# Identification of Environmental Fungal Species in Clinical Services of University Hospital of Angre, Abidjan (Cote d'Ivoire)

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## Abstract

**Introduction:** In hospital's environment, hygiene of premises and ambient air quality are essential for healthcare. Airborne fungal agents constitute a health concern for patients in these premises.

**Objective:** To identify the fungal agents of the environment in the clinical services of University Hospital of Angre.

**Materials and Methods:** This was a descriptive cross-sectional study was carried out in the clinical services of the University Hospital of Angre from February to May 2024. Samples were taken by swabbing and exposing sterile Petri dishes to the air; then they were plated on Sabouraud-Chloramphenicol medium and incubated in oven at 37° C. Identification of yeast strains was performed using chromogenic medium and the VITEK 2□ Compact. Molds identification was based on the macroscopic characteristics and microscopic examination of colonies.

**Results:** Among 429 samples, 206 positive cultures (48%) with a higher positivity rate in the surgical emergency service (93.1%). The most isolated fungal agents were molds (84%) with *Aspergillus niger* at 41.4%. *Candida parapsilosis and Candida krusei* species were the most prevalent yeasts isolated respectively at 42.3% and 23.1%. The frequency rate of fungal species was correlated to clinical services and sampling sites (p<0.001). The implementation of rigorous hygiene measures and regular decontamination of fungal niches should be systematically to avoid possible nosocomial infections.

**Keywords:** Fungal Agents; Environment; Clinical Services; University Hospital of Angre; Cote d'Ivoire

## Introduction

Nosocomial fungal infections or more broadly, fungal infections related to healthcare and the hospital environment, remain dreaded diseases [1,2]. These diseases are common complication among hospitalized patients especially between who is immunocompromised [3,4]. Environmental fungal contamination has been associated with health consequences including infectious diseases, toxic effects and allergies. The great diversity of fungal agents constitutes a

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risk of environmental contamination and the onset of human diseases; they may also come from clinical waste samples [1,5].

The last decade has seen the emergence of new opportunistic fungal agents, including yeasts and molds. Research has shown that filamentous fungi with translucent mycelia of the *Aspergillus* genus are the most frequently observed airborne and hospital environmental fungal flora [4,6]. These fungal agents represent a major concern for intensive care units and a significant challenge for physicians and microbiologists [7].

Furthermore, the emergence of these fungal agents in the environment and their predominance in certain departments such as hospitalization and intensive care, combined with their variable resistance to certain antifungal agents, is one of the causes of therapeutic failure. This makes it difficult to manage patients in these services [7,8].

In Cote d'Ivoire, as little is known on a national scale, the impact of these fungal agents causing nosocomial infections in immunocompromised patients is estimated by healthcare staff for better management of nosocomial infections in hospital wards [6]. Hygiene in health establishments is defective, and the prevalence of nosocomial infections is alarmingly high. Thus, the prevalence of nosocomial infections is estimated 9% and 12% in the university hospital of Cocody and Yopougon [9]. Therefore, measures must set up to prevent the contraction of nosocomial fungal infections within the various health structures. However, there is no data on environmental fungal contamination of clinical services and consequently no knowledge of the likely fungal species present. This study aimed to determine the biodiversity of environmental fungal flora in clinical services of the University Hospital of Angre.

## Material and Methods

### Study design and areas

This was a descriptive cross-sectional study carried out over 4 months from February to May 2024 in clinical services at University Hospital of Angre, Abidjan. The services involved in this study were intensive care, neonatology, gynecological consultation and hospitalization, operating room, surgical, gynecological and medical emergencies. The hospital receives patients from all the municipalities of Abidjan and other cities. It boasts high-quality technical facilities, modern health infrastructures and a wide range of services.

### Samples collection

Samples were taken from the ambient air, main door, air-conditioner, incubator the nursing staff's bench, chariot,

storage cabinet, etc. A sheet was drawn up for data collection. Samples were collected by swabbing (surfaces) and exposure in Petri dishes containing Sabouraud-Chloramphenicol agar.

