Assessment of Bioaerosol Contamination (bacteria and fungi) in Operating Rooms of The Largest Educational Hospital in Shiraz, Iran

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ABSTRACT
There is a concern about exposure to bio-aerosols due to their harmful health effects. Bio-aerosols in surgery environment can potentially cause infection in susceptible and unsusceptible patients. The present study aimed to evaluate bacterial and fungal densities and species in surgery rooms in an educational hospital in Shiraz.
A total of 168 samples were collected. Active sampling was done by using a single-stage Anderson sampler on plates including blood agar and sabouraud dextrose agar for 10 minutes for bacteria and fungi, respectively.
The mean densities of fungi and bacteria were respectively 233.23±2.024 and 232.6±1.383 CFU/m^3 before sterilization compared to 233.01±2.041 and 233.57±1.324 CFU/m^3 after sterilization of the surgery rooms. No significant difference was observed between the densities of bacteria and fungi before and after sterilization in all surgery rooms ($P>0.05$). However, it was a significant difference between the total densities of bio-aerosols after sterilization compared to the suggested value. The density of bio-aerosols was greater than 30 CFU/m^3 ($P<0.01$) in 100% of the cases, but did not exceed 500 CFU/m^3 in any of the cases ($P<0.01$ in 71.42% of the cases). Moreover, *Penicillium*, *Fusarium*, *Cladosporium*, and *Aspergillus* were the most common fungi and gram-positive bacteria, including *Staphylococcus aureus*, *Bacillus*, and *Staphylococcus epidermidis*, were the most dominant types of bacteria in surgery rooms.
Quantitative and qualitative findings of this study revealed high densities of bacteria and fungi in surgery rooms. Thus, effective strategies have to be proposed to control bio-aerosols and their related health effects.
Keywords: Air pollution; Bio-aerosols; Bacteria; Fungi; Operating room

LIST OF ABBREVIATIONS
CFU: colony-forming unit
HEPA: high-efficiency particulate air
IAQ: Indoor Air Quality

INTRODUCTION
Bio-aerosols are airborne particles including microorganisms, such as bacteria, viruses, and fungi, or organic compounds derived from microorganisms, such as endotoxins, metabolites, toxins, and other microbial fragments. Bio-aerosols vary in size from 20 nm to more than 100μm [1]. Hospital environment is an ideal 'reservoir' for a variety of microorganisms, such as bio-aerosols [2].
Recently, health effects of bio-aerosols have attracted a lot of attention [3,4,5]. Bio-aerosols may cause infectious diseases [6], acute poisoning [7], allergies, stress, and cancer [1]. Therefore, assessing the type and density of airborne microorganisms is essential for ambient, indoors, and workplaces air [3,8].
In operating rooms, contamination of surgical suction tips with bacteria leads to development of surgical site infections [9]. Monitoring bio-aerosols in hospitals can provide useful data for epidemiological investigation of nosocomial infectious diseases [3,10]. Different sampling and detection methods, such as active and passive air sampling, are used to determine bio-aerosols level by counting colony numbers per cubic meter of air or square meter of floor area.
Mirzaei et al. compared the quantity and quality of bio-aerosols in operating rooms and emergency department of an educational hospital in Zahedan,

Iran. In that study, 17 types of bacteria were detected and the mean number of bacteria was 103.88±33.84 CFU/m^3 (colony forming units per cubic meter) in the emergency department compared to 63.32±32.94 CFU/m^3 in the operating rooms [1].

In addition, Kim *et al.* showed that the main airborne fungi identified in the study hospital were *Cladosporium spp.*, *Penicillium spp*., *Aspergillus spp*. and *Alternaria spp*. [11].

In recent years, hospital infections significantly increased the duration of hospitalization in Iran and created tremendous risk of transmitting ***infectious*** disease for the patient as well. Therefore, the present study aims to (i) determine the density and the types of airborne bacteria and fungi by measuring bio-aerosols using the active method and (ii) assess the effect of sterilization in operating rooms in one of the largest educational hospitals in Shiraz, Iran.

