Assessment of airborne fungi in the emergency and traumatology departments of University Hospital Centre, Sidi Bel-Abbes, Algeria

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ABSTRACT

Introduction: Airborne fungi in hospital environments represent a significant risk, particularly in wards housing immunocompromised patients, where they may contribute to nosocomial infections, including hospital-acquired mold diseases. This study aimed to assess the presence of airborne fungi in operating theatres, waiting areas, and corridors within the emergency and traumatology departments of the University Hospital Centre in Sidi Bel-Abbes, Algeria, and to examine environmental and human-related determinants.

Method: Seventy-four samples were collected using passive air sampling. Temperature, relative humidity, and occupant density were recorded. Fungi were identified by macroscopic and microscopic characteristics.

Results: Operating theatres showed the lowest contamination 18 (28.10%), whereas corridors and waiting rooms each accounted for 23 (35.9%). Occupant density was significantly associated with fungal levels ($p = 0.05$). *Aspergillus* and *Penicillium* predominated.

Conclusion: The findings indicate that the operating theatres, waiting rooms, and corridors of the emergency and traumatology departments exhibited relatively low levels of fungal contamination. Airborne fungal concentrations, expressed as Colony Forming Units (CFU)/m³ based on agar plate exposure, were within limits; a few specific high-risk samples (12%) in operating theatres exceeded the threshold.

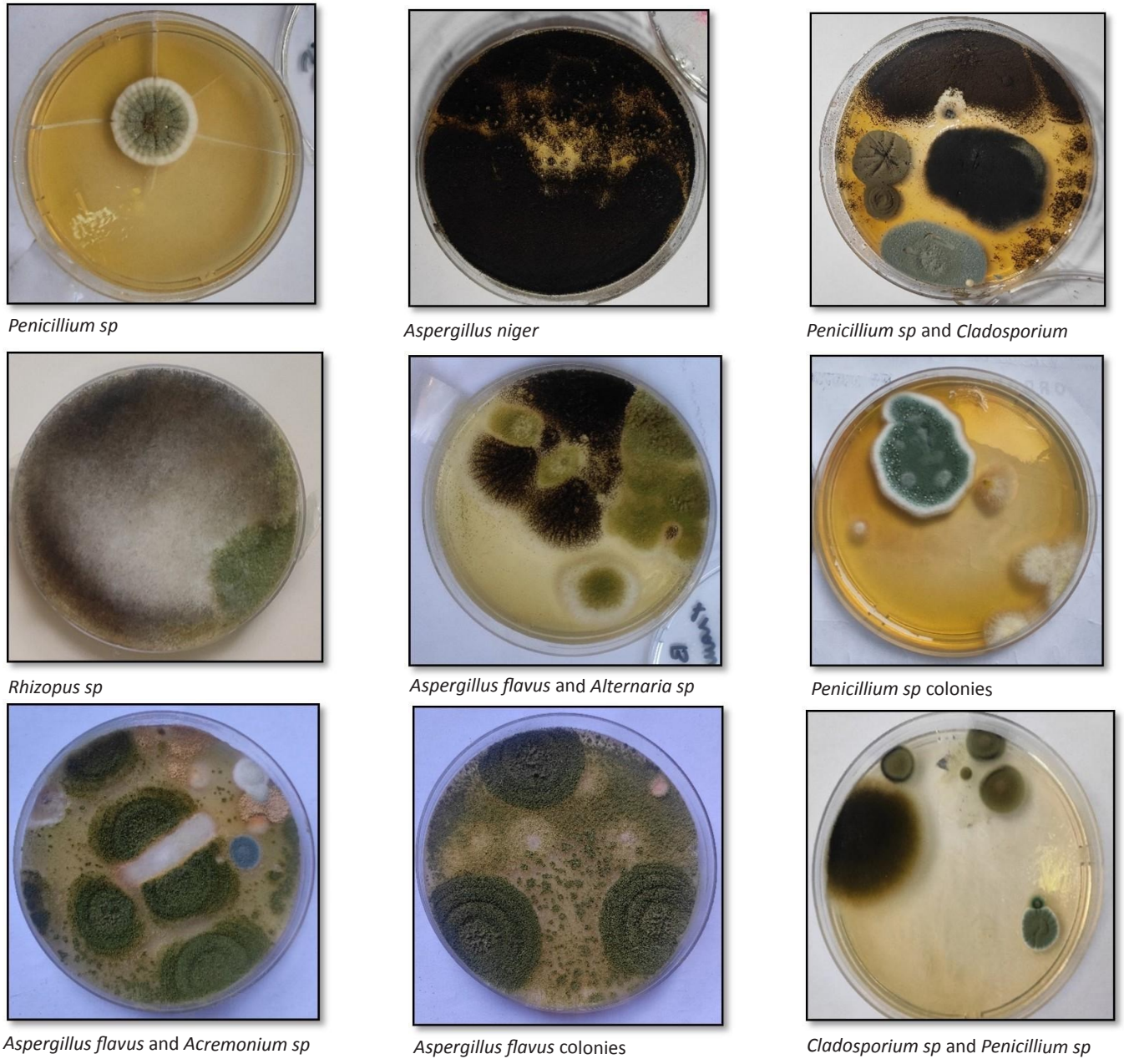
Keywords: indoor air quality; airborne fungi; fungal assessment; operating theatres.

