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REDUCING AIR AND SOIL POLLUTION IN CHINA: ISSUES IN ENVIRONMENTAL TECHNOLOGIES AND MANAGEMENT

The effect of temperature on airborne filamentous fungi in the indoor and outdoor space of a hospital

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Abstract

Fungi are one of the bioaerosols in indoor air of hospitals. They have adverse effects on staff and patients. The aim of this study was to investigate the effects of three incubation temperature on the density and composition of airborne fungi in an indoor and outdoor space of hospital. Sabouraud dextrose agar was used for culture the fungi. For improvement of aseptic properties, chloramphenicol was added to this medium. The density of airborne fungi was less than 282 CFU/m^3. The highest density was detected in emergency room and the lowest of them was in neonatal intensive care unit (NICU) and operation room (OR). Results showed that fungi levels at 25 °C were higher than 37 and 15 °C ($p = 0.006$). In addition, ten different genera of fungi were identified in all departments. The predominant fungi were *Fusarium* spp., *Penicillium* spp., *Paecilomyces* spp., and *Aspergillus niger*. Moreover, the density and trend of distribution of *Fusaruim* spp. in the indoor space was directivity to outdoor space by ventilation system. The present study has provided that incubation temperature had effect on airborne fungi remarkably. We are suggested that more studies would be conducted on incubation temperature and other ambient factors on airborne fungi.

Keywords Fungi · Temperature · Incubation · Hospital · Shiraz

Introduction

Indoor air quality (IAQ) is a critical factor in indoor space (Azimi et al. 2013) because the improper IAQ may lead to infections, hospital syndrome, and various occupational risks (Wan et al. 2011). Density of bioaerosols including bacteria, fungi, viruses, and pollens is known as an important factor that influence on IAQ (Naddafi et al. 2011). Their high density is infectious, allergenic, and toxic (Mandal and Brandl 2011). Among different types of bioaerosols, fungi plays an important role in human health (Hoseini et al. 2013), especially in hospital environments where patients are vulnerable to these infections (Weaver et al. 2010). Therefore, the monitoring of them is essential. Various factors such as the relative humidity, temperature, intensity of UV and visible radiation, and wind speed influence on the airborne fungi. Those moisture, nutrients, and temperature are the most important factors (Haleem Khan and Mohan Karuppayil 2012). Since the temperature in buildings is usually 20–25 °C, the growth of mesophilic fungi will be promoted. Moreover, the conidia germinate at temperatures between 12 and 37 °C (Piontek et al. 2016). Those *Aspergillus*, *Cladosporium*, and *Penicillium* were the most common isolated at 27 °C for 3 to 7 days (Hedayati et al. 2011). However, the temperature less than optimum level caves a decrease in growth of fungi (Haleem Khan and Mohan Karuppayil 2012). Moreover, in summer, the relative humidity (70–80%) and temperature (12–15 °C) promote bioaerosol survival and higher emissions (Szyłak-Szydłowski et al. 2016).

Various studies have investigated the fungal air quality in hospital environments and other occupational works. In these studies, the average density of airborne fungi was 19 ± 19 CFU/m^3 (Perdelli et al. 2006), 5.5–10.6 CFU/m^3 (Panagopoulou et al. 2002), and 0–319 CFU/m^3 (Li and Hou 2003). But in other space such as composting plant,

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airborne fungi was very high (4000–5000 CFU/m^3) (Abbasi et al. 2016). In other studies, the effect of sampling method and temperature, and relative humidity (Hwang et al. 2016) on airborne fungi in air of animal facility was investigated (Nieguitsila et al. 2011). In general, fungi can be useful indicator for indoor air quality (Cabral 2010).

The fungal growth is attributed to the variations in the relative humidity, ambient temperature, and others (Tanaka et al. 2016). The optimum temperature for growth, activation, and survival of any species is different (Piontek et al. 2016). Moreover, there have not been many studies concerned to the effects of environmental factors such as temperature on airborne fungi. Moreover, the relative importance of incubation temperature on bioaerosols is poorly characterized. In the present study, we proposed a hospital offering childcare and pregnant women services. Moreover, there is not appropriate data from air quality and indoor exposure to airborne filamentous fungi has an increased risk of developing asthma in infants and asthma morbidity in individuals who have asthma (Baxi et al. 2016). Even some of fungi were identified as non-pathogenic, they have high risk of several pathogenic diseases such as mycotoxicosis and ear, urinary, nail, and eye infections in hospital environments (Aquino et al. 2013).

