

ASSESSMENT OF MICROBIAL LOAD IN INDOOR ENVIRONMENT OF UNIVERSITY AND HOSPITALS OF HAIL, KSAs

Mohd A. Kausar*, Jamal M.Arif, Sultan M.M.Alanazi, Aqeel M. A.Alshmmry, Yazid A. A.Alzapni, Fahad K. B. Alanazy, Syed M. A. Shahid and Ashfaque Hossain¹

Department of Biochemistry, College of Medicine, University of Hail, Hail, Kingdom of Saudi Arabia.

¹Department of Pathology, College of Medicine, University of Hail, Hail, Kingdom of Saudi Arabia.

*e-mail: adnankausar1@gmail.com

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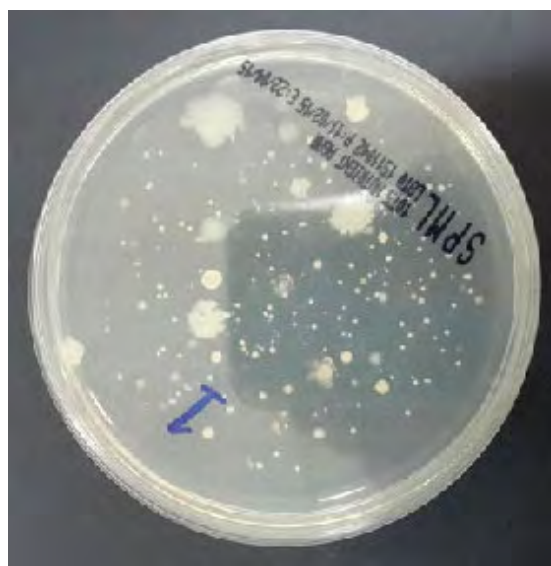
ABSTRACT : Indoor air may contain pathogenic and non-pathogenic live or dead bacteria, fungi, viruses, allergens, endotoxins, pollen, plant fibres, etc. Microorganisms (bacteria and fungi) are considered significant part of the indoor air as causal agents of respiratory disorder and lung dysfunction in occupational (indoor) and non-occupational environment (Douwes et al, 2003). The measurement of microbial load in indoor environment samples is of great importance due to their potential negative impact on occupational safety & health. The present study was undertaken to isolate and identify different bacteria present in the indoor environment of different colleges of University of Hail, Hail, KSA and hospital of Hail, KSA. Air samples were collected using Spin Air 5500 (Air sampler, IUL Instruments, Barcelona, Spain) on bacteriological culture media (Nutrient gar plate and chocolate agar plate) from the different location (ICU, OPD, Laboratory, office) of King Khalid Hospital, Hail and Some colleges of University of Hail, Hail, KSA. Media plates were incubated at 37°C overnight. For identification of microbes, microbial colonies obtained from air samples were sent to Molecular Diagnostics and Personalized Therapeutics Unit (MPTU), College of Applied Medical Sciences, University of Hail. Hail, KSA. By using MALDI-TOF Biotyper, we identified the following microbes in the air samples of different locations of Hail: *Bacillus pumilus*, *Staphylococcus hominis*, *Exiguabacterium aurantiacum*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Staphylococcus haemolyticus*, *Lactococcus lactis*, *Escherichia coli*, *Penicillium*, and *Aspergillus* species, *Staphylococcus epidermidis*, , *Staphylococcus hominis*, *Acinetobacter pitti*, *Bacillus simplex*, *Staphylococcus warneri*, *Staphylococcus pasteur*, *Bacillus mojavensis*, *Staphylococcus capitis*, *Exiguabacterium aurantiacum*, *Corynebacterium efficiens*, *Aspergillus* species. Both pathogenic and nonpathogenic bacteria were present in the indoor air of the hospitals. Patients, staff and doctors in hospitals are exposed to different airborne bacteria, which may lead to variety of infections including respiratory diseases.