Introduction

The World Health Organization (WHO) identifies air pollution as the world's largest single environmental health risk.[1] The presence of airborne fungi in hospital air was first demonstrated by Noble and Clayton in 1963,[2] and

later in a study by Lidwell and Nobe in 1975.[3] This raises significant concerns, particularly in wards with immunocompromised patients, as a source of nosocomial infections or Hospital-Acquired Mold Infections (HAMI), which represent a major challenge and concern for hospitals, especially with the high costs they generate and the consequences for public health.[3]

The operating theatre environment plays an important role in the causation of post-operative wound infections or surgical site infections (SSI), constituting a third of all nosocomial infections and 20% of all hospital-acquired infections. Several studies have shown that 80 to 90 % of contaminants found in the wound after surgery emanate from the air.[4,5]



Penicillium sp

Aspergillus niger

Penicillium sp and *Cladosporium*

Rhizopus sp

Aspergillus flavus and *Alternaria sp*

Penicillium sp colonies

Aspergillus flavus and *Acremonium sp*

Aspergillus flavus colonies

Cladosporium sp and *Penicillium sp*

Figure 1. Different types of samples collected on SDA media.

The objective of this study was to provide data on fungal concentrations in the indoor air of operating theatres, waiting rooms, and corridors within the traumatology and emergency departments of the University Hospital Centre of Sidi Bel-Abbès, Algeria.

Method

This case study was conducted over a 13-week period, from 1 September to 31 December 2023. The assessment was undertaken at the University Hospital Centre of Sidi Bel-Abbes, Algeria. Two departments were selected: emergency and traumatology

They were selected on the grounds that endo-orthopaedic procedures—i.e., those involving the implantation of prosthetic or artificial components—present a heightened susceptibility to contamination by airborne microorganisms. In addition, the emergency department hosts a diverse array of surgical procedures, each associated with distinct risk profiles and varying levels of sensitivity to microbial contamination.

Sampling was carried out in the following locations: waiting rooms, corridors, and operating theatres. The operating theatre within the emergency department was selected at random. The passive air sampling method was selected due to its practicality, as it utilizes readily available resources. This technique relies on the natural settling of airborne particles by gravity onto the surface of a culture medium. Petri dishes containing 4% Sabouraud dextrose agar supplemented with chloramphenicol were exposed, face upwards, to the ambient air for 15 to 20 minutes, and positioned on the floor at the centre of each sampling location.

Sabouraud dextrose agar is suitable for isolating airborne fungi due to its acidic pH and high dextrose content, which favour fungal growth while inhibiting many bacteria. The addition of chloramphenicol further suppresses bacterial contamination, preventing overgrowth on the culture medium. Together, they ensure selective, reliable detection and enumeration of fungal populations in air samples.

An observational checklist was completed concurrently during each sampling session to record indoor air temperature, relative humidity, the number of occupants present, and the presence or absence of a source of outdoor air.

After sampling, Petri dishes were promptly transported to the laboratory and incubated at 24 °C in an inverted position to prevent condensation, a condition suitable

Table 1. The characteristics of fungal contamination in operating theatres.

Characteristic		Proportion of contamination n (%)
Operating room	Emergency	10 (55.6)
	Traumatology	08 (44.4)
Environmental conditions	Temp < 26 °C	10 (56.0)
	Temp > 26 °C	08 (44.0)
	RH < 60 %	16 (88.9)
	RH > 60 %	02 (11.1)
Surgeries	During surgery	11 (61.0)
	Out of surgery	07 (39.0)
Number of individuals during sampling	≤ 5	12 (66.7)
	≥ 5	06 (33.3)
Type of surgery	Orthopaedic	06 (33.3)
	Digestive	05 (27.8)
	None	07(38.9)
Mold presence	Presence	17 (94.4)
	Absence	01 (05.6)
Yeast presence	Presence	01 (05.6)
	Absence	17 (94.4)
Microbial contamination	Mono-microbial	15 (83.3)
	Multimicrobial	03 (16.7)
Isolated genera	*Candida*	01 (05.6)
	Alternaria	01 (05.6)
	Cladosporium	01 (05.6)
	Penicillium	02 (11.1)
	Aspergillus	13 (72.1)
CFU/m^3 in operating rooms	< 180 CFU/m^3	37 (88.0)
	>180 CFU/m^3	05 (12.0)