With regard to this situation, this study aimed to determine the fungal density level and identify the fungal species in different incubation temperatures in indoor and outdoor of this hospital.

Materials and methods

Selection of sampling location

This study was a cross-sectional study in one of Shiraz educational hospitals, Iran. This hospital specialized for children and mothers. It was conducted between Novembers until mid-December, 2015. Air samples were taken from the emergency room, biomedical laboratory, Obstetrics (OB), Maternity ward, NICU, very important person (VIP) department, children surgery, OR, radiology, children recovery, Gynaecology (GYN), and hospital outdoor space. This hospital is located on the mountain hillside (Fig. 1).

Sampling and measurement equipment

Air sampling was conducted at the respiratory height of about 1.5 m above the ground (Abbasi et al. 2016). Measurements were taken between 9 am and 3 pm. Samples were taken from various indoor and outdoor spaces. Anderson single-step model (10-710) with a flux of 28.3 L/min was used to sample airborne fungi. Duration of sampling in each space was 10 min. Before each sampling, the inside of the sampler was disinfected with 70% alcohol. During sampling period, temperature was measured by a thermometer. All materials purchased from Merck (Germany). All experiments performed thrice.

Isolation and identification of fungal flora

For fungal culture, Sabouraud dextrose agar (SDA) (Merck, Germany) was used. For improvement of the aseptic properties of media, the chloramphenicol (0.2 g/L) was used (Chang et al. 2014; Rangaswamy et al. 2013) because it restricts bacterial growth. After air sampling, the culture media were immediately carried to the laboratory and were cultured in an incubator for 3–7 days at 15 °C (sychrophill species) then at 25 °C (mesophilic species) and finally at 37 °C (thermotolerant species) for fungi. The density of airborne fungi was calculated by dividing the value obtained from counting the colonies formed on the culture medium after the process of culturing (CFU) per air volume (m^3). Airborne fungal genus was identified according to slide culture.

Description of slide culture

Identification of fungal species is done by several methods. One of the best methods is slide culture, because the application of this method is inexpensive and easy. This technique was described by Riddell (1950) and it is the best method for observing sporulation. In this method, an agar media such as Sabouroud dextrose agar was transferred to a glass slide and placed in a wet chamber. Moisture is provided by water layer in chamber (Riddell 1950). The schematic of slide culture was shown in Fig. 2.

The steps of this technique include the following: (1) cutting out a piece of 1.5 × 1.5 cm from Sabouroud dextrose agar and placing it onto the sterilized glass slides, (2) inoculation of agar media with a loopful of fungal conidia, (3) placing a sterile cover slip onto their surface of media, and (4) placing the prepared slide glass in a chamber containing the water layer. After their incubation, the fungal species was identified by microscopic method (model of dark field).

Statistical analysis

In this study, data were analyzed with Kruskal–Wallis test and statistical significance with Mann–Whitney *U* test by SPSS version 19 to evaluate the relationship between temperature and the density of airborne fungi (as CFU/m^3). A significance level of $p < 0.05$ was used.

Results and discussion

Comparison of fungi in difference incubation temperature

In this study, the average density of airborne fungi was compared at three incubation temperatures (Fig. 2). The mean of