Key words : Indoor Environment, Bio-contamination, MALDI-TOF.

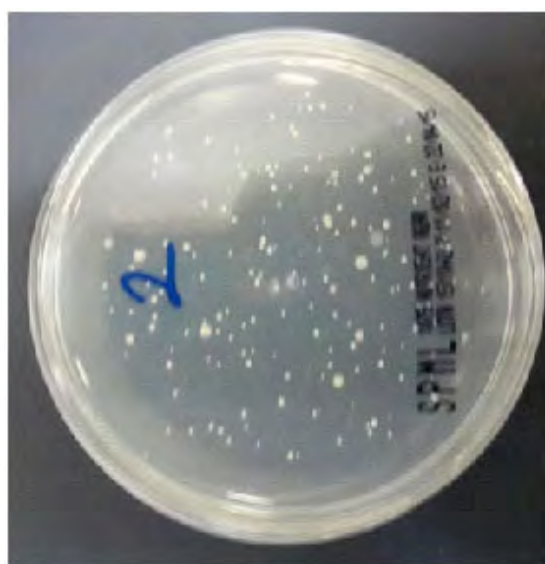
INTRODUCTION

In our daily life, we are working, meeting, living, inside buildings which are mostly closed one and the microorganisms are living with us their too! which can leading from serious infections to seasonal influenza (Molhave, 2011; Nakayama and Morimoto, 2007). Presence of microorganisms (bacteria, moulds and viruses) in indoor environment is one of the problems of indoor air quality and people spends 80%-90% of their time in indoors environments by breathing on average 14 m³ of air per day (Hayleeyesus and Manaye, 2014). During a breathing and sneezing, millions of droplet of water and mucus which contain many micro-organism, makes people highly exposed to indoor air environments and causes different types of infection to human being. It is bluster the individuals health microbes are cause of respiratory disease of humans, causing allergies, asthma and pathogenic infection of respiratory tract (Adgate *et al*, 2008).

Evidence of adverse effects of indoors and outdoors air pollution on human health substantially increased in recent years (Brunekreef and Holgate 2002; Pope and Dockery, 2006). Indoor environments are beset with various biocontaminant such as bacteria, bacterial endotoxins, fungi, fungal spores, mycotoxins, viruses, algae and parasites, each adversely affecting the health of the human health. Biocontamination is an ongoing process and with modern, air-tight construction and air recirculation systems, these contaminants have nowhere to escape. World Health Organization (WHO) suggested that poor indoor air quality associated with various symptoms of malaise which includes; eye, nose and throat irritation; sensation of dry mucous membranes; headache; high frequency of airway infection and cough; wheezing, itching and unspecific hypersensitivity; nausea, dizziness; asthma; breathing difficulties; chest infections; colds; cough etc. (WHO, 2009). It is therefore important to assess the indoor air quality which adversely effects on human health (Bakke *et al*, 2008).