### Mycological analysis

Surface samples were taken with two sterile swabs: one for direct examination and the other for culture on Sabouraud-chloramphenicol medium poured into tubes. Exposed Petri dishes and inoculated tubes were incubated at 37°C for 3 to 4 days. Fungal agents isolated after culture were identified macroscopically by the appearance of their colonies. Yeast colonies were whitish, smooth, hairless, moist, shiny or matte. In the case of molds, downy, woolly or cottony colonies of varying colors were observed, depending on the species. Yeasts were identified using chromogenic medium method (CHROMagar™ Candida). The Vitek 2 Compact® (Biomerieux) was used according to manufacturer's procedures in case of yeast diagnosis difficulty.

Molds isolated from culture were identified by their microscopic appearance in lactophenol blue between slide and coverslip at x40 magnification. Thus, for *Aspergillus* genus, ´identification was based on the identification of conidiophores, of variable length depending on the species, which terminated in aspergillate heads (uniseriate or biseriate heads) consisting of´a set of vesicles, phialides and conidia.
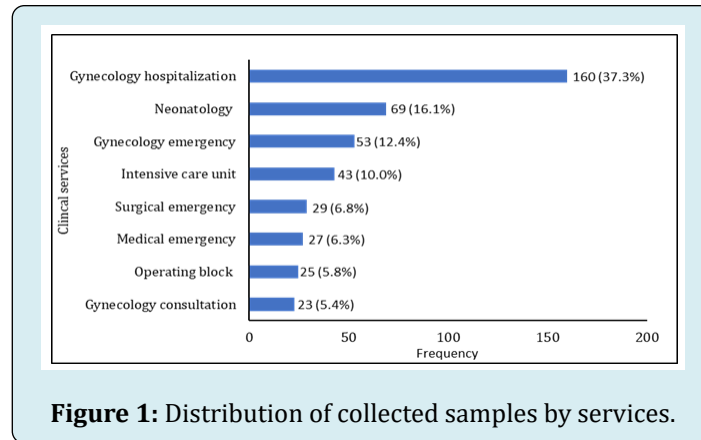
### Statistical analysis

Data analysis was performed using SPSS 21 software (Statical Package for Social Science, IBM SPSS Statistics). All variables were classified in a group and the Fisher exact test was used to compare proportions. P-value < 0.05 was considered significant.
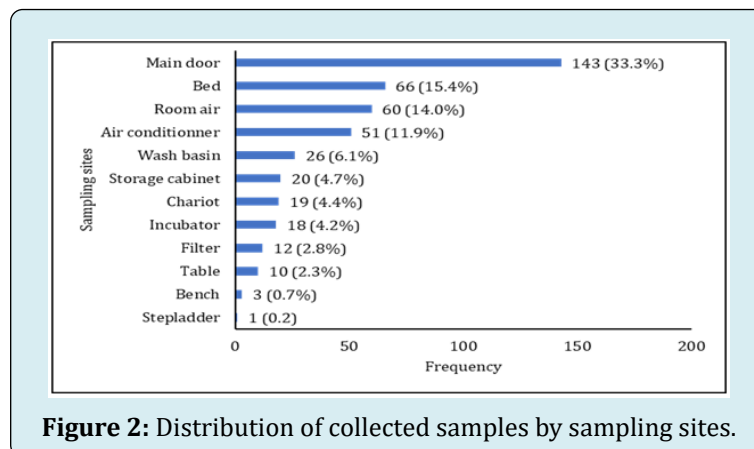
## Results

### Overall results

A total of 429 samples were collected from eight clinical services and 12 sampling sites at University Hospital of Angre, Abidjan. Samples were distributed unevenly between the various clinical services. The gynecological hospitalization service has the largest proportion with 37.3%, followed by neonatology with 16.1%. Gynecological emergencies and intensive care have less significant proportions, with 12.3% and 10% respectively. Medical emergencies, surgical emergencies, operating block and gynecological consultations have the lowest frequencies (Figure 1).

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**Figure 1:** Distribution of collected samples by services.

Door handles are the most frequent sampling sites, accounting for 33.3%. Beds followed with 66 samples (15.4%), indicating a high level of potential contamination. Air conditioners and room air show significant proportions of 11.9% and 14% respectively. Sinks and cupboards had proportions of 6.1% and 4.7% respectively. Other sites, such as tables, benches, carts, filters, incubators and slings, represent lower percentages, indicating varying levels (Figure 2).



