
Opportunistic Pathogenic Fungi from the Dust in Sebha Medical Centre, Libya

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Summary:

Fungi are one of the major biological components of the dust. Exposure to these fungi and them particles can cause respiratory disorders and fungal mycoses to human. The indoor environments of a hospital can affect on the health of patients, staff, and visitors and increase patient mortality, morbidity, and length of hospital stay and overall costs.

A total 50 duplicated dust samples were collected during the period March - June 2012 from operating theatres (OT), intensive care units (ICU) and neonatal wards (NW) and sending for identification of microbiological agents.

A total of 202 fungal colonies were isolated and identified. 83.7 % were molds and 16.3 % were yeasts. The average concentration per gram of dust in OT, ICU and NW was 65 CFU/g, 89 CFU/g and 48 CFU/g, respectively, with significant difference between the three units ($P < 0.001$). Diversity of fungal types showed no significant difference between the three units.

From all isolated fungi, the major present types was *Aspergillus spp.* with 52.47%, *Penicillium spp.* with 22.27%, *Candida spp.* with 16.3%, and the other isolated fungal types were present in low percentages.

Candida spp. was isolated during the study, four isolates (11.76 %) were identify as *Candida albicans* (Germ tube test positive), while 30 isolates (88.24 %) were *Candida non-albicans spp.* from total isolated yeast.

Aspergillus, *Penicillium*, *Candida*, *Bipolaris*, *Graphium*, *Curvularia*, *Fusarium*, *Epicoccu* and *Exophilia* were identified in all studied units with different percent.

Key Words: Dust, Fungi, Sebha, Hospital, Operating Theaters, Intensive Care Units, Neonatal Wards

Introduction:

Fungi are one of the major biological components of the dust.¹ Dust fungi play an important role in the dissemination of human diseases.² Exposure to these fungi including the airborne fungal particles can cause a variety of respiratory disorders and fungal mycoses especially in the immunocompromised patients.^{3,4}

Hospitals and other healthcare facilities are complex environments.^{5,6} They act as a potential reservoir of infectious agents since it houses both patients with diverse pathogenic microorganisms and a large number of susceptible immunocompromised individuals.^{7,8} The indoor environment of a hospital could influence the health of patients, staff, and hospital visitors and increase patient mortality, morbidity, and length of hospital stay and overall costs.^{2,7,8}

Hospitalized immunocompromised patients are susceptible to infections from naturally occurring airborne fungi that can grow at body temperature. Hospital staff, other patients,

visitors, soil, food, water, formites, urinary catheter, intravenous devices, respiratory equipment and other prostheses are the suspected source of hospital environments contamination.^{9,10}

In the last year's, hospital infections caused by opportunist micro-organisms in immunosuppressed patients groups have become increasingly important.^{11,12}

Moreover, the biological quality of hospital environments is of particular concern as infected patients may serve as a source of pathogenic microorganisms to staff and hospital visitors, in addition to fellow patients.^{13,14}

In the present study attempts were made to study fungi in the dust of operating theatres (OT), intensive care units (ICU) and neonatal ward (NW) of Sebha Medical Center, which could be risk factors for patients and hospital employees.

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Materials and Methods:

Collection of samples

Over a three-month period from 12 March to 15 June 2012, a total 50 of microbiological dust samples were collected from the hospital units, 16 samples from (OT), 18 samples from (ICU) and 16 samples from (NW) of the hospital. Duplicated samples were collected from each visit at different sites of each unit. Samples from the OT were collected before the operation when the room was not in use. Each dust sample was kept in clean plastic bag at 4-6° C until processing.

Samples processing

Each dust sample were weighing, then 0.25 gram were suspended and transferred aseptically to 9 mm sterile petri dish contain Sabouraud dextrose agar (SDA) (Oxoid, UK) supplemented with 10 mg/L Chloramphenicol (14). The calculated concentrations of dust borne fungi were colony forming units (CFU)/g of dust. Samples were incubated at 25° C with daily observation of the plates for fungal growth for 5-7 days. Diversity and total counts of fungal colonies on SDA plates were recorded. Pure cultures were made from all morphologically different colonies.

The fungal colonies were identified to genus (subgenus in the case of *Aspergillus* isolates) according to the available manuals. The tests were based mainly on growth colonial morphology, reverse and surface coloration. Microscopic examination of the spore and hyphal characteristics of the stained preparations by Lactophenol-cotton-blue solution and scotch tap technique. *Candida albicans* identify by Germ tube test.¹⁵⁻¹⁹

Statistical analysis

The data were analyzed by student t-test. A P-value more than 0.05 was considered as significant.

Results:

From March to June 2012 a total of 50 dust samples were collected from the OT, ICU and NW of the Sebha Medical Centre (Table 1). A total of 202 fungal colonies were isolated and identified. Molds were comprised 83.7% while yeasts were comprised of 16.3 %.

The results show that the average concentration of fungi per gram of dust in OT, ICU and NW was 65 CFU/g, 89 CFU/g and 48 CFU/g, respectively, with significant difference ($P < 0.05$) between the three units. Diversity of fungal types in ICU, NW is ranged between 0-5, and in OT is 0- 4 (Table 1). There was no

significant difference between the three units ($P > 0.05$).