Site-1



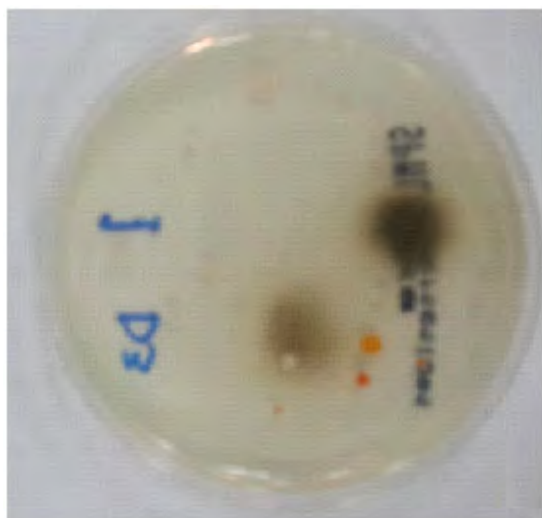
Site-2



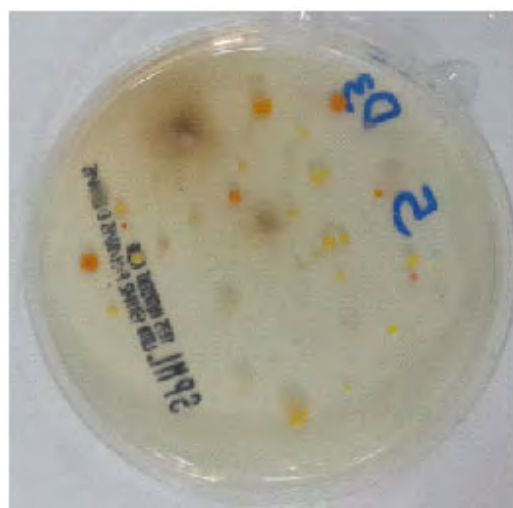
Site-3



Site-4

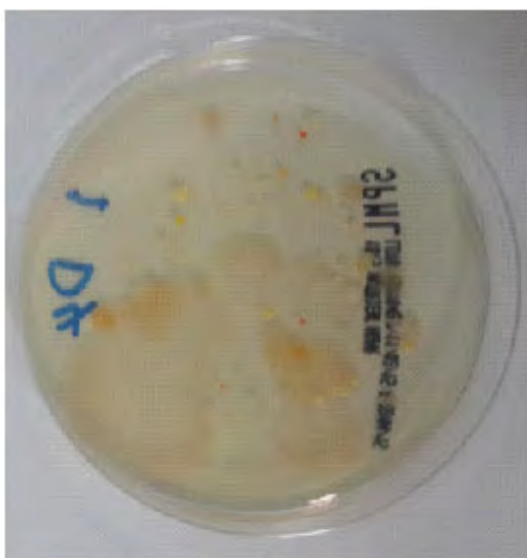


Site-5



Site-6

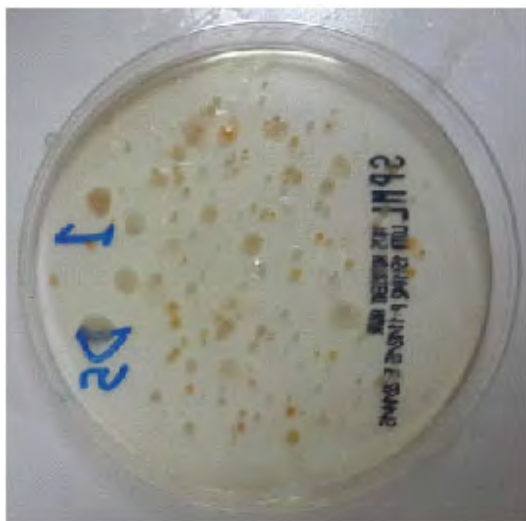
Fig. 1 : Representative plates of microbiological colony obtained from Air samples of different site (site 1-6).



Site-7



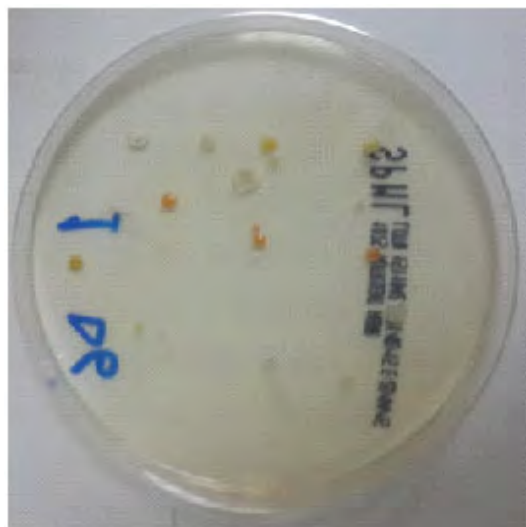
Site-8



Site-9



Site-10



Site-11



Site-12

Fig. 1 : Representative plates of microbiological colony obtained from Air samples of different site (site 7-12).