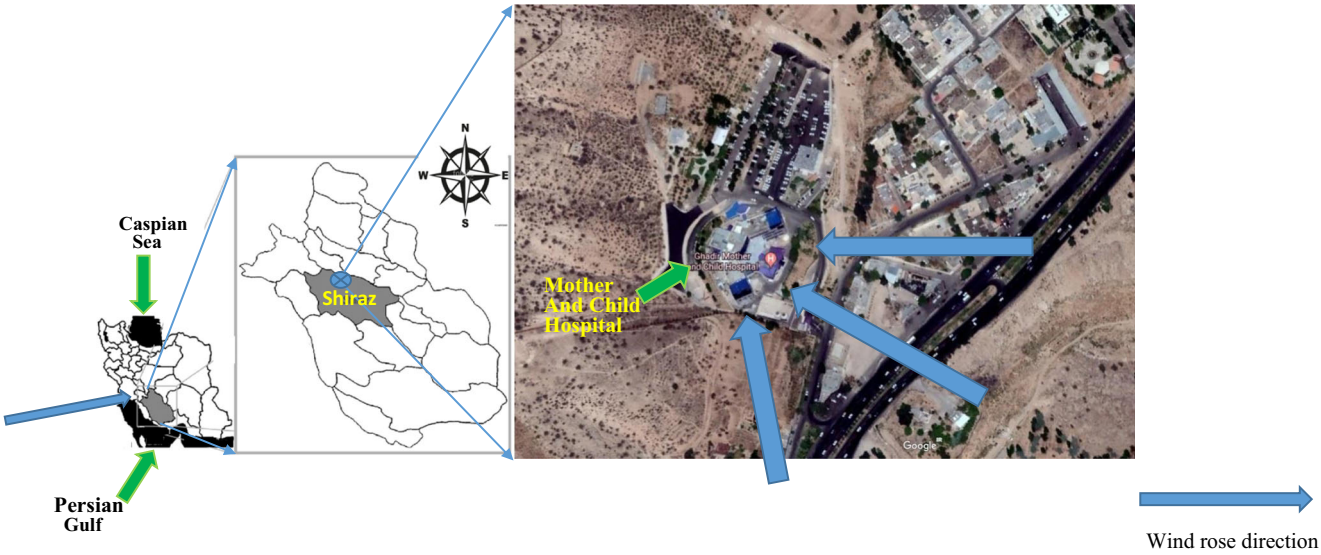

Fig. 1 Location map of hospital

airborne fungal density was 90, 113, and 24 CFU/m^3 at 15, 25, and 37 °C, respectively, in the indoor hospital space respectively that this difference was significant ($p = 0.006$). The highest density was in the emergency room whereas the lowest was attributed to operating rooms (without operation) at 15 °C (17 CFU/m^3). At 25 °C, the highest was emergency room and the lowest was VIP (22 CFU/m^3). But at 37 °C, the surgical child room and NICU and OR had the highest and the lowest density, respectively. Therefore, the trend of effective temperature on fungal growth was varied in any department and both incubation temperature and nature of department can be effective. But the effect of incubation temperature on airborne fungi was investigated rarely.

Furthermore, the highest density was detected at 25 °C (Fig. 2). In other facilities such as poultry farmhouse, the density of fungi under incubation at 25 °C was more than at 37 °C (Nieguitsila et al. 2011), because they are mesophilic genera and the growth rate of bioaerosol was higher at mild temperature (Soleimani et al. 2016).

Although the results of this study was lower than guidelines such as ACGIH, NIOSH and EU (500 CFU/m^3 for total fungi or *A. fumigatus*), and IRSST (2000 CFU/m^3) (Abbasi et al. 2016), the risk assessment of this hospital is essential. Moreover, the investigation of other conditions such as incubation temperature, sampling methods, and media type can improve the content of similar studies.

Results of the present study compare the density and species of airborne fungi at specific temperature (Fig. 3). As shown in Fig. 3, 52.8% of the total airborne fungi was *Fusarium* (1234.844 CFU/m^3). Moreover, their density of was 106.06 CFU/m^3 in the emergency room at 15 °C and 151.515 CFU/m^3 at 25 °C, but the density of *A. niger* was 26.515 CFU/m^3 in children's surgical at 37 °C.

