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Short communication

Assessment of microbiological indoor air quality in a public hospital in the city of Agadir, Morocco

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Abstract

Background and Purpose: *Air is the greatest dissemination environmental media of pathogenic microbes, which cause significant problem in the indoor hospital environment, in particularly in terms of nosocomial infections. In this context, it is important to know the ypes of microorganisms present in suspended matter in an air sample to assess the initial situation and the effectiveness of corrective measures.*

Materials and Methods: *This study aimed to assess of microbiological indoor air quality ina three hospital units: central resuscitation, neonatal resuscitation and operating room, using a passive sampling method.*

Results: *Findings of this study indicated that the central resuscitation recorded the highest bacterial counts population 3.33 10^2 CFU/m^3. Total viable count of fungi was recorded high in neonatal resuscitation. This research showed that* Staphylococcusnon aureus *and* Staphylococcus aureus *were the most predominant among isolated bacteria. The percentage was 70% -21% in central resuscitation and 79%-13% in operating room. In neonatal resuscitation* Staphylococcus non aureus *represent (29%) followed by* Staphylococcus aureus *(19%),* Pseudomonas aeruginosa *(17%) and* Pasteurella pneumotropica *(16%). Thus, the fungal and species identified in operating room were* Penicillium *spp (61%),* Aspergillus niger *(20%) and* Candida glabrata *(19%). In neonatal resuscitation, we found* Penicillium *spp 51%,* Candida glabrata *25% and* Aspergillus niger *20%. In central resuscitation, the most predominant fungi were* Cladosporium *spp (30%),* Penicillium *spp (28%) and* Candida glabrata *(13%).*

Conclusions: *Microorganisms isolated from indoor air constitute microbial reservoirs that may present a risk of infection for both patients and staff. In this light microbiological monitoring of the environment in health facilities is a topic that is part of the new approach in the prevention of nosocomial infections.*

INTRODUCTION

Hospital indoor air contains a diverse range of microorganisms (bacteria, yeasts, fungi, viruses and parasites). The transmission of these germs to humans by air is due to bioaerosols, which are a colloidal suspension formed of liquid droplets and solid particles in air, containing microorganisms. It has been suggested that many pathogens can survive as bioaerosol, spread considerable distances, and result in infection (1).The hospital can be considered a dynamic environment influenced by seasons (2), weather, ventilation systems (3),and moisture intrusion. In addition, the sources of hospital airborne infection or

contamination could include the patient's own normal flora, linens, bed sheets, staff clothes, visitors and the materials. Activity of patients (sneezing, coughing, talking, yawning) and the number of patients per room may likewise be the sources of hospital infection (4, 5).

Airborne microflora in hospital rooms was the subject of numerous studies as a potential cause of hospital infections(2, 6). Among all of the microorganisms, bacteria and fungi are of great concern as the leading airborne pathogens that can lead to large economical as well as ecological consequences (7). Kim et *al.,* (2010) (8) reported that air samples from hospital (main lobby, intensive care unit, surgical ward and biomedical laboratory) were processed and the isolates were *Staphylococcus spp*, *Bacillus spp*, *Micrococcus* spp and *Corynebacterium* spp Javel et *al.,* (2008) (9) showed that *S. aureus* were isolated from all the air samples obtained from the various operation theatres except (ear, nose and throat). Several studies have shown that hospital infections are also caused by fungi, such as *Candida spp* and various species of *Aspergillus*, *Cladosporium* and *Penicillium* (10). Even in samples from the ventilator system (HEPA filter and common filter), air canal, air and hospital instruments, fungi such as *Penicillium*, *Aspergillus*, *Cladosporium*, *Trichoderma*, *Stereptomyses, Chrysosporium*and *Rhizopus* have been isolated(11).In other study, the profile of air samples showed that *P. aeruginosa* was the predominantly isolated bacteria from thoracic surgery ward, *S epidermidis* from bone marrow transplantation ward and neonatal ward; *Enterococcus*from intensive care unit and *Acinetobacter* from operating room. Other miroorganisms were also isolated from these wards such as *Proteus*, *Stenotrophomonas maltophilia, Enterobacter, S. aureus, Streptococcus group* D*, E coli, Klebsiella* and *Candida albicans. Cladosporium* was the most frequent fungi found(12).The presence of these germs in hospitals is generally linked to several types of infections. It has been observed that exposure to certain pathogenic microorganisms in hospitals is associated with an increased risk of nosocomial infections. Such infections constitute a major concern for public health because of the increased length of stay of patients and the cost of hospital care they may cause(13).