**Figure 2:** Distribution of collected samples by sampling sites.

### Mycological results

From 429 collected samples, 206 gave a positive culture with an environmental fungal colonization with a rate of 48%. A statistically significant difference between services and culture results was observed (p=0.0001). Surgical emergency service had the high frequency in positive samples (93.1%) following by medical emergency (85.2%) service and operating block (80%). The lowest positive frequency was observed in intensive care unit at 25.6%. The distribution of positive samples by clinical services is shown in Table 1.

According to positive samples number by sampling site, main door had the highest number of positive samples (63), followed by ambient air (41) and air conditioners (34). But in terms of frequency rate, filter and wash basin were the most contaminated sites with 83.7% and 76.1% respectively. The difference between sampling sites and positive samples is statistically significant (p-value = 0.0001). Table 2 shows the distribution of positive samples by sampling sites.

| Clinical services | Negative culture | | | Positive culture | |
|---|---|---|---|---|---|
| | Total | Frequency (n) | Percentage (%) | Frequency (n) | Percentage (%) |
| Gynecology hospitalization | 160 | 93 | 58.1 | 67 | 41.9 |
| Neonatology | 69 | 40 | 58 | 29 | 42 |

| | | | | | |
|---|---|---|---|---|---|
| Gynecology emergency | 53 | 37 | 69.8 | 16 | 30.2 |
| Intensive care unit | 43 | 32 | 74.4 | 11 | 25.6 |
| Surgical emergency | 29 | 2 | 6.9 | 27 | 93.1 |
| Medical emergency | 27 | 4 | 14.8 | 23 | 85.2 |
| Operating block | 25 | 5 | 20 | 20 | 80 |
| *p-value* | *0.0001* | | | | |

**Table 1:** Distribution of negative and positive samples by clinical services.

| Sampling sites | Negative culture | | | Positive culture | |
|---|---|---|---|---|---|
| | **Total** | **Frequency (n)** | **Percentage (%)** | **Frequency (n)** | **Percentage (%)** |
| Main door | 143 | 81 | 56.7 | 63 | 43.3 |
| Bed | 66 | 46 | 69.7 | 20 | 30.3 |
| Room air | 60 | 19 | 31.7 | 41 | 68.3 |
| Air conditioner | 51 | 17 | 33.3 | 34 | 66.7 |
| Wash basin | 26 | 6 | 23.1 | 20 | 76.1 |
| Storage cabinet | 20 | 15 | 75 | 5 | 25 |
| Chariot | 19 | 15 | 78.9 | 4 | 21.1 |
| Incubator | 18 | 13 | 72.2 | 5 | 27.8 |
| Filter | 12 | 2 | 16.7 | 10 | 83.7 |
| Table | 10 | 7 | 70 | 3 | 30 |
| Bench | 3 | 1 | 33.3 | 2 | 66.7 |
| Stepladder | 1 | 1 | 100 | 0 | 0 |
| *p-value* | *0.0001* | | | | |

**Table 2:** Distribution of negative and positive samples by sampling sites.

In positive culture, molds were the most frequently identified at 84% following by yeasts (11.2%). Association between molds and yeasts was found at 4.8%. In the molds group, *Aspergillus niger* is the most frequently isolated species, accounting for 41.4% of cases, followed by *Aspergillus flavus* with 22.1%. Other *Aspergillus* species are also present, such as *Aspergillus fumigatus* (7.1%). In addition to *Aspergillus* species, other molds were identified, such as *Fusarium oxysporum* (7.5%), *Mucor sp.* (10.7%) and *Penicillium sp.* (10.4%). *Alternaria sp.* was the least frequent molds with 0.7% of cases (Figure 3).