Predominant airborne fungal species in each incubation temperature was different, so that *Fusarium is* 63% and *Penicillium* is 18% at 15 °C and they were 66 and 15% at 25 °C but at 37 °C, the predominant was *A. niger* (47%) and *A. flavus* (21%). Moreover, there were significant difference among the density of predominant fungal genera at all temperatures ($p = 0.001$). In previous studies, the density of fungi usually was determined in limit incubation temperature and the result of them was dissimilar, so that the density of the

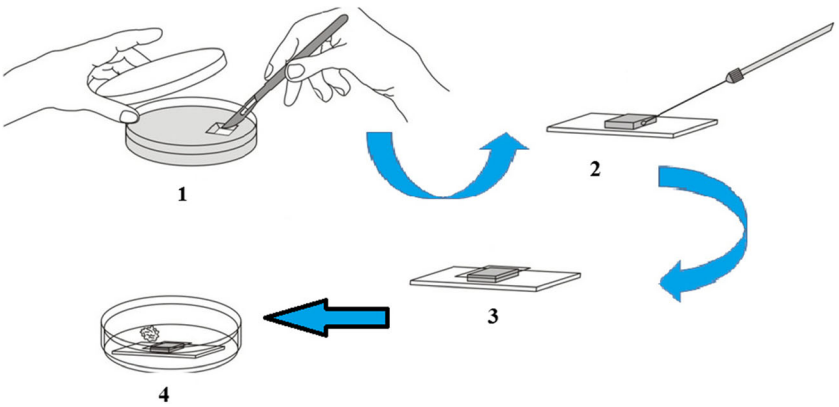

Fig. 2 The schematic of slide culture

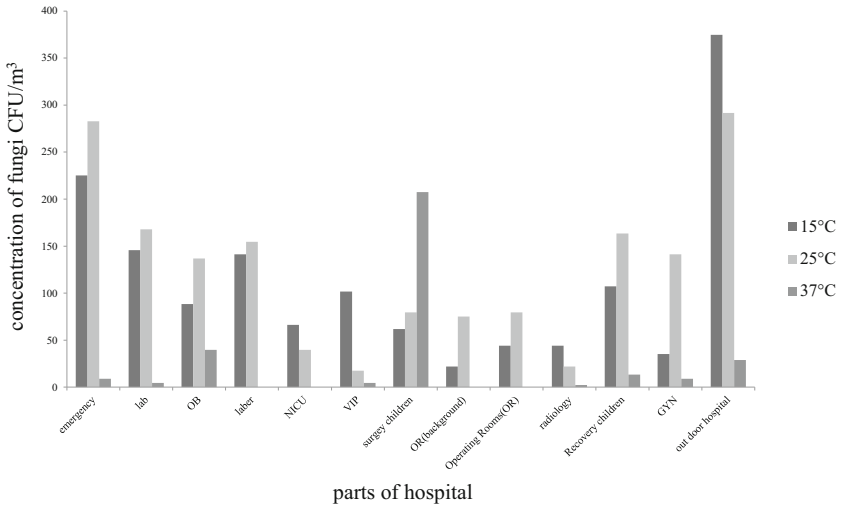

Fig. 3 Mean density of fungi in three incubation temperature

predominant species was varied in each paper (Verde et al. 2015; Azimi et al. 2013; Rangaswamy et al. 2013; Okten and Asan 2012; Kim et al. 2010; Aquino et al. 2013; Cordeiro et al. 2010; Darvishzadeh et al. 2013; Meheust et al. 2013; Fournel et al. 2010; Dehdashti et al. 2012; Choobineh et al. 2008; Soleimani et al. 2016).

With regard to these results, it was reported that incubation temperatures, culture duration, culture number, and media choice were identified as effective agents on distribution on bioaerosols (Sanjay and Noel 2014). In addition, the published data in various studies are rarely comparable, because their samples were incubated at different temperatures. Moreover, different procedures of air sampling and analysis have been used. Based on the results of this study, there was a significant difference between distinct temperatures ($p < 0.05$), so that it is better to set and publish the expected guidelines such as ACGIH and IRSST based on incubation temperature.

Even though *Aspergillus* spp. have aerophilic property which might enable them to survive in the air for relatively long time (Kim et al. 2010) and in broad range of temperatures such as 37, 45, and 50 °C (Zafar et al. 2014), the predominant species was usually *Fusarium*. Because *Fusarium* can grow at different temperature such as 4, 25, 35, 42, and 46 °C (Steinberg et al. 2015), that could produce fusarioses (Georgiadou et al. 2014; Greece et al. 2014) and it creates fatal fungal infection in immunosuppressed patients that it have high mortality and morbidity rates in some of them. Moreover, it may be transmitted in the hospital by air flow. Therefore, even air must be considered as possible reservoir for patient infection (Scheel et al. 2013). With regard to these results, future research is essential for control of environmental reservoirs for *Fusarium* spp. and the identification of the implications and risk of them in all of the parts.

