.

2.2. Sampling duration and frequency

Seasonal (winter and spring) assessment of airborne bacteria and fungus was performed covering one month of each season. Winter sampling covered month of January 2017 when average outdoor temperature and relative humidity, recorded from nearest weather station (33.62°N, 73.10°E), were 11 °C and 74% respectively while the spring sampling was carried out during April 2017 when average outdoor temperatures and relative humidity were 26 °C and 46.5% respectively.

Airborne microbial samples were collected twice a week during the peak hours of each sampling location using personal air sampler (Gilian 5000) operating at flow rate of 5 l/min for 10 min. To represent breathing zone, sampling height was kept at 1.5 m above ground. Cellulose nitrate filter paper (Sartorius, 13107-47-CAN) with a pore size of 0.45 μm and diameter 47 mm was used as a collecting medium for microbes. Tryptone soy agar (TSA) (OXOID CM0131) for bacterial colonies and potato dextrose agar (PDA) (OXOID CM0139) for fungal colonies, autoclaved at 121 °C for 15–20 min, were used as culture media for the sampled microbes. After sampling, filter papers were placed directly [23] on the respective growth medium on plates under sterile conditions. Plates were then sealed and transferred to laboratory where bacterial colonies were incubated at 37 °C for 24–48 h while fungal colonies were incubated at 28.5 °C for 3–5 days. Colonies obtained were then counted and expressed as CFU/m^3 [11,24]. Samples were collected in triplicate to ensure reproducibility of results and average values are reported.

2.3. Isolation and identification of bacteria and fungi

Airborne bacterial colonies obtained on TSA plates were initially separated on the basis of their morphological characteristics (shape, size, color) and then identified up-to the genus level on the basis of their microscopic appearance and results of biochemical tests. Microscopic appearance of bacterial colonies was observed under a microscope with oil immersion (1000× magnification) after gram-straining. Colonies were grouped in classes of gram-negative and gram-positive according to Bergey's Manual of determinative bacteriology [25]. Further biochemical characterization of bacterial colonies was then performed by modified oxidase test and catalase test. The most frequently observed colonies were then identified up-to the specie level by sequencing the amplified regions of extracted 16s ribosomal DNA gene with paired primers [26].

Airborne fungal colonies recovered on PDA plates were also initially classified morphologically by their spores' color and shape. Identification of dominant colonies up-to genus level was performed by preparing wet-mount slides using lacto-phenol blue and then observed under microscope of magnification 400×. Fungi were then identified according to their microscopic appearance.

2.4. Statistical analysis

The data was analyzed in MS Excel (Microsoft Corporation, USA) and SPSS 14 (IBM Corp., USA). Normality of data was checked by Kolmogrov-Smirnov test and Shapiro-Wilk test. One-Way ANOVA was used to analyze the statistical difference among different sampling sites and *t*-test was used to analyze the statistical difference between observations of two seasons for same site.

3. Results and discussion

3.1. Airborne bacterial and fungal concentrations

Indoor bacterial concentration in a hospital is supposed to be affected by the type and number of patients in that particular area. Moreover, indoor fungal concentration depends on the indoor moisture conditions, cleaning frequency and outdoor atmospheric conditions of that particular area. Table 2 shows descriptive statistics of indoor concentration of airborne bacteria and fungus in six sites of the hospital.

Results showed higher range and mean values of airborne bacteria