*Candida parapsilosis* was the most frequently isolated species at 42.3% of cases. Other *Candida* species were also present, such as *C. krusei* (23.1%), *C. guilliermondii* (15.4%), and *C. lusitaniae* at 11.5%. *Candida albicans* and *Candida famata* are the least frequent species, each accounting for 3.8% of cases (Figure 4).
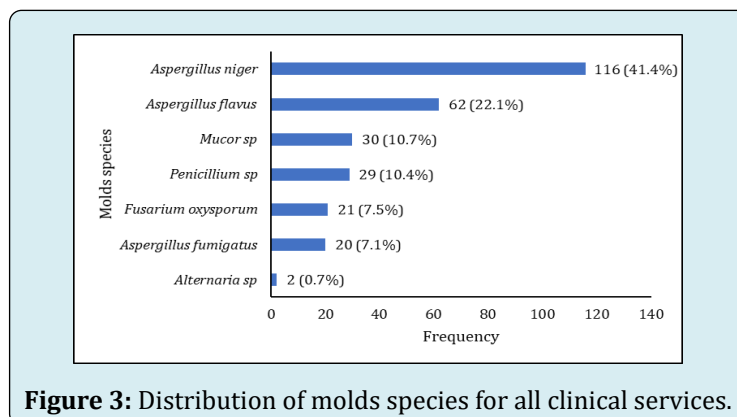


**Figure 3:** Distribution of molds species for all clinical services.
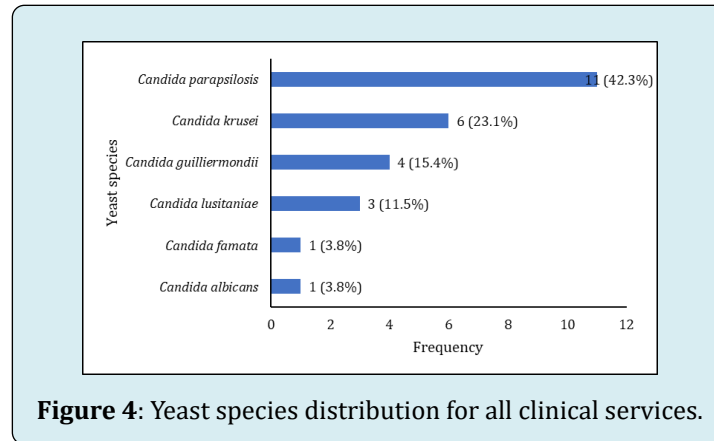
**Figure 4**: Yeast species distribution for all clinical services.

The distribution of isolated mold species by clinical services shows that *A. niger* and *A. flavus* were identified from all services with a high frequency in gynecology hospitalization service at 43.1% and 30.6% respectively. *Penicillium sp.* and *Mucor sp.* were also observed in this service (Table 3). In the same time, surgical emergency, gynecological hospitalization and medical emergency were the services with the most yeast species, such as *C. parapsilosis*, *C. guilliermondii* and *C. krusei* (Table 4).

| Clinical services | *A. niger* | *A. flavus* | *A. fumigatus* | *Alternaria sp* | *Penicillium sp* | *Mucor sp* | *Fusarium oxysporum* |
|---|---|---|---|---|---|---|---|
| Operating block | 2 | 8 | 0 | 2 | 8 | 0 | 9 |
| Intensive care unit | 4 | 3 | 1 | 0 | 0 | 1 | 0 |
| Surgical emergency | 10 | 11 | 1 | 0 | 0 | 0 | 10 |
| Medical emergency | 15 | 1 | 4 | 0 | 0 | 0 | 1 |
| Neonatology | 11 | 6 | 3 | 0 | 9 | 0 | 0 |
| Gynecology consultation | 10 | 2 | 0 | 0 | 0 | 1 | 0 |
| Gynecology hospitalization | 50 | 19 | 5 | 0 | 11 | 28 | 1 |
| Gynecology emergency | 14 | 12 | 1 | 0 | 1 | 0 | 0 |
| **Total** | 116 | 62 | 15 | 2 | 29 | 30 | 21 |

A : Aspergillus
**Table 3:** Distribution of mold species by clinical services.