Almost one billion people, mostly women and children, are exposed to levels of indoor air pollution that exceed the World Health Organization guidelines. The highest exposures occur in the indoor environments of developing countries mainly from cooking and heating activities, which use solid fuels, like wood, dung, crop residue, charcoal etc. Inefficient stoves and poor ventilation in rural homes further aggravate this situation. For example in India, where 80% of the households use solid fuels, there are estimates that a half a million children die annually from indoor air pollution (Balakrishnan *et al* 2011). Nearly three fifths of the total global exposure to particulate matter occurs in the rural areas of developing countries. On a global scale this amounts to three million deaths a year from indoor air. While ambient air quality has been a matter of debate and concern in the developing world, very little attention has been paid to indoor air quality in Saudi Arabia. It should be noted that the country faces other pressing health issues that compete for both resources and manpower.

Inside the university and hospitals students, doctors and staff they stay a long time in the classes, libraries, laboratories and office and they are exposed to many microorganisms in indoor environment (Haleem and Mohan, 2012). They inhale air and some microbes every moment. Even most of the microbes present in air are harmless but there is less than 1% of the airborne bacteria is serious and pathogenic. Indoor air has more chances of infections especially in labs and in small classes with large number student.

Therefore, this study to see the indoor air microbial load inside the hospital and University of Hail, Hail in Saudi Arabia. The result of this study are helpful to improved environmental control of microorganism and reduced the health risk to individuals.

METHODS

Sampling site

The study was conducted from October to December, 2015 in different colleges of Hail University and different wards King Khalid Hospital Hail. Hail University has capacity of 37000 students and a total of more than 5000 staffs and more than 50 buildings. The samples were collected from King Khalid hospital (ICU, OPD and Laboratory area etc) and ten buildings of University of Hail *viz.* college of medicine, science medical college, nursing, college of health information, college of pharmacy, college of business, college of computer engineering, central library and college of science and its library.

Sampling procedure

Air samples and swab samples were collected using Spin Air 5500 (Air sampler, IUL Instruments, Barcelona, Spain) on 60 culture media plates (Nutrient agar, Blood Agar and chocolate agar plates) from the different location of King Khalid Hospital and different Colleges of University of Hail, Hail, KSA. The sampling height which approximated to human breathing zone was 1.5 m above the floor and at the center of the room. To obtain the appropriate surface density for counting and to determine the load with respect to time of exposure, the sampling times were determined on the basis of room size. Moreover, samples were collected every Tuesday from 10:00 AM to 12:00 Noon. After exposure, the sample were taken to the laboratory (Department of Biochemistry, College of Medicine, University of Hail) and incubated at 37°C for 24 hours for bacteria and at 25 °C for 3 days for fungi.

For identification of microbial colonies obtained from air samples were sent to Molecular Diagnostics and Personalized Therapeutics Unit (MPTU), College of Applied Medical Sciences, University of Hail. Hail, KSA. Microbiological Colonies were identified MALDI-TOF Biotyper.

RESULTS

By using MALDI-TOF Biotyper, we identified the following microbes in the air samples of different location such as King Khalid hospital and University of Hail, Hail, KSA *Bacillus pumilus*, *Staphylococcus hominis*, *Exiguabacterium aurantiacum*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Staphylococcus haemolyticus*, *Lactococcus lactis*, *Escherichia coli*, *Penicillium* and *Aspergillus* species, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Acinetobacter pittii*, *Bacillus simplex*, *Staphylococcus warneri*, *Staphylococcus pasteur*, *Bacillus mojavensis*, *Staphylococcus capitis*, *Exiguabacterium aurantiacum*, *Corynebacterium efficiens*, *Aspergillus* species. The details of sample sites and types of microbes identified were described in Tables 1 and 2. Representative media plates of microbiological colony obtained from samples of different site were presented in Figure 1.