According to the literature, some hospital departments are more exposed to airborne infections than others, especially the operating room and the neonatal service, given the specificity of their patients. Indeed, microbiological monitoring of the environment in healthcare facilities is a topic that is integral to current events in the prevention of nosocomial infections. This study aimed to characterize microbial and fungal contamination levels in the indoor air of three units of a Regional Public Hospital in the city of Agadir, Morocco as well as to identify the air born bacteria and fungi that maybe associated with nosocomial infections.

MATERIAL AND METHODS

Study area

Hassan II hospital, a government hospital was chosen for the study due to its high number of patients, visiting both from Agadir and distant villages of Agadir for their treatment. The units concerned are: central resuscitation, neonatal resuscitation and operating room.

Sampling and culture media

Sampling was carried on five points for each unit, with a frequency of eight samples per point per day (one sampling in the morning 9:00 and one sampling in afternoon 15:00). For technical reasons, the samples made at the operating room were carried out only in the morning. Air specimens were collected using the settle plate method for the enumeration of bacterial and fungal isolates. Petri dishes containing the Trypto-casein soybean agar medium for bacteria, while Sabouraud's chloramphenicol agar medium was used for the fungi sampling. Petri dishes are placed one meter above the ground and one meter from the obstacles and walls and left open to the air for 1 hour.

Enumeration and isolation of bacteria and fungi

The Petri dishes previously exposed to the air in the three units were incubated at 37°C for 24 hours for the bacteria and at 25°C for 5 to 7 days for the fungi. After the incubation, the developed colonies were counted and converted into colony forming unit per cubic meter of air (CFU/m^3) using Omeliansky formula (14). Indeed, purification of different types of colonies was performed by exhaustion on Trypto-casein soybean agar medium. All strains purified were stored frozen (–20 °C) in Trypto-casein soybean broth-glycerol 50% (vol/vol). The pure cultures of fungi were stored as tube slants at 4°C.

Identification of isolated bacteria and fungi

Purified bacterial colonies were subject to identification by both Gram stain and by classical biochemical strips and BioMérieux API strips. Identification of fungi was made according to their macroscopic and microscopic morphological characteristics of the vegetative mycelium and the reproductive structures by standard mycological methods.

RESULTS

Airborne microbial concentrations of Bacteria and Fungi

The microbial load of indoor airsamples collected from three hospital unitsare presented in Tables 1. These results

Table I: *Microbial load of indoor air samples collected from three hospital units by passive air sampling.*

Sampling site	Bacterial count (CFU/m³)		Fungal count (CFU/m³)	
	Sampling time		Sampling time	
	Morning	Afternoon	Morning	Afternoon
Central resuscitation	3.93 10^2	1.09 10^2	54	29
Neonatal resuscitation	3.33 10^2	87	1.76 10^2	66
Operating room	2.61 10^2	–	1.09 10^2	–

indicated that the highest concentration of bacterial air contamination was detected during the morning. In the central resuscitation, the average of the total bacterial colony counts was 3.93 10^2 CFU/m³ followed by neonatal resuscitation 3.33 10^2 CFU/m³ and operating room 2.61 10^2 CFU/m³. While the lowest average of the total bacterial colony counts were recorded in the afternoon with 1.09 10^2 and 87 CFU/m³ respectively in central resuscitation and neonatal resuscitation.

In respect to the levels of airborne fungi (Table 1), these results further confirm the results of the bacteriological analysis. In the morning, the average of the total fungal colony counts of central resuscitation, neonatal resuscitation and operating room were 54, 1.76 10^2 and 1.09 10^2 CFU/m³ respectively. In the afternoon neonatal resuscitation recorded the highest bacterial counts 66 CFU /m³.

Profile of airborne bacteria

Frequency of bacteria identified in indoor air of three-studied units are presented in fig. 1.Results indicated that all air samples collected from different units were contaminated with different types of microorganisms. Indeed, *Staphylococcus*non *aureus* and *Staphylococcus aureus* were the most predominant among isolated bacteria from air samples collected from central resuscitation, neonatal resuscitation and operating room. The percentage of *Staphylococcus*non *aureus* and *Staphylococcus aureus* in air of central resuscitation (Fig. 1A) was 70% and 21% respectively. Whereas in operating room (Fig. 1C) the percentage of this microorganism was 79% for *Staphylococcus*non *aureus* and 13% for *Staphylococcusaureus*.