| Clinical services | *C. parapsilosis* | *C. guilliermondii* | *C. lusitaniae* | *C. krusei* | *C. famata* | *C. albicans* |
|---|---|---|---|---|---|---|
| Operating block | 0 | 0 | 0 | 0 | 0 | 0 |
| Intensive care unit | 1 | 0 | 1 | 2 | 0 | 0 |
| Surgical emergency | 0 | 5 | 0 | 0 | 1 | 0 |
| Medical emergency | 0 | 0 | 0 | 4 | 0 | 1 |
| Neonatology | 2 | 1 | 2 | 0 | 0 | 0 |
| Gynecology consultation | 3 | 1 | 0 | 0 | 0 | 0 |
| Gynecology hospitalization | 4 | 0 | 0 | 0 | 0 | 0 |
| Gynecology emergency | 1 | 0 | 0 | 0 | 0 | 0 |
| **Total** | 11 | 7 | 3 | 6 | 1 | 1 |

*C : Candida*
**Table 4:** Distribution of yeast species by clinical services.

We recorded 77 mixed species in our study with a rate of 37.6%. Table 5 shows the fungal species isolated in mixed cultures for all sectors sampled from clinical services. So, for all sampling sites, *Aspergillus niger - Aspergillus flavus* association predominated at a frequency of 20.8%, followed by *Aspergillus niger - Aspergillus flavus - Mucor sp.* (11.6%), *Aspergillus niger - Mucor sp.* (10.4%) and *Aspergillus niger-Aspergillus fumigatus* (7.8%). Also, association with *Fusarium oxysporum - Penicillium sp.* and *Aspergillus niger- Aspergillus flavus- Fusarium oxysporum* were observed at 5.2%°.

| Fungal species | Frequency (n) | Percentage (%) |
|---|---|---|
| *Aspergillus niger - Aspergillus flavus* | 16 | 20.8 |
| *Aspergillus niger-Aspergillus flavus-Mucor sp.* | 9 | 11.6 |
| *Aspergillus niger - Mucor sp.* | 8 | 10.4 |
| *Aspergillus niger - Aspergillus fumigatus* | 6 | 7.8 |
| *Fusarium oxysporum - Penicillium sp.* | 4 | 5.2 |
| *Aspergillus niger-Aspergillus flavus-Fusarium oxysporum* | 4 | 5.2 |
| *Aspergillus niger- Candida krusei* | 3 | 3.9 |
| *Aspergillus niger - Candida guilliermondii* | 3 | 3.9 |
| *Penicillium sp. -Aspergillus flavus -Mucor sp* | 3 | 3.9 |
| *Aspergillus niger-Penicillium sp. -Aspergillus flavus* | 3 | 3.9 |
| *Aspergillus niger- Penicillium sp.* | 2 | 2.6 |
| *Aspergillus niger - Fusarium oxysporum* | 2 | 2.6 |
| *Fusarium oxysporum - Aspergillus flavus* | 2 | 2.6 |
| *Aspergillus niger- Aspergillus flavus -Candida parapsilosis* | 2 | 2.6 |
| *Aspergillus niger-Mucor sp. - Aspergillus fumigatus* | 2 | 2.6 |
| *Aspergillus niger- Aspergillus fumigatus- Aspergillus flavus* | 2 | 2.6 |
| *Penicillium sp. - Aspergillus fumigatus* | 1 | 1.3 |
| *Aspergillus flavus - Mucor sp.* | 1 | 1.3 |
| *Penicillium sp. - Mucor sp.* | 1 | 1.3 |
| *Aspergillus flavus - Aspergillus fumigatus* | 1 | 1.3 |
| *Penicillium sp. -Aspergillus flavus-Fusarium oxysporum* | 1 | 1.3 |
| *Aspergillus niger-Aspergillus fumigatus-Fusarium oxysporum* | 1 | 1.3 |

**Table 5:** Distribution of fungal species in mixed culture.

## Discussion

This cross-sectional study carried out in eight clinical services at the University Hospital of Angre in Cote d'Ivoire showed unequal distribution of samples between clinical services. The gynecological hospitalization service has the largest sample size. The global frequency rate of environmental fungal colonization was 48%. Higher results than ours were reported in Senegal [4] and Burkina Faso [10]. This high frequency in his study could be explained by a smaller sample size, which was 73 samples. Furthermore, results similar to ours were observed in Cote d'Ivoire at the University Hospital of Bouake, central part of Cote d'Ivoire with a fungal colonization rate of 48.4% [6].