DISCUSSION

In the present study, we demonstrated considerable numbers of microorganism in the air of the King Khalid Hospital and different Colleges of University of Hail, Hail, KSA. This is the first study carried out in King Khalid Hospital comparing the microbial contamination of air in the ICU, OPD and Laboratory area etc and University

Table 1 : Sample sites and types of microbes identified.

S. No	Date	Place	Type of Sample	Microbes
1	13 Oct 2015	OPD, King Khalid Hospital, Hail, KSA	Indoor environment (Air sample)	<i>Bacillus pumilus</i>
2	13 Oct 2015	ICU, King Khalid Hospital, Hail, KSA	Indoor environment (Air sample)	<i>Staphylococcus hominis</i>
3	13 Oct 2015	Laboratory, King Khalid Hospital, Hail, KSA	Indoor environment (Air sample)	<i>Exiguabacterium aurantiacum</i>
4	20 Oct 2015	Office, King Khalid Hospital, Hail, KSA	Indoor environment (Air sample)	<i>Acinetobacter baumannii</i> ,
5	20 Oct 2015	King Khalid Hospital, Hail, KSA	Indoor environment (Air sample)	<i>Enterobacter cloacae</i>
6	20 Oct 2015	King Khalid Hospital, Hail, KSA	Indoor environment (Air sample)	<i>Staphylococcus haemolyticus</i> ,
7	20 Oct 2015	King Khalid Hospital, Hail, KSA	Indoor environment (Air sample)	<i>Lactococcus lactis</i>
8	20 Oct 2015	King Khalid Hospital, Hail, KSA	Indoor environment (Air sample)	<i>Escherichia coli</i>
6	15 Dec 15	University of Hail, Hail, KSA	Surface swab	Not Reliable Identification
7	4 Dec 15	University of Hail, Hail, KSA	Surface swab	<i>Staphylococcus epidermidis</i>
8	9 Dec 15	King Khalid Hospital, Hail, KSA	Surface swab	<i>Staphylococcus pateuri</i> <i>Staphylococcus epidermidis</i>
9	9 Dec 15	Dialysis Center, King Khalid Hospital, Hail, KSA	Surface swab	Not Reliable Identification
10	9 Dec 15	Patient Bed, Hospital King Khalid Hospital, Hail, KSA	Surface swab	<i>Bacillus mojavensis</i> <i>Staphylococcus warneri</i>
11.	8 Dec 15	College of Business, University of Hail, Hail, KSA	Surface swab	<i>Staphylococcus epidermidis</i>
12.	8 Dec 15	College of Business, University of Hail, Hail, KSA	Surface swab	Not Reliable Identification
13.	20 Dec 15	College of Nursing, University of Hail, Hail, KSA	Surface swab	<i>Staphylococcus hominis</i>
14.	24 Dec 15	College of Health, University of Hail, Hail, KSA	Surface swab	Not Reliable Identification
15.	01 Dec 15	College of Science, University of Hail, Hail, KSA	Surface swab	<i>Acinetobacter pittii</i>
16.	01 Dec 15	College of Science, University of Hail, Hail, KSA	Surface swab	<i>Staphylococcus hominis</i>
17.	01 Dec 15	College of Science, University of Hail, Hail, KSA	Surface swab	Not Reliable Identification
18	08 Dec 15	College of Business, University of Hail, Hail, KSA	Surface swab	Not Reliable Identification
19	08 Dec 15	College of computer engineering, University of Hail, Hail, KSA	Surface swab	Not Reliable Identification
20	08 Dec 15	College of computer engineering, University of Hail, Hail, KSA	Surface swab	Not Reliable Identification
21	15 Sept 2015	College of science, University of Hail, Hail, KSA	Surface swab	Not Reliable Identification
22	9 Dec 15	Pediatric ward, King Khalid Hospital, Hail, KSA	Surface swab	Not Reliable Identification
23	9 Dec 15	Teaching center, King Khalid Hospital, Hail, KSA	Surface swab	Not Reliable Identification
24	24 Nov 2015	College of health information, University of Hail, Hail, KSA	Indoor environment (Air sampler)	<i>Staphylococcus hominis</i>
25.	24 Nov 2015	College pharmacy, University of Hail, Hail, KSA	Indoor environment (Air sampler)	Not Reliable Identification
26.	10 Nov 15	College of Medicine, University of Hail, Hail, KSA	Surface swab	Not Reliable Identification
27.	10 Nov 15	College of Medicine, University of Hail, Hail, KSA	Surface swab	<i>Staphylococcus hominis</i>
28.	10 Nov. 15	College of Medicine, University of Hail, Hail, KSA	Surface swab	Not Reliable Identification
29.	10 Nov 15	College of Medicine, University of Hail, Hail, KSA	Surface swab	Not Reliable Identification
30.	8 Nov 15	Computer Science, University of Hail, Hail, KSA	Indoor environment (Air sample)	Not Reliable Identification
31.	15 Nov 15	Library, University of Hail, Hail, KSA	Surface Swab	Not Reliable Identification
25.	8 Dec 15	College of computer and engineering	Surface Swab	Not Reliable Identification