Table 2. The analytical section of the study

Characteristic		Contamination (-)	Contamination (+)	Prevalence n (%)	P-value
Sampling locations	Emergency	05	34	39 (87.2)	
	Traumatology	05	30	35 (85.7)	0.854
	Total	10	64	74 (86.5)	
Operating rooms	Outside	03	46	49 (93.9)	
	Inside	07	18	25 (72.0)	0.009
	Total	10	64	74 (86.5)	
Waiting rooms	Outside	08	41	49 (83.7)	
	Inside	02	23	25 (92.0)	0.322
	Total	10	64	74 (86.5)	
Corridors	Outside	09	41	50 (82.0)	
	Inside	01	23	24 (95.8)	0.103
	Total	10	64	74 (86.5)	
Ventilation conditions	Not ventilated	10	43	53 (81.1)	
	Ventilated	00	21	21 (100.0)	0.322
	Total	10	64	74 (86.5)	
Air temperature	< 26 °C	06	36	42 (85.7)	
	> 26 °C	04	27	31 (87.1)	0.865
	Total	10	63	73 (86.3)	
Air humidity level	< 60 %	10	60	70 (85.7)	
	> 60 %	00	04	04 (100.0)	0.416
	Total	10	64	74 (86.5)	
Number of individuals during sampling	≤ 5	07	24	31 (77.4)	
	≥ 5	03	40	43 (93.0)	0.05
	Total	10	64	74 (86.5)	
Surgeries	Absence	03	07	10 (70.0)	
	Presence	07	57	64 (89.1)	0.101
	Total	10	64	74 (86.5)	
CFU/m^3	<1000	07	38	45 (84.4)	
	>1000	00	05	05 (100.0)	0.206
	Total	07	43	50 (86.0)	
Mold contamination	Absence	10	02	12 (16.7)	
	Presence	00	62	62 (100.0	0.0001
	Total	10	64	74 (86.5)	

Yeast contamination	Absence	10	61	71 (85.9)	0.485
	Presence	00	03	03 (100.0)	
	Total	10	64	74 (86.5)	
Multi-microbial contamination	Mono-microbial	10	33	43 (76.7)	
	Multi-microbial	00	31	31 (100.0)	0.004
	Total	10	64	74 (86.5)	

for the growth of most fungal species. The cultures were maintained for 7–10 days with daily observations.

The grown colonies were identified depending on morphological properties (shape, size, colour, elevation and edge shape), as well as microscopic examination (40X) to identify their proliferative parts, conidia shape, size, situation and mycelium segmentation (Figure 1).

Fungal isolates were subjected to microscopic examination using Lactophenol Cotton Blue (LPCB) staining following tape preparation/tease mount techniques. Identification was based on both macroscopic and microscopic characteristics, including colony texture and pigmentation, as well as the morphology of conidiophores, vesicles, phialides, and conidial arrangements observed under light microscopy.

Specifically, *Aspergillus* species were differentiated according to standard mycological criteria:

Aspergillus niger was identified by the presence of large globose vesicles with biseriate phialides and black radiate conidial heads.

Aspergillus flavus was characterized by rough conidiophores, predominantly uniseriate/biseriate phialides covering the vesicle, and yellow-green conidial heads. »

Following enumeration, airborne fungal concentrations were expressed as CFU/m³ using Omeliansky's equation. [6]

$$N = \frac{5a \times 10^4}{bt}$$

N: microbial (CFU/m3) of indoor air.

a: number of colonies per Petri dish.

b: dish surface (cm2).

t: exposure time (minutes).

CFU/m³ (Colony-Forming Units per cubic meter) is a standard unit used to quantify the concentration of viable microorganisms in air. It represents the number of microbial colonies that can grow on a culture medium from a defined volume (1 m³) of air, providing an estimate of airborne microbial load.

To minimize misidentification, presumptive *Candida* isolates were further examined microscopically after Gram staining. The isolates showed oval to budding yeast cells consistent with **Candida** morphology. In addition, colony texture and creamy appearance on Sabouraud agar were considered during preliminary identification. No chromogenic media or germ tube testing was performed in the present study, and this limitation has been acknowledged in the revised manuscript.