From all isolated fungi, the major present types was *Aspergillus spp.* with 52.47%, *Penicillium spp.* with 22.27%, *Candida spp.* with 16.3%, and the other isolated fungal types were present in low percentages. Also, some of isolated fungi 2.97% were recorded as unknown mold and yeast, because there were difficulties to identify by available diagnostic techniques (Table 2).

34 colony of *Candida spp.* were isolated during the present study. Four isolates (11.76 %) from total *Candida spp.* were identified as *Candida albicans* (Germ tube test positive), while 30 isolates (88.24 %) were identified as *Candida non-albicans spp.*

Eight types of fungi were identified in all studied units in Sebha Medical Centre; Four types of fungi frequent isolated in operating theatres (OT), *Penicillium spp.*, *Aspergillus niger*, *A. fumigates* and *Candida spp.* were isolated with 43.1%, 36.9%, 12.3% and 7.7%, respectively. In intensive care units (ICU) ten fungal types were isolated; the four previous types were present in high percentage than other types of fungi in this unit. While *Aspergillus niger*, *A. terreus*, *Penicillium spp.* and *Candida spp.* were present in high percentage from eight isolated types of fungi in neonatal ward (NW). *Aspergillus niger* was present in highest percentage in ICU and NW than the other fungal types were isolated, while, the unknown mold and yeast absent in OT and present in ICU and NW (Table 3).

Discussion:

About 20% of airborne contaminations were biological materials.⁹ Most of them originate from natural sources such as soil, air, and humans.¹³ Many dangerous elements found in hospital indoor environment including microorganisms. Contamination by these biological agents especially the opportunistic microbes may colonize and transmitted in several areas of hospitals and consider as a potential source of a hospital infection.^{2,20,21}

Microbial populations present in different quantity and types through the Hospital indoor environments.²² That may be generated from human related organisms and external outdoor contamination.^{8,23} The effect of hospital indoor environment contamination on the patients can related to air quality, number of occupants and their physical activities, degree of contamination and the rate of ventilation.²²

Based on the results obtained from the present study, molds is the major part of isolated fungi, which include different type that produce a large number of spores, they can be transmitted by the air and isolated from outdoor and indoor environment.^{23,24} While the yeast is comprised of 16.3% from total isolated fungi, these results are constant with those reported by Overberger *et al*²⁵ and Manuel *et al*.²⁶

Results in Table (1) show that fungal count on OT and NW is significantly low than that in ICU, this may due to the high sanitary standards in this hospital area. In general, ICU had the highest total count of microorganisms. These finding could be explained by many factors including the number of visitors, human activities, building design, opened doors and the amount of material brought from outside, such as flowers and fruits. These are recognized as sources of hospital contamination.^{23,27,28}

The fungal count in this three units in general is low than this reported in outdoor environment.^{23,29,30-32}

Diversity of bacteria and fungi is usually related to the counts.^{20,26} In this study, the fungal diversity (Figure 1) was similar per each sample in all units of the hospitals, while the numbers of types were high and similar in ICU and NW and low in OT.^{5,23,28,29} Ross *et al*³³ and Rainer *et al*¹⁴ approved that the high microbial biodiversity is associated with high temperature and relative air humidity that favor microbial growth.

The common genera of fungi that were identified from the hospital dust in this study were *Aspergillus*, *Penicillium*, *Candida*, *Bipolaris*, *Graphium*, *Curvularia*, *Fusarium*, *Epicoccu* and *Exophilia*. From these nine genera *Aspergillus spp.*, *Penicillium spp.*, *Candida spp.* was the most common fungi isolated, respectively (Table 2), *Aspergillus* and *Penicillium* spores are the most widespread in the world, and they are one of the most common indoor moulds, because they have good ability to survive in the air for long time.^{26,34-36}

In addition, *Candida spp.* was reported in dust with variable percentage in different studies

from different hospital units and indoor environments,^{24,25,37-39} and was considered as the major source of hospital fungal infections. Ahmad *et al*³⁷ reported that 62% of all *Candida* samples collected from ICU patients and nurseries were *C. albicans*. Weinberger *et al* (38) reported that 54% of *Candida spp.* were *C. albicans* from ICU patient and staff. In this study, low percent *C. albicans*. were isolated that may be due to low humidity in desert conditions.

The results of distribution for different isolated fungi in three hospital units (Table 3) showed that the *A. niger* is predominant species in all studied units, while *A. fumigatus* is present in high percent in ICU, and *A. terreus* present only in NW, *A. fumigatus* is the most common species found in invasive aspergillus infections, although *A. terreus*, *A. niger* and other species also frequently cause invasive aspergillosis.⁴⁰⁻⁴²

The high percent of *A. niger* and the variable percent of other species of *Aspergillus* that may due to combination of each indoor and outdoor contamination (Figure 2). These results were similar to the results of Curtis *et al*,⁴² Obbard,¹⁴ Richards⁴³ and Lugauska.³⁶ On other hand, high percent of *Penicillium spp.* were isolated from OT dust than ICU and NW; it might give a signal for external contamination and poor ventilation and disinfectant process.