MATERIALS AND METHODS

Sampling site

This descriptive-analytical study was conducted in one of the largest educational hospitals in Shiraz, southwest of Iran, during 2013-2014. This hospital has 18 operating rooms of which 14 are active. Almost 40-50 operations are done in each operation room. They were equipped with air conditioning systems with no HEPA filters

Sampling method

In this study, types and densities of bio-aerosols containing bacteria and fungi were investigated. In order to determine the effect of sterilization, samples were collected before and after sterilization. In addition, samples were taken from three points in each room to increase the accuracy. Moreover, the ventilation system in operation rooms were on before and after of sampling.

Two different types of media were used; i.e., blood agar for bacteria and sabouraud dextrose agar for fungi. A total of 168 samples including 84 fungal samples and 84 bacterial samples, were collected biweekly in the 14 active ***operating*** rooms.

The bio-aerosol sampling strategy was designed to best represent the effect of sterilization in the operating rooms environment. An Anderson single-stage Anderson sampler (IAQ, Model; 8762, TSI, Shoreview, MN, USA) was used with a constant flow rate of 28.3 L/min for sampling time of 10 min. The air samples were collected at the human breathing zone (approximately 1.8 m from the floor) at a distance of 2 m from the walls of the operating room. Air temperature and humidity were monitored simultaneously during the sampling.

The airborne microorganisms were sampled using a 20 mL blood agar for bacteria and sabouraud dextrose agar with chloramphenicol for fungi. When changing the collection plates, the stage hole was sterilized with a 70% ethanol solution and dried to prevent cross-contamination. After sample collection, the plates were closed by Para-film and transported to the laboratory using a cold box. Then, the samples were incubated at 37°C for 1-2 days for bacteria and at 25°C for 4-7 days for fungi, respectively. The minimum detectable number of CFU was 30 per sample. The concentration of bioaerosols was reported in terms of CFU per unit volume of air (m^3). The slide culture method was used for fungal species identification. The fungal colonies were identified by slide culturing simple method and using a microscope at the magnification of ×40. The bacterial were identified using the Gram staining technique, biochemical differential and serological tests and standard methods were used for bacterial species identification [12, 13].

Data analysis

All the data were analyzed using SPSS statistical software, version 16. Wilcoxon signed ranks test and the ***t-test*** statistic were used to determine the differences between densities of bacteria and fungi before and after sterilization.

RESULTS

In this study, the average densities of fungi and bacteria before sterilization were 233.23±2.024 and 232.6±1.383 CFU/m^3, respectively compared to after sterilization 233.01±2.041 and 233.57±1.324 CFU/m^3, respectively (Figs. 1 and 2).

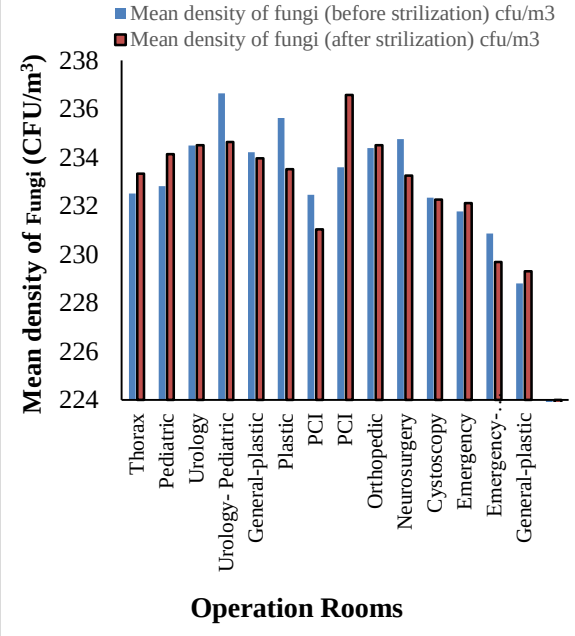

Fig. 1: Comparing the average density of fungi (CFU/m^3) in **operating** rooms before and after sterilization