Table 1 continued...

Table 1 continued...

26.	8 Dec 15	Central Library, University of Hail, Hail, KSA	Surface Swab	<i>Staphylococcus warneri</i>
27.	15 Dec 15	Central library, University of Hail, Hail, KSA	Surface Swab	<i>Exiguabacterium aurantiacum</i>
28.	15 Sept 15	Library, College of Science, University of Hail, Hail, KSA	Surface Swab	Not Reliable Identification
29.	27 Oct 15	College of Medicine, University of Hail, Hail, KSA	Surface Swab	<i>Bacillus simplex</i>
30.	27 Oct 15	College of Medicine, University of Hail, Hail, KSA	Surface Swab	<i>Exiguabacterium aurantiacum</i>
31.	27 Oct 15	College of Medicine, University of Hail, Hail, KSA	Surface Swab	<i>Staphylococcus epidermidis</i>
32.	24 Nov 15	College of Health, University of Hail, Hail, KSA	Surface Swab	Not Reliable Identification
33.	24 Nov 15	College of pharmacy, University of Hail, Hail, KSA	Surface Swab	<i>Bacillus endophyticus</i>
34.	15 Dec 15	Central Library, University of Hail, Hail, KSA	Surface Swab	<i>Staphylococcus haemolyticus</i>
35.	15 Dec 15	Central Library, University of Hail, Hail, KSA	Surface Swab	Not Reliable Identification
36.	15 Dec 15	Central Library, University of Hail, Hail, KSA	Surface Swab	<i>Corynebacterium efficiens</i>
37.	15 Dec 15	Central Library, University of Hail, Hail, KSA	Surface Swab	Not Reliable Identification
38.	15 Dec 15	Central Library, University of Hail, Hail, KSA	Surface Swab	Not Reliable Identification

Table 2 : List of Microorganism.

S.No	Microorganism
1.	<i>Bacillus pumilus</i> ,
2.	<i>Staphylococcus hominis</i> ,
3.	<i>Exiguabacterium aurantiacum</i> ,
4.	<i>Acinetobacter baumannii</i> ,
5.	<i>Enterobacter cloacae</i> ,
6.	<i>Staphylococcus haemolyticus</i> ,
7.	<i>Lactococcus lactis</i>
8.	<i>Staphylococcus epidermidis</i> ,
9.	<i>Staphylococcus hominis</i>
10.	<i>Acinetobacter pittii</i>
11.	<i>Bacillus Simplex</i>
12.	<i>Staphylococcus warneri</i>
13.	<i>Staphylococcus pasteurii</i>
14.	<i>Bacillus mojavensis</i>
15.	<i>Staphylococcus capitis</i>
16.	<i>Exiguabacterium aurantiacum</i>
17.	<i>Corynebacterium efficiens</i>
18.	<i>Aspergillus</i>