In addition, *Bipolaris*, *Graphium*, *Curvularia*, *Fusarium*, *Epicoccu* and *Exophilia* were consider as opportunistic fungi and they have different toxic and allergic effects in human health.^{3,44-46}

However, the evolution of microbiological quality of indoor environments are very important especially in health care facilities, in order to prevent hospital infection, determine the degree of cleanliness and to save the health of patients, staff and visitors.

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Table1: The average count of fungal colonies (CFU/g) and the fungal diversity per each sample (* significant value)

	OT	ICU	NW
Number of samples	16	18	16
Average Count of colonies (cfu/g)	65*	89*	48*
Number of types / units	4	10	8
Fugal Diversity			
Range / sample	0-4	0-5	0-5
Average / sample	1.37	1.94	1.62

OT: operating theatres, ICU: intensive care units, NW: neonatal wards

Table 2: Total number and percentage of isolated fungi from all studied units

Isolated fungi	Total Number	Total Percentage (%)
<i>Aspergillus spp</i>	106	52.47
<i>Penicillium sp</i>	45	22.27
<i>Candida spp</i>	33	16.33
Yest, Mold, Unknown	6	2.97
<i>Bipolaris spp</i>	3	1.48
<i>Curvularia spp</i>	3	1.48
<i>Fusarium spp</i>	2	0.99
<i>Epicoccum spp</i>	2	0.99
<i>Exophilia spp</i>	2	0.99

Table 3: The percentage of fungal genera and species in hospital units.

	OT	ICU	NW
Type of isolated Fungi	Percentage of isolated fungi		
<i>A. niger</i>	36.9	33.65	39.5
<i>Penicillium</i>	43.1	10	16.5
<i>Candida</i>	7.7	23.7	14.5
<i>A. fumigatus</i>	12.3	18	4.2
<i>Curvularia</i>	0	2.25	2
<i>A. terreus</i>	0	0	14.5
<i>Bipolaris</i>	0	2.25	2.8
<i>Exophilia</i>	0	2.25	0
<i>Fusarium</i>	0	2.25	0
<i>Epicoccum</i>	0	2.25	0
Mold, Yest, Unknwon	0	3.4	6



Figure 1: Fungal diversity in one sample.

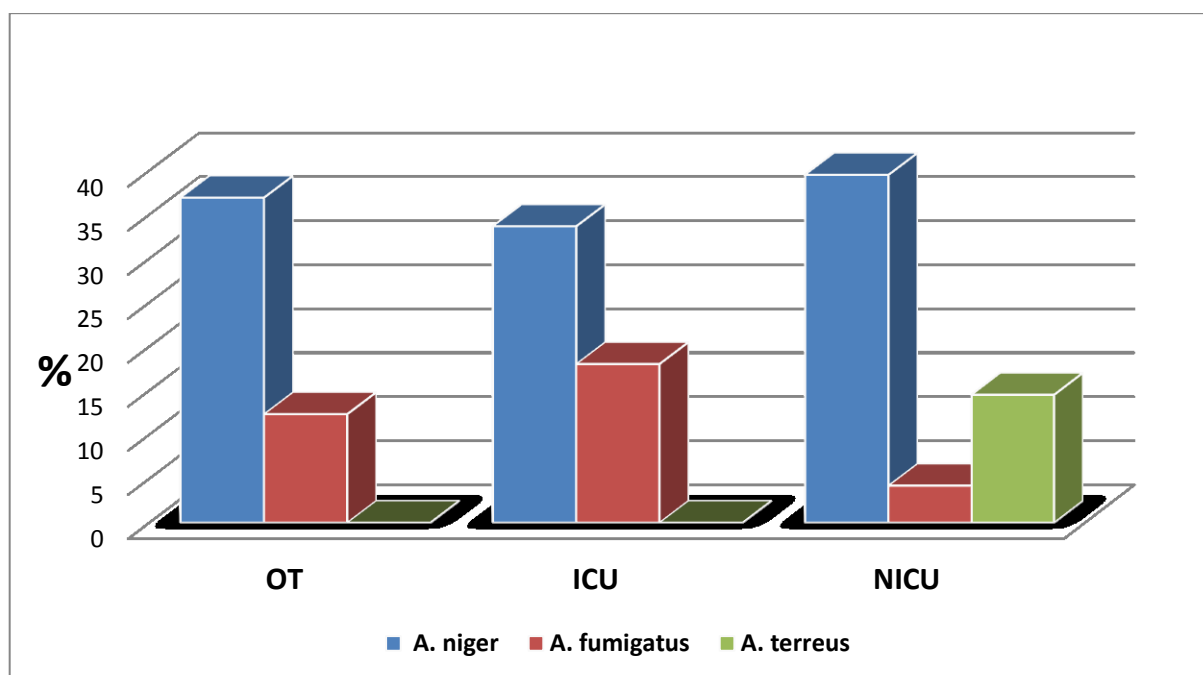


Figure 2: The percentage of *Aspergillus* species on hospital units.

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