Moreover, most pathogenic fungi are thermotolerant because they grow at 37 °C and they easily survive in human body because the natural temperature of human body is 37 °C (Zeini et al. 2013). These species including *Paecilomyces*, *A. niger*, *A. fumigatus*, and *Penicillium* (Guido et al. 2000; O'Gorman and Fuller 2008) are considered as main indoor allergens. *A. niger* is one of the most common indoor environmental fungal species (Fukutomi and Taniguchi 2015; Vermani et al. 2011) and *A. flavus* produces aflatoxin (Freitas-Silva and Venâncio 2011). In the present study, the density of *A. niger* was more than others (26.5 CFU/m^3). Due to pathogenicity, this specious should be given more attention and the necessary measures should be taken by the hospital authorities.

According to results, the density of thermotolerant fungi was lower than other all departments (see Fig. 3). Only the result for pediatric surgery department was different. Since the immune system of infant is more sensitive than adult, the risk of infectious can be high, because *Aspergillus* spp. can produce opportunistic respiratory allergy that sinusitis effects usually observed in adolescents or young adults (Schomberg et al. 2013). Since some of the fungal species have synergistic characteristics, their risk is higher. Moreover, *Aspergillus* is one of the most common fungi in samples with positive *Legionella* (Alum and Isaacs 2016). But this hypothesis required further researches on prevalence and burden of disease in hospital, especially sensitive departments such as NICU.

The correlation between airborne fungi and ambient temperature

The correlation between ambient temperature and fungal density is shown in Fig. 4.

According to this figure, indoor temperature ranged from 23 to 29 °C and average of outdoor temperature was 16 to 18 °C, respectively. Based on this figure, the correlation of fungi density and indoor air temperature was significant ($p < 0.001$, $r^2 = 0.047$), so that the density of fungi could be related to ambient temperature. Only in outdoor, the results were differing. Therefore, this hypothesis is not acceptable in outdoor space. In other studies, temperature was from 19 to 26 °C (Kim et al. 2010), 23.4 to 25 °C (Guadalupe Marı´a et al. 2016), and 15 to