For both services, bacteria such as *Acinetobacter baumanii, Pasteurella pneumotropica, Stenotrophomonas maltophilia, Enterobacter sakazakii, Pasteurelleoryzihabitans*and *Klebsiella* as the least abundant microorganisms. In the neonatal resuscitation unit (Fig. 1B), it was noted that Staphylococcus non *aureus* represent the highest percentage 29%, followed by *Staphylococcus aureus* 19%,

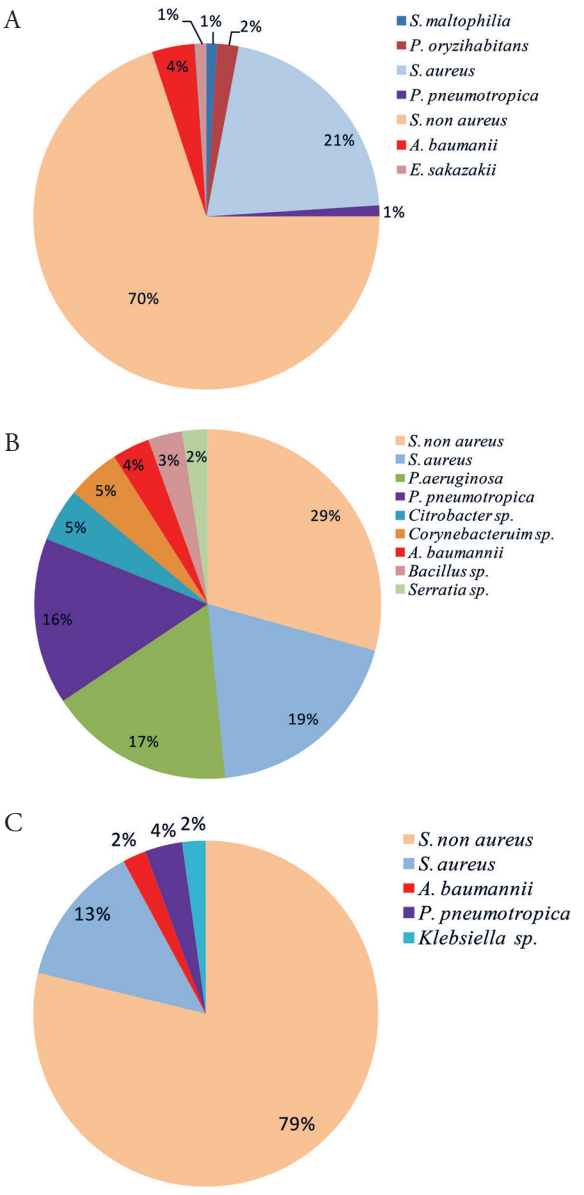

Figure 1: *Distribution of bacterial species isolated from indoor air in the various studied hospital units using a passive sampling method. A: central resuscitation, B: neonatal resuscitationand C: operating room.*

Pseudomonas aeruginosa and *Pasteurella pneumotropica* with, 17%, and 16% respectively. The least represented bacteria are *Citrobacter* and *Corynebacterium* 5%. While *Acinetobacter baumannii*, *Bacillus* and *Serratia* represent a percentage less than 5%.

Profile of airborne fungi

The findings of this research (fig. 2) showed that indoor air of each studied hospital units was contaminated to various fungi. The frequency of the fungi isolated from the neonatal resuscitation (Fig. 2B) showed a predominance of *Penicillium* 51% followed by *Candida glabrata*