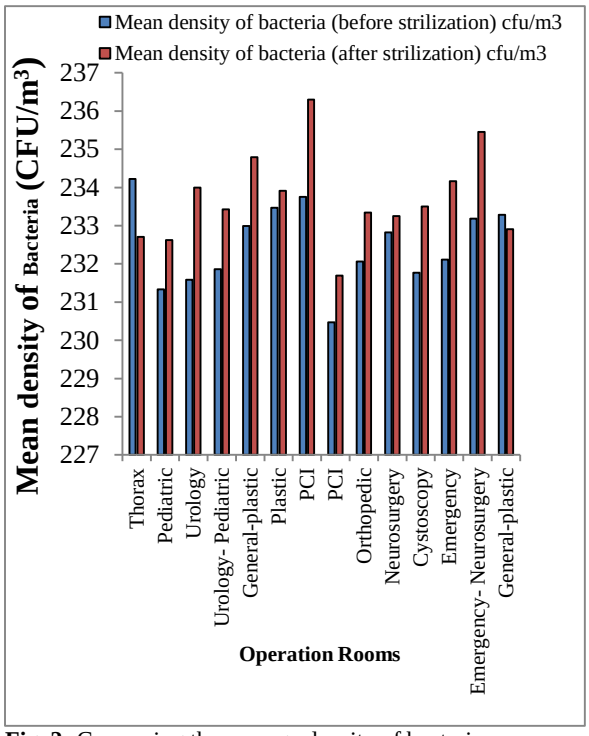

Fig. 2: Comparing the average density of bacteria (CFU/m^3) in **operating** rooms before and after sterilization

The average densities of bacteria and fungi in all the 14 ***operating*** rooms before and after sterilization have been presented in Table 1. Also, total densities of bio-aerosols after sterilization compared to the suggested value showed in Table 2.

Table 1: Mean density of bacteria and fungi in operating rooms before and after sterilization

Mean density / Status of sterilization	Mean density of bacteria (CFU/m^3)	Mean density of fungi (CFU/m^3)
Before sterilization	232.6±1.383	233.23±2.024
After sterilization	233.57±1.324	233.01±2.041
P value	P>0.05	P>0.05

Table 2: The comparison of total densities of bio-aerosols after sterilization to the suggested value

Suggested value (CFU/m^3)	30 (CFU/m^3)	500 (CFU/m^3)
Total densities of bio-aerosols after sterilization (CFU/m^3)	466.58	466.58
P value	P<0.01	P<0.001

DISCUSSION

According to our results, the average densities of bacteria before and after sterilization were 230.47-234.22 CFU/m^3 to 231.69-236.3 CFU/m^3, respectively. Moreover, the average densities of fungi varied from 228.81- 236.64 to 229.3-236.58 CFU/m^3 before and after sterilization, respectively. The study of Bolookat *et al.* showed that mean concentrations of bacterial and fungal bioaerosol (CFU m^{-2} h^{-1}) were 919 and 796, respectively [14]. Mirzaei *et al.* also monitored the density of bio-aerosols in operating rooms of an educational hospital. In that study, the mean number of bacteria in the operating rooms was 63.32±32.94 CFU/m^3 [1], which was much lower compared to our study. Pastuszka *et al.* reported the average density at different sections of Silesian hospitals was 100-1000 CFU/m^3 [15]. Another study showed that bacterial and fungal concentrations in hospital clean rooms were 1-423 and 0-319 CFU/m^3, respectively [3]. Jaffal *et al.* study also showed that the average densities of microbial pollution in the Intensive Ca Unit (ICU) and the operating room were 687 CFU/m3 and 473 CFU/m^3, respectively [16]. Results of the current study were in agreement with the majority of other studies**.**

According to data obtained in the current study, before the sterilization the maximum and minimum number of bacterial colonies was determined in thorax operation and Percutaneous Coronary Intervention (PCI) rooms, respectively. However, the maximum and minimum number of fungi was observed in urology-pediatric operation and general operating rooms, respectively. The results revealed no significant difference between densities of bacteria and fungi in operating rooms before and after sterilization (P>0.05). Therefore, it can be assumed that that sterilization was not at all effective in controlling the bioaerosol in the operating rooms. Also, the results show no relation between the bacteria and fungi densities with type of operation and number of operations in operation rooms. This may be due to regular sterilization of the operating room before and after of each operation.