of Hail, KSA. We performed the study in October to December 2015. In the literature there is clear evidence of seasonal differences in the numbers of microbes in indoor air. For example, Lumpkins *et al* (1976) reported that numbers were higher in summer and autumn than in spring and winter. King Khalid Hospital and different Colleges of University of Hail, Hail, KSA, we detected the following microorganism.

Bacillus pumilus, *Staphylococcus hominis*, *Exiguabacterium aurantiacum*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Staphylococcus*

haemolyticus, *Lactococcus lactis*, *Escherichia coli*, *Penicillium* and *Aspergillus* species, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Acinetobacter pittii*, *Bacillus simplex*, *Staphylococcus warneri*, *Staphylococcus pasteurii*, *Bacillus mojavensis*, *Staphylococcus capitis*, *Exiguabacterium aurantiacum*, *Corynebacterium efficiens*, *Aspergillus* species isolated from air and swab samples.

In contrast, in the present study we found significant number of *Staphylococcus* species. Air microbial load was lower in winter and higher in summer and autumn but seldom above acceptable levels (Panagopoulou *et al* 2002). *Staphylococcus hominis* was the most prevalent species in the air of all the hospitals followed by *Staphylococcus epidermidis*. The least microbial load were detected in the College of Medicine, University of Hail, whilst most microbial load were found in King Khalid hospital, Hail, KSA. Our findings, suggest that hospitals have more microbial load due presence of various types of patients. are accordance with these findings, in the present study we also detected high number of *Staphylococcus* species. No correlation between microbial species, season, hospital or departments was observed.

The presence of *Staphylococcus*, may pose a potential threat to the health of patients of these rooms [Hahn *et al*, 2002; Menotti *et al*, 2005; Hay *et al*, 1995]. Bacteria in these and other genera affect humans in complex ways and are capable of causing a variety of diseases, such as infection, allergy and irritation, and toxicosis. Exposure to microorganism has been unequivocally associated with exacerbation of asthma.

Bacteria, especially *Staphylococcus*, can cause devastating infections in these high-risk patients (Hay *et al*, 1995; Blum *et al*, 2008; Alhambra *et al*, 2008). Moreover, surface contamination with settled microbes could also present a source of potential health risk (Blum *et al*, 2008). In our study, we found a high occurrence of microorganism in the air and settle dust in the King Khalid Hospital and different Colleges of University of Hail, Hail, KSA.

CONCLUSION

Both pathogenic and nonpathogenic bacteria were present in the indoor air of the hospital, Colleges of Hail University, Hail. Patients, staff and doctors in hospitals are exposed to different airborne bacteria, which may lead to variety of infections including respiratory diseases. Diagnostic tests should be developed and recommended to determine the nature of building-related illness, e.g. allergy, hypersensitivity pneumonitis, encephalopathy, fungal infections, bacterial infection, etc. The medical profession must recognize the importance of immediate removal of occupants from the toxic environment. Government agencies and medical universities need to increase research to continue to further solidify knowledge regarding health impacts that multi biocontaminants have on human and animal occupants. Increased awareness of the potential health hazards of indoor biocontaminants is the first step in managing – and ultimately reducing – the illnesses they induce.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

There are no financial/commercial conflicts of interest.

REFERENCES

- Adgate J L, Ramachandran G, Cho S J, Ryan A D and Grengs J (2008) Allergen levels in inner city homes: baseline concentrations and evaluation of intervention effectiveness. *J>f Exposure Analysis and Environmental Epidemiology* **18**, 430–440.
- Alhambra A, Catalán M, Moragues M D, Brena S, Pontón J, Montejo J C and del Palacio A (2008) Isolation of *Aspergillus lentulus* in Spain from a critically ill patient with chronic obstructive pulmonary disease. *Rev. Iberoam. Micol.* **25**(4), 246–9.
- Bakke J V, Norback D, Wieslander G, Hollund B E, Florvaag E, Haugen, E N and Moen B E (2008) Symptoms, complaints, ocular and nasal physiological signs in university staff in relation to indoor environment – temperature and gender interactions. *Indoor Air* **18**, 131–143.
- Balakrishnan K, Ramaswamy P, Sambandam S, Thangavel G, Ghosh S, Johnson P, Mukhopadhyay K, Venugopal V and Thanasekaraan V (2011) Air pollution from household solid fuel combustion in India: an overview of exposure and health related information to inform health research priorities. *Glob. Health Action* **4**.
- Blum G, Perkhof S, Grif K, Mayr A, Kropshofer G, Nachbaur D, Kafka-Ritsch R, Dierich M P and Lass-Flörl C (2008) A 1-year *Aspergillus terreus* surveillance study at the University Hospital of Innsbruck : molecular typing of environmental and clinical isolates. *Clin. Microbiol. Infect.* **14**(12), 1146–51.
- Brunekreef B and Holgate S T (2002) Air pollution and health. *Lancet* **360**, 1233–1242.
- Douwes J, Thorne P, Pearce N and Heederik D (2003) Bioaerosol health effects and exposure assessment : progress and prospects. *Ann. Occup. Hyg.* **47**(3), 187–20
- Hahn T, Cummings K M, Michalek A M, Lipman B J, Segal B H and McCarthy P L Jr. (2002) Efficacy of high-efficiency particulate air filtration in preventing aspergillosis in immunocompromised patients with hematologic malignancies. *Infect Control Hosp. Epidemiol.* **23**(9), 525–31.
- Haleem Khan A A and Mohan Karuppaiyil S (2012) Fungal pollution of indoor environments and its management. *Saudi J. Biol. Sci.* **19**(4), 405–26.
- Hay R J, Clayton Y M and Goodley J M (1995) Fungal aerobiology: how, when and where? *J. Hosp. Infect.* **30** Suppl, 352–7.
- Hayleeyesus S F and Manaye A M (2014) Microbiological quality of indoor air in university libraries. *Asian Pac. J. Trop. Biomed.* **4**(Suppl 1), S312–7.
- Lumpkins E D Sr and Corbit S (1976) Airborne fungi survey: II. Culture plate survey of the home environment. *Ann. Allergy* **36**(1), 40–4.
- Menotti J, Waller J, Meunier O, Letscher-Bru V, Herbrecht R and Candolfi E (2005) Epidemiological study of invasive pulmonary aspergillosis in a haematology unit by molecular typing of environmental and patient isolates of *Aspergillus fumigatus*. *J. Hosp. Infect.* **60**(1), 61–8.
- Molhave L (2011) Sick building syndrome. *Encyclopedia of Environmental Health*, 61–67.
- Nakayama K and Morimoto K (2007) Relationship between, lifestyle, mold and sick building syndromes in newly built dwellings in Japan. *Int. J. Immunopathology Pharmacol.* **20**, 35–43.
- Panagopoulou P, Filioti J, Petrikos G, Giakouppi P, Anatoliotaki M, Farmaki E, Kanta A, Apostolakou H, Avlami A, Samonis G and Roilides E (2002) Environmental surveillance of filamentous fungi in three tertiary care hospitals in Greece. *J. Hosp. Infect.* **52**(3), 185–91.
- Pope C A and Dockery D W (2006) Health effects of fine particulate air pollution: lines that connect. *J Air Waste Manag Assoc.* **56**, 709–742.
- WHO Guidelines (2009) for *Indoor Air Quality: Dampness and Mould*. World Health Organization Regional Office for Europe: Copenhagen, Denmark.