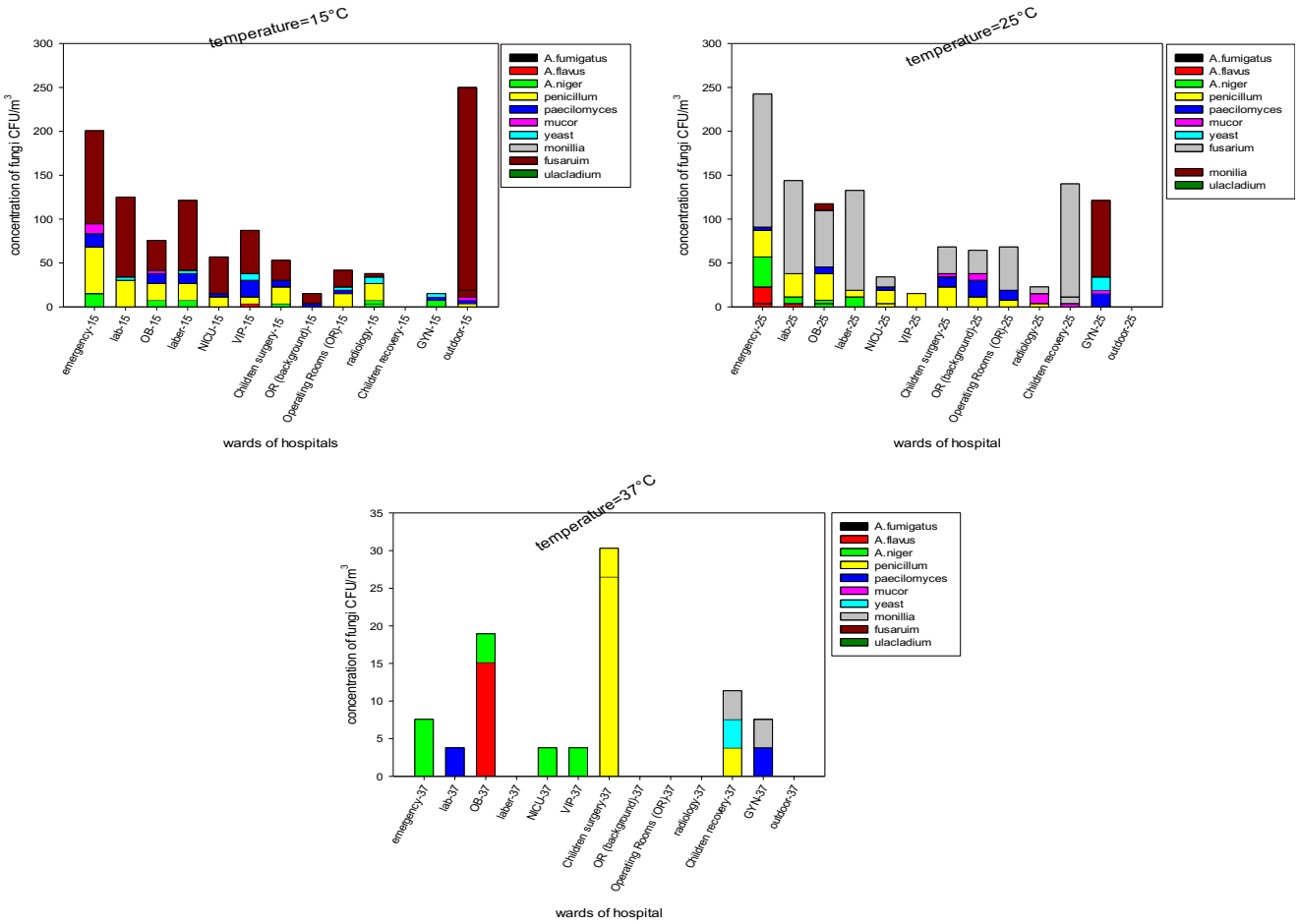

Fig. 4 The mean density of airborne fungal species in different incubation temperature

25 °C (Okten and Asan 2012). Although there is not specific standard density for ambient temperature in hospitals of Iran, European Standards recommend that the temperature of 18 to 24 °C is comfortable for human. But to avoid hypothermia, the ambient temperature in the OR should be between 24 and 26 °C (Nastase et al. 2016). Moreover, the optimum temperature for sporulation is 25–30 °C (Cabral 2010) and the highest density of this mycotoxin is produced in these ranges (Arrus et al. 2005). Therefore, indoor temperature in majority of departments of this hospital was suitable for sporulation and the risk of airborne fungi was higher than outdoor because human sensitivity to spore formulation is higher (Zeini et al. 2013). Airborne fungi can be related to ambient agents such as ambient temperature, humidity, sections of hospital, number and frequency of operation and visitors, and length of exposure to environmental air (Tanaka et al. 2016; Niazi et al. 2015). According to our study, temperature of indoor air can be one of the important factors influencing bioaerosols (see Fig. 5) as seen in other studies (Cordeiro et al. 2010; Soleimani et al. 2016; Cabral 2010; Niazi et al. 2015). Overall, observed difference in the present study can be the result of an indoor environmental temperature.

Predominant species was *Aspergillus* and *Penicillium* that require high humidity. But some *xerophilic* species are able to survive in a dry environment (Fukutomi and Taniguchi 2015) and it was reported that air temperature was identified as an effective parameter on airborne fungi (Ijaz et al. 2016; Sattar and Bact 2016). But there was not significant relationship between *Penicillium* or *Aspergillus* and ambient temperature (O'Gorman and Fuller 2008). Therefore, other meteorological agents (i.e., ambient temperature) seem be more effective. According to results in any section of hospital, the highest density was related to each incubation temperature that it was consistent with environmental temperature, so that it is better than the incubation temperature set on environmental temperature to show the real conditions. Moreover, the threshold levels was expressed for *Aspergillus* conidia in air but there is still a gap in previous studies in assessing the fungal infection risk (Méheust et al. 2013).

