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Fungal biodiversity of the IFAN beach sand in Dakar, Senegal

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ABSTRACT

Background: From a recreational point of view, the sandy beaches are much frequented because they represent a space for relaxation and leisure. Due to the large number of visitors, a possible contamination of the sand by fungal microorganisms could constitute a source of transmission by potentially pathogenic saprophytic fungi. The aim of this work is to determine the biodiversity of the sand of the IFAN (Fundamental Institute of Black Africa) beach in Dakar, Senegal. **Methods:** We conducted a cross-sectional and descriptive study during the period from August 1, 2017, to April 1, 2018 (8 months). For every month, five sand samples of about 70 g collected at different locations on the beach were cultured on Sabouraud medium using the washing technique for yeasts and non-dermatophytic filamentous fungi isolation and the Vanbreusghem trapping technique for dermatophytes isolation. **Results:** Out of 45 sand samples collected, all contained at least one fungal species (100% positivity). Fifty-three (53) fungal strains composed of 12 micromycetes belonging to nine (09) different genera were isolated: *Aspergillus* (41.51%), *Cladosporium* (26.42%), *Penicillium* (15.09%) were the most predominant. Other species recovered comprise *Cryptococcus neoformans* and *Candida albicans* with 1.89% each. **Conclusion:** According to our results, the sand of IFAN beach contains a great diversity of fungal species, including fungi incriminated in human pathology. Thus, it seems that the coastal sand represents an important reservoir of fungi whose role is poorly elucidated.

Introduction

Beach attendance has become an increasingly pressing social need. Beaches are a place of relaxation, recovery, sport, well-being with undeniable advantages for physical and mental health but also with some disadvantages. Indeed, it has been shown that the sand of sea, river or lake beaches is home to microorganisms (bacteria and parasites) that can affect human health [1-3]. It should also be added that the accumulation of data on possible fecal contamination of beaches has led to the introduction of regulatory measures to monitor sand/water contamination by checking for the presence of *Escherichia coli* and *Enterococci* as indicators of fecal contamination [4,5].

In addition to other beach microorganisms, fungal species can also contaminate beach sands [4,6]. Indeed, beach sands are eukaryotes with a worldwide distribution and a rich enzymatic arsenal allowing them to survive in different ecological and environmental conditions [7]. There are a large number of potentially or conditionally pathogenic

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micomycetes that can be contracted through beach sand. Among these micomycetes some are frequently found in human pathology. They are dermatophytes which are responsible for 10 to 40% of superficial mycoses, of which 2 to 3% are onychomycoses [8]. Yeasts such as *Candida* that cause dermatomycosis with 5 to 40% nail damage and molds that are increasingly described in the literature as agents of superficial mycoses such as onychomycoses. These infections generally occur on nails already weakened by primary pathogens such as *C. albicans* or dermatophytes. However, these infections are often very difficult to treat because these molds are resistant to most of the available antifungal agents [9,10]. These superficial mycoses are generally favoured by the human concentrations that ensure the transmission and diffusion of fungal species essentially anthropophilic, and this through the generally wet and dirty soils of infected dander or hair [8].

However, beaches with a large number of visitors and constituting a meeting place could constitute a favorable place for contamination via the sand hosting keratin fragments