Kim *et al.* study revealed that the average density of airborne bacteria in one of the general hospitals in Korea was in the following order: the main lobby > surgical ward> biomedical laboratory> ICU. In addition, the average density of fungi in the different parts of the hospital was as follows: the main lobby>biomedical laboratory>surgical ward> ICU [11]. The study of Bolookat *et al.* showed that the total fungal bioaerosols were higher than the bacterial bioaerosols in the CCU, MS, and WS, whereas total bacterial bioaerosols were higher than the fungal bioaerosols observed in the NICU, GICU, ICU, and NS [14].

According to guidelines, bio-aerosols' concentration should range from 30 CFU/m^3 for modern surgeries to 500 CFU/m^3 [17,18]. The results of the present study demonstrated a significant difference between the total densities of bio-aerosols after sterilization compared to the suggested value. The density of bio-aerosols was greater than 30 CFU/m^3 (P<0.01) in 100% of the cases,

but did not exceed 500 CFU/m^3 in any of the cases ($P<0.01$ in 71.42% of the cases).

Moreover, *Penicillium*, Fusarium, *Cladosporium*, and *Aspergillus* were the most common fungi in the operating rooms. Similar results have been obtained by many studies [11,15]. Kim *et al.* showed that the predominant genera of airborne fungi identified in the general hospital were *Cladosporium, Penicillium, Aspergillus,* and *Alternaria* [11]. Pastuszka *et al.* also demonstrated that Penicillium accounted for 90% of the total fungi in moldy homes [15]. Li *et al.* also reported that *Penicillium* was the predominant in hospital clean rooms [3]. Also, another study showed that Aspergillus spp. (49.9%) were the most dominant fungal genera [14].

Furthermore, our study results showed that most of the detected bacteria were gram-positive and *Staphylococcus aureus*, *Bacillus* Spp., and *Staphylococcus epidermidis* were the dominant bacteria types. Similarly, Mirzaei reported *Micrococcus*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, and *Staphylococcus epidermidis* as the dominant bacteria types [1]. Another study demonstrated that Micrococcus luteus (43.4%) > Staphylococcus epidermidis (15.5%) > Streptococcus spp. (12.5%) > Diphtheroid spp. (11.3%) > Micrococcus roseus (10.8%) > Bacillus subtilis (6.4%) in the all samples were dominated and all the bacterial bioaerosols in this study were gram-positive [14]. Tolabi *et al.* showed that all bacterial species observed in his study (except E. coli) were gram-positive and the gram-positive bacteria were the most frequent species of bacterial [19].

CONCLUSSION

According to data obtained in the current study, the densities of bacteria and fungi in all the operating rooms were higher than the suggested value (30 CFU/m^3). The highest and lowest number of bacterial colonies before sterilization was detected in thorax operation and PCI rooms, respectively. Also, the highest and lowest number of fungal colonies was related to urology-pediatric operation and general operating rooms, respectively. In spite of sterilization, many different types of fungi and bacteria were detected. The predominant genera of airborne bioaerosol isolated in the air of ORs were gram-positive bacteria including: *Staphylococcus aureus*, *Bacillus* Spp., *Staphylococcus epidermidis*, *Penicillium*, Fusarium, *Cladosporium*, and *Aspergillus*. An increase in the concentration of bioaerosols after sterilization might be related to high humidity while disinfection and not enough UV irradiation. Therefore, designing well-constructed ventilation systems, implementing more stringent disinfection procedures, and controlling humidity are essential in reducing bioaerosol in the operating rooms in the hospital under.

ETHICAL ISSUES

Ethics approval and consent to participate were not applicable for this study, because no human sampling was performed.

CONFLICT OF INTEREST

The authors declare that they have no competing interests**.**

AUTHORS' CONTRIBUTIONS

Mansooreh Dehghani: Study concept and design, Critical revision of the manuscript for important intellectual content, Administrative, technical, and material support

Samaneh Shahsavani: Analysis and interpretation of data, Drafting of the manuscript: Acquisition of data.

Narges Shamsedini: Study concept and design, Statistical analysis

fatemeh dehghani: Study supervision.

maryam gholamzadeh: Statistical analysis

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