Comparison of fungal load in indoor and outdoor spaces

The density of fungi in outdoor air was more than indoor spaces (Fig. 4) but there was no significant difference between them ($p = 0.126$). Moreover, the predominant species was *Fusarium* spp. (92% of total fungal species). Therefore, the

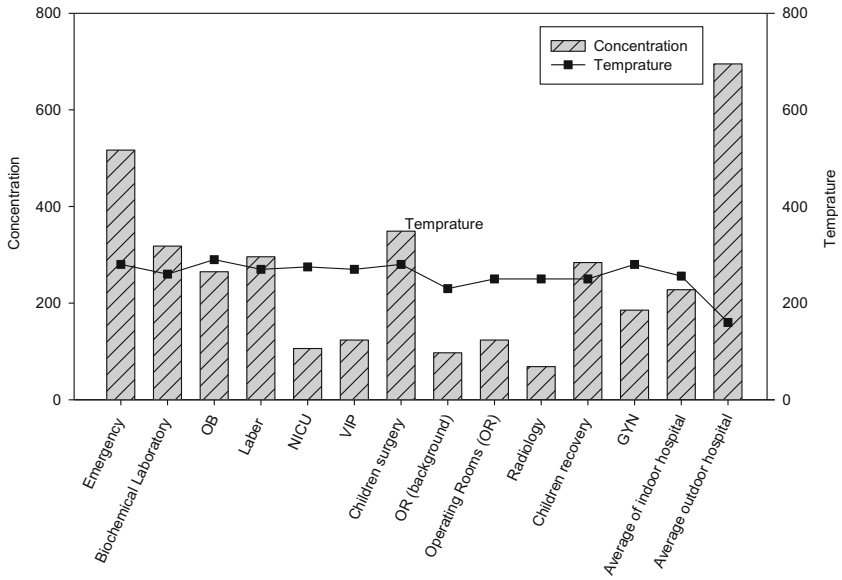

Fig. 5 The correlation between fungal density and air temperature of indoor of the hospital

distribution of indoor predominant airborne microorganisms was similar to the outdoor space. Even though their temperature was different, these results were consistent with the report in previous studies (Pastuszka et al. 2000; Costa Baquião et al. 2012; Rafael et al. 2012).

Moreover, there are many trees and plants in campus of hospital (Fig. 1). *Fusarium* spp. is genus that grows on the agricultural environment (Weikl et al. 2015) and dust (Al-Bader et al. 2016). The epidemiology of *Fusarium* spp. varies with geography, climate, and level of immune suppression (Douglas et al. 2016). Therefore, it was expected that *Fusarium* spp. was originated from outdoor; then, it was transformed to indoor. Although *Fusarium* rarely produces fusarioses in human (Ma et al. 2013), some of *Fusarium* spp. are known as opportunistic human pathogens and cave infections in the skin, nail, or eye (Novak Babic et al. 2015). Moreover, they produce diverse toxic secondary metabolites (mycotoxins) that they also have broad resistance to fungicides drugs (Ma et al. 2013). This matter produces skin and soft tissue infections in the immune-competent host and lung in the immune compromised host (Douglas et al. 2016). Some of *Fusarium* spp. are fungal keratitis that causes blindness. This disease was observed in developing countries (Zhang et al. 2013).

Of course, the identified fungal species can be the result of the type of hospital and location of this hospital. Because it was located in the direction of dust storm from Iraq and downwind of a highway, dust was transmitted to indoor hospital (Fig. 1). In this highway, heavy vehicles with diesel combustion were transported mostly which incorporated fungi to nanoparticles from diesel motors and current air was emitted (Colls 2002; Fröhlich-Nowoisky et al. 2016; Gaoa et al. 2015) and it may penetrate to respiratory tracts.