Results

Characteristics of fungal contamination in operating theatres

Table 1 summarizes the characteristics of fungal contamination in operating theatres. The highest proportion of contamination, 10 (55.60%), was observed in the emergency operating theatre. Operating theatres with relative humidity below 60% exhibited the highest contamination rate at 16 (88.90%).

During surgical procedures, contamination was recorded in 11 (61%), representing the highest rate. The highest contamination rate was also observed in operating theatres with fewer than five occupants, at 12 (66.70%). Among contaminated samples in operating theatres, 17 (94.40%) contained mould.

Samples exhibiting single-species contamination (Mono-microbial) were predominant, accounting for 15 (83.30%). Aspergillus was the most frequently isolated

genus, present in 13 (72.10%). Notably, 5 samples (12%) in operating theatres exceeded 180 CFU/m³, indicating significant contamination.

Analytical section

Table 2 summarizes the analytical findings of the study.

Regarding sampling locations, the correlation between contamination and the department type (emergency versus traumatology) was not statistically significant (p = 0.854). In contrast, a significant correlation was observed between contamination and operating theatres (p = 0.009).

Concerning environmental conditions, no significant correlation was found between contamination and natural ventilation, temperature, or relative humidity (RH), with p-values of 0.322, 0.865, and 0.416, respectively.

However, significant correlations were identified between contamination and the number of occupants during sampling, the presence of mould, and the occurrence of multi- microbial contamination, with p-values of 0.05, 0.0001, and 0.004, respectively.

Discussion

The emergency department showed a slightly higher, though not statistically significant, contamination prevalence (39 [87.2%] vs 35 [85.7%] in traumatology; p = 0.854) (Table 2). Within operating theatres, contamination was greater in the emergency theatre (10 [55.6%] vs 8 [44.4%]) (Table 1), likely reflecting higher patient turnover and the variety of acute and contagious cases managed.

The incubation temperature of 24°C was selected because it is commonly recommended for the cultivation and enumeration of environmental airborne fungi, particularly filamentous molds, in indoor air quality studies. This temperature favours the growth and sporulation of a broad spectrum of environmental fungal species while limiting excessive bacterial proliferation. In addition, several previous studies investigating fungal air contamination in hospital environments have used incubation temperatures close to 25°C for environmental monitoring purposes.

Contamination prevalence was significantly higher outside operating theatres (49 [93.9%]; p = 0.009) (Table 2), while operating theatres showed the lowest levels, likely due to restricted access, strict hygiene protocols, and controlled locations. These results align with findings from Nigerian teaching hospitals, where operating theatres also exhibited the lowest fungal concentrations (10–15 CFU/m³).[7]

Contamination was more prevalent in areas outside waiting rooms, affecting 41 (64.1%) of samples. Within waiting rooms, a higher, though not statistically significant, prevalence was observed (92% vs 83.7% outside; p = 0.322), consistent with findings from the Nigerian study.[7]

The prevalence of contamination was higher inside corridors, with 24 (95.80%), compared to 50 (82%) outside corridors; however, this difference was not statistically significant (p = 0.103) (Table 2).

No significant association was observed between contamination and the presence of natural ventilation (Table 2). Nevertheless, ventilation—natural or mechanical—can influence indoor fungal levels by diluting, removing, and replacing air, as supported by previous studies.[4,7]

In operating theatres, contamination was 10 (56%) at temperatures below 26 °C (Table 1), with a slight, non-significant increase in warmer areas (p = 0.865) (Table 2), indicating no clear temperature effect. While an Iranian study observed a similar non-significant rise in fungal growth at higher temperatures,[8] other research has reported a strong correlation between indoor fungal load and ambient temperature.[9]

Contamination in samples collected at ambient humidity below 60 % was 60 (93.8%), with 42 (85.7%) in operating rooms. This difference was not statistically significant (p = 0.416), consistent with a Portuguese maternity unit study,[10] although other research, such as in Southern Thailand hospital wards, reported a significant correlation between relative humidity and fungal contamination.[11]

Overall contamination at relative humidity below 60 % was 60 (93.8%) (Table 2), with 16 (88.9%) in operating theatres (Table 1). This difference was not statistically significant (p = 0.416), aligning with a Portuguese maternity unit study,[10] although other research, such as in Southern Thailand,[11] has reported a significant correlation between humidity and hospital fungal contamination.