The fungal colonization frequency was highest in the gynecological and neonatology services, and lowest in the intensive care unit. These findings could be explained by the high number of workers in the services which can lead to the surfaces contamination. In contrast, studies carried out in Dakar showed a high frequency in pediatric oncology service and a low positivity rate in neonatology [4]. The difference in frequencies observed may be due to the small sample size in these different studies. We noted a high frequency of colonization at doors and in ambient air. These Similar results were reported in the environment of intensive care units in Bobo-Dioulasso [10]. These results justify to the dissemination of fungal spores in hospital services which penetrate inside buildings. The presence of these fungal

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agents is a major public health concern, economic and social implications [3,11].

According to the distribution of the different fungal groups observed, molds were the main ones isolated. Filamentous fungi, such as molds were isolated in tertiary care hospital in previous studies [12,13]. The predominance of these fungal groups has also been described in the literature [4,8,14]. Furthermore, when conditions are favorable, molds produce a large number of mature spores that can be transported by air currents or by humans, and find their way into homes and buildings, including hospitals. The production of spores aerosolized by air can disperse over great distances and to their diversity [10,15]. In the current study, *Aspergillus* and *Penicillium* were the most frequently isolated mold genus. The predominance of the genus *Aspergillus* was also observed in Cote d'Ivoire [6]. Elsewhere in Brazil, similar studies were conducted in various cities where *Penicillium sp.* was more identified [16,17]. *Aspergillus* and *Penicillium* genus are ubiquitous, cosmopolitan filamentous fungi that are very abundant in the environment. *Aspergillus niger* was the most prevalent species. In general, this species is widespread in the outdoor environment as a saprophyte [18,19]. The spores were associated with positive reactions allergen in human body [20]. In another study, *A. niger* was among the most prevalent fungal agents in samples from houses of mold-allergic patients and hospital air samples [21]. *Aspergillus flavus* and *A. fumigatus* identified in our study were also observed in air in different areas of a hospital [10,22]. Studies conducted in hospitals have shown that *A. flavus* can be present in dust inside homes, in ventilation ducts and on contaminated building materials. In a study carried out in Nigeria, *A. flavus* was identified in wall surface samples taken from damp homes [23]. *Aspergillus flavus* has also been identified in filters, which can serve as a source of indoor contamination in hospitals [10]. *Aspergillus fumigatus* identified in our study has been reported elsewhere. The small size of *A. fumigatus* spores may favor their deposit in the upper respiratory tract [24].

Molds in hospital environment have been observed to exacerbate respiratory presentations with *Aspergillus sp.*, *Penicillium sp.*, and *Alternaria sp.* found to comprise the predominant allergenic airborne spores [25,26]. These fungal agents produce numerous small, light spores that remain in the air for a long time, dispersing more rapidly. This can be life-threatening for severely immunocompromised patients [27,28].

As for the yeast species isolated in the current study, *Candida parapsilosis* and *C. guilliermondii* were the predominant species, followed by *Candida albicans*, *C. lusitaniae* and *C. krusei*. *Candida parapsilosis* is a fungal agent that lives on human body without causing clinical symptoms. If it gets into a wound, surgical incision or implanted medical device, this species could be involved in a serious infection [29]. In addition, the isolation of *Candida* non-*albicans* species in our study confirms the emerging nature of these yeasts, which is linked to the increase in the number of immunocompromised patients as a result of the growing number of risk factors (HIV, corticosteroid therapy, chemotherapy, diabetes, etc.). The presence of yeasts in the hospital environment could be associated with care (fibroscopy and oxygen therapy); with the use of medical devices (catheter); with patients and/or nursing staff [30].

Mixed culture in the current study was observed with the association between molds species. These results indicated the potential of hospital services as reservoirs for fungal species [31,32].

## Conclusion

These results highlight the need for measures to prevent the spread of fungal agents in the hospital environment, particularly in clinical wards where patients are at high risk. These measures can include air renewal, disinfection of work surfaces and raising staff awareness of practices such as hand washing and managing mobility between wards. This study is very important for the implementation of rigorous hygiene measures to avoid possible nosocomial infections.

## Acknowledgments

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