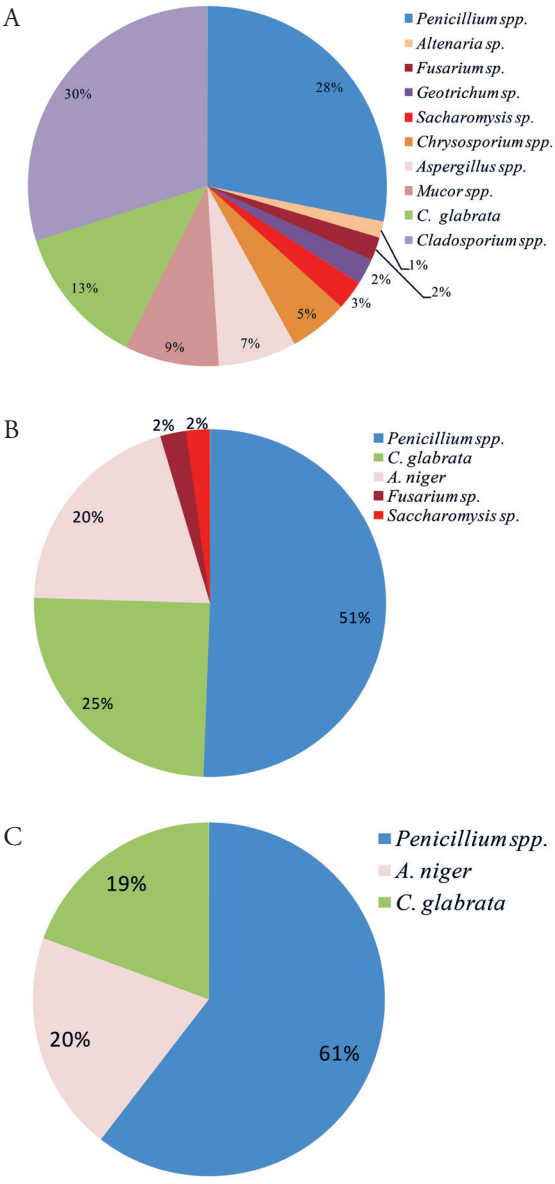

Figure 2: *Distribution of fungal species isolated from indoor air in the various studied hospital units using a passive sampling method. A: central resuscitation, B: neonatal resuscitationand C: operating room.*

(25%) and *Aspergillus niger*(20%). Whereas, *Fusarium* and *Sacharomysis* were found in a small proportion. In operating room (Fig. 2C)*Penicillium*, *Aspergillus niger*and *Candida glabrata* were the most frequently fungal genera isolated with a percentage of 61, 20 and 19 % respectively. Central resuscitation (Fig. 2A) showed a predominance of *Cladosporium* (30%) followed by *Penicillium* (28%) and *Candida glabrata* (13%). Indeed, fungal genera such as *Mucor, Aspergillus* and *Chrysosporium* were found with a percentage less than 10%, and others germs such as *Sacharomysis,Geotrichom*, *Fusarium* and *Alternaria*were found with a percentage less than 5%.

DISCUSSION

The aim of this work was to assess microbiological indoor air quality in a three hospital units: central resuscitation, neonatal resuscitation and operating room, using a passive sampling method. Results obtained in this study showed that microbial indoor air pollution was observed at all the sampling of the three monitored units. The Gram-positive bacteria were isolated with a percentage of 81.7%, while Gram-negative bacteria represent a percentage of 18.7%. This result consistent with precious study (15, 16, 17), which showed that the majority of bacterial findings in the indoor air were bacteria Gram-positive. These results can be explained that Gram-positive bacteria survive longer in the form of aerosol than Gram-negative bacteria. This is mainly due to the composition of their wall, which contains peptidoglycan resistant to many environment factors. Thus, findings of this research showed that the highest bacterial and fungal population was recorded in morning compared to afternoon. On the other hand, quality of indoor air in relation to microbial contamination at a given time period is determined by the quality of air entering into the building, the number of occupants, their activities, cleaning procedures, resultant aerosol generation and efficiency of ventilation (18, 19).The microorganisms load found in the air of these units could be the cause of the increased risk of infection in hospitalized patients. Presence of certain pathogenic microorganisms in the air of the hospital, especially in the operating room could be the cause of severe postoperative infections. In one study, relationship between airborne pathogen levels and nosocomial infections is not known yet, but it could be hypothesized that decreasing the level of these pathogens in the air would result in providing an environment that would help lower the risk of hospital acquired infection (20, 21). In this context, it is important to know the types of microorganisms present in suspension in an air sample to assess the initial situation and the effectiveness of corrective measures. Results from this study showed that indoor hospital air was contaminated to some extent with different types of microorganisms among which *Staphylococcus* non *aureus* was the most predominant followed by *Staphylococcus aureus.* For the three studied units, bacteria such as *Pseudomonas aeruginosa, Acinetobacter baumanii, Pasteurella pneumotropica* and *Klebsiella* sp. were also isolated. The results of fungi identification indicate that the operating room showed a predominance of *Penicillium* spp. 61% followed by *Aspergillus niger* (20%) and *Candida glabrata* (19%). While in neonatal resuscitation the dominant fungal were *Penicillium* spp, *Candida glabrata* and *Aspergillus niger* with the respective frequencies 51%, 25% and 20%. In central resuscitation the frequency of the fungal isolated from air samples were *Cladosporium* spp. (30%) followed by *Penicillium* spp. (28%) and *Candida glabrata* (13%). According to the literature, previous results show that the dominant bacterial species identified from seven different operation theatres were Coagulase negative staphylococci (22). Fur-