In operating theatres, contamination increased with more than five individuals present during sampling, reaching 12 (66.7%) (Table 1). Overall, areas with over five occupants showed higher contamination (43 [93%] vs 31 [77.4%] (Table 2) in areas with five or fewer), with a significant correlation between fungal levels and occupant density (p = 0.05), consistent with previous findings from a Saudi Arabian university hospital.[12]

Contamination prevalence was higher in active operating theatres (64 [89.1%]) (Table 2) compared with idle

theatres (10 [70%]), possibly reflecting the impact of staff activity and patient-related exposure. However, this difference was not statistically significant (p = 0.101), in contrast to findings reported in other studies.[13]

Mould was detected in 97% of positive samples, while yeasts were found in 5%, with 48% showing mixed growth; in operating theatres, mould predominated (94%), and contamination was strongly associated with mould (p = 0.0001) but not with yeasts (p = 0.485), likely reflecting the limited sensitivity of passive air sampling for yeasts (Table 2).

A significant correlation was observed between multi-species contamination and overall contamination (p = 0.004), with higher microbial loads in such environments, suggesting that these settings may favour microbial proliferation and reflect potential shortcomings in hygiene practices.

Aspergillus spp. was the most frequently detected genus 56 (33.7%), followed by *Aspergillus flavus* 47 (28.3%), Penicillium spp. 21 (12.7%), and *Aspergillus niger* 14 (8.4%). In operating theatres, *Aspergillus spp.* predominated 13 (72.2%), followed by *Penicillium spp.* 2 (11.1%), while *Cladosporium, Alternaria*, and *Candida* were each detected in a single case (5.6%).

Although direct comparisons are limited by methodological and environmental differences, numerous studies consistently report these genera as the most prevalent airborne fungi in hospital indoor environments.[14]

Across multiple studies, the most frequently reported genera were *Aspergillus spp.* (24 studies), *Penicillium spp.* (23 studies), *Cladosporium spp.* (17 studies), and *Fusarium spp.* (10 studies), while *Candida spp.* was the most common unicellular fungus. Within *Aspergillus*, the most prevalent sections were Niger (18 of 24 studies), followed by *Fumigatus* (14 of 24) and *Flavus* (9 of 24).[15]

A study conducted across five educational hospital wards in Iran reported that the highest fungal populations were *Penicillium spp*. (32.06%), *Cladosporium spp.* (20.5%), *Aspergillus fumigatus* (14.61%), and *A. niger.*[13]

Similarly, a study carried out in the Paediatric Unit of Edirne Government Hospital in Turkey found that the most frequent genus was *Cladosporium*, with 462 colonies (33.58%), followed by *Alternaria* with 310 colonies (22.53%) and *Penicillium* with 280 colonies (20.35%).[16]

A higher, though not statistically significant, prevalence was observed in samples with CFU/m³ exceeding 1000 5 (100%) compared with those below this threshold (p = 0.206) (Table 2), consistent with the World Health Organization's recommended limits.

In operating theatres, 37 (88%) of CFU/m³ values were below 180 (Table 1), corresponding to levels associated with a lower risk of postoperative wound infections.[5]

Although passive air sampling is practical and inexpensive for routine environmental surveillance, it is less sensitive and less quantitative than active volumetric sampling methods.

Future investigations should incorporate molecular identification methods, such as PCR and sequencing, to improve the accuracy of fungal identification at the species level. The inclusion of antifungal susceptibility testing would also be highly relevant, particularly for the detection of emerging antifungal-resistant strains, including azole-resistant *Aspergillus spp.*, which represent an increasing concern in hospital-associated infections and infection control programs.

Conclusion

Most fungal isolates were detected at levels below 180 CFU/m³ in operating theatres and below 1000 CFU/m³ in waiting rooms and corridors, remaining within internationally accepted limits and indicating effective infection control measures. However, the presence of *Aspergillus* underscores the need for improved cleaning and disinfection practices.

Overcrowding should be minimized, as occupant density is positively associated with airborne fungal levels; restricting visitors may therefore help reduce indoor microbial load in hospital settings.

Future studies should incorporate molecular identification techniques and antifungal susceptibility testing to better characterize airborne fungal species and detect emerging resistant strains, particularly azole-resistant *Aspergillus spp*. of clinical importance in hospital environments.

Conflict of interest: None

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Authors' contributions: DM and SM contributed to study conceptualization, methodology, data interpretation, and drafting of the original manuscript. ZB and AB contributed to the methodology. YM, MAB, and OG conducted sampling and contributed to data interpretation.

Ethics approval and consent to participate:

The study protocol was approved by the Ethics Committee of the Sidi Bel-Abbes University Hospital Centre (Ref: Opinion No. 34, 17 June 2023). Informed consent was obtained from all participants.

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