As direction of wind rose was outdoor space to indoor, therefore, potential risk of eye disease may be present in this hospital. With regard to these results, outdoor air plays an important role on emission of bioaerosol in indoor (Faridi et al. 2014) and the isolation of hospital may be appropriate. Even though almost all sections are equipped with air conditioning systems, insufficient operation or inadequate maintenance of the air conditioning system can allow unfiltered outdoor air to enter indoor. As other studies have shown, high density of fungi in indoor was directly correlated to outdoor levels (Cabral 2010; Rafael et al. 2012; Soleimani et al. 2016; Okten and Asan 2012); however, there are some contradictory studies (Verde et al. 2015). Therefore, outdoor air and ventilation system were a potential threat to public health (Shams-Ghahfarokhi et al. 2014; Weikl et al. 2015; Fukutomi and Taniguchi 2015; Noman et al. 2016) and transition of fungi from outdoor to indoor can be related to technology and geography (O'Gorman and Fuller 2008). It is suggested that proper location be made before building a hospital. Moreover, it should create positive pressure and appropriate ventilation system such as the high efficiency particulate air (HEPA) and laminar air filtration for reduction of contamination especially airborne *Aspergillus* (Fournel et al. 2010). Even though it is expected that the reason of the OR at 15 °C and VIP at 25 °C and OR, NICU, and labor had the lowest level of airborne microorganisms at 37 °C, it might be due to the fact that it was a clean room with high ventilation rate and air conditioning system checked regularly. While for physical structure, human activity, outdoor air current, ventilation efficiency effect on indoor air quality of this hospital, and physical situation, renovation at hospital campus should be optimized (Sattar and Bact 2016; Borrego and Perdomo 2016).

Average density of total fungi

In this study, the density of airborne fungi in each section of this hospital was investigated. As mentioned, total density of fungi in the emergency room was the highest ($p = 0.041$) and in the radiology, it was the lowest ($p = 0.393$) (Fig. 4).

However, there were no significant differences between the total density of airborne fungi in various parts of the hospital ($p = 0.185$). Total mean density of fungi was 227.7 CFU/m^3 in an indoor space of the hospital and predominant fungal species were *Fusarium* spp., *Penicillium* spp., *A. niger*, and *Paecilomyces* spp. But this result was inconsistent with other studies (Azimi et al. 2013; Kim et al. 2010), so that the average density of fungi was 4.2, 26 to 78, and 2 CFU/m^3 in indoor (Sautour et al. 2007; Gorny and Dutkiewicz 2002; Oberle et al. 2015; Rainer et al. 2001) and the predominant fungal species were *Penicillium* spp. and *Aspergillus* spp., *Chaetomium* spp., *Alternaria* spp., *Fusarium, Cladosporium* spp. (Sautour et al. 2007; Li and Hou 2003; Kim et al. 2010). According to the results, total density of fungi in this study was more than previous studies and the predominant species was not similar, due to management and specious condition of this hospital.

With regard to these results, the lowest risk of airborne fungi relates to radiology section. In this part, hospitalization of patient was rarely. It seems that hospitalization of patients was one of the main agents of bioaerosols. With regard to these results, further studies is required in order to determine the correlation between fungal level and other factors as well as temperature, humidity, and culture media, moreover, the optimization of the ambient structure. Therefore, it provides optimum condition such as incubation temperature for growth of colonies and promote of physical structure to reach suitable temperature for patient.

Conclusion

Airborne fungi is one of the indicators for indoor air quality especially in the hospital. According to these results, the mean of airborne fungal density was different at 15, 25, and 37 °C in indoor space. Moreover, there was a significant difference among incubation temperature ($p = 0.006$), so that incubation temperature was an important factor on fungal level that the guideline should be set on this and other parameters. The highest and the lowest density of fungi was attributed to emergency room and operation room at 15 °C, emergency room and VIP at 25 °C, and surgical child room and NICU at 37 °C, respectively. İncubation temperature was an effective agent on growth specious of airborne fungi, so that the predominant species was *Fusarium* at 15 and 25 °C while there was not stable pattern for them at 37 °C. Moreover, there was significant difference among the density of predominant fungal genera at all temperature ($p = 0.001$). In addition to, the correlation of fungi density and indoor air temperature was significant ($p < 0.001$, $r^2 = 0.047$), so that the density of fungi could be related to ambient temperature. Therefore, it is better that the incubation temperature was selected based on the hospital's internal temperature. Moreover, the airborne fungi transmit from outdoor to indoor space by ventilation, door, and window. With regard to this result, it is suggested that further studies should be conducted on the effects of temperature and others environmental parameters on the density and fungal species in an indoor air.

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