thermore, Qudiesat et *al*. (23) noted that, in both hospitals, (a private and a public) in Jordan, *S. aureus*, *Micrococcus luteus* and Coagulase-negative staphylococci (CoNS) were among the most common bacteria identified whereas fungal species *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp. and *Alternaria* spp. were identified in both hospitals. In one study, research from 30 wards in five educational hospitals, coagulase-negative staphylococci (32.49%), *Bacillus* spp. (14.74%), *Micrococcus* spp. (13.68%) and *Staphylococcus aureus* (11.34%) were the highest bacterial population identified. The highest fungal populations were *Penicillium* spp. (32.06%), *Cladosporium* spp. (20.5%), *A. fumigates* (14.61%) and *A. niger* (7.43%) (16). On the other hand, in office building equipped with a heating, ventilation and air conditioning (HVAC) system Brandal *et al*., (2014) (24) reported that *Bacillus* and *Staphylococcus* are the most frequent in airborne microflora whereas Bonetta *et al*., (2010) (25) found *Staphylococcus* and *Micrococcus* as the most common bacterial genera in indoor air. Literature reports that the most frequently isolated bacteria from autopsy room air were Coagulase-negative staphylococci CoNS, *Micrococcus* spp., *Bacillus* spp. and diphtheroid bacillus for the Gram-positive, and *Acinetobacter* spp., *Proteus mirabilis* (*P. mirabilis*) and *E. coli* for the Gram-negative groups. Most frequently isolated fungi were *Penicillium* spp., *Alternaria* spp. and *A. flavus* (18). In one study, air samples from eight selected rooms in the Hospital Sultanah Nur Zahirah were processed and the most dominant fungi were identified as *Cladosporium* sp. (85%), *Penicillium* sp. (48%), *yeast* sp. (28%), *Mucor* sp. (25%), and *Aspergillus* sp. (12 %). This indicated that indoor environment of each selected location in the hospital provides more favorable conditions for the survival of fungi (26). This result is in line with the finding of earlier researcher by Guiamet et *al*. (2012) (27), who studied in indoor air sampling showed the similar identification of fungi that were *Aspergillus* sp. and *Penicillium* sp. as the most abundant microorganisms.

There are two common techniques used for monitoring and controlling of aireborne contamination in hospitals and medical centers, the passive sampling using the sattle plate the active method using a microbiological sampler (28). The advantages of passive method over active method were reported as cheap, easy to perform, available everywhere, ability to detect and mesure harmful part of aireborne contamination, many samples can be taken at the same time from different locations, significant outcomes concerning critical surfaces, comparable and usually reliable outcomes, the airflow is not disturbed, and microorganisms growth under the natural conditions. The disadvantage of this method can be listed as unknown volume of samped air, long sampling time, insufficient for fungal spore evaluation (29, 30). Earlier studies have been done to compare between the values of microbial loads obtained by both passive and active methods. In some cases there was a significant correlation between the results of these methods (31), while in others there was no correlation (32). In this perspective the present study can be completed by using the active sampling method to futher assess the correlation between the results of the different sampling methods.

Whatever the method used, the presence of these germs in hospitals is generally linked to several types of nosocomial infections such as urinary tract infection, severe pneumonia, tuberculosis and gastroenteritis (33). Other examples are described in the literature by different authors. Indeed, presence of many virulence factors in *S. aureus* strains resistant to methicillin an oxacillin gives this microorganism an advantage to cause acute to chronic infections, such as boils, deep tissue abscesses, enterocolitis, bacteriuria, osteomyelitis, pneumonia, carditis, meningitis, septicemia and arthritis (34). *A. baumannii* causes outbreaks of nosocomial infections because of its multidrug-resistance patterns and its resistance to desiccation. A specific epidemic strain of *A. baumannii* causes infection or colonization of numerous patients (35).Compared with other *Enterobacteriaceae*, *K. pneumonia* is the most concerning pathogen for its severe morbidity and mortality. Tsukadaira et *al*. (2004) (36), reported four cases of *K. pneumonia* infections, which were typical lobar pneumonia (Friedlander pneumonia), acute bronchopneumonia with subclinical aspiration, and chronic *K. pneumonia* with typical cavitary lung abscesses.

CONCLUSION

Results from this study showed that microorganisms isolated from indoor air constitute microbial reservoirs that may present a risk of infection for both patients and staff. In this light, efforts are needed to improve hospital hygienic environment and it is recommended to raise the awareness and educational status of medical workers to reduce the hazards of air-borne transmission of such potentially pathogenic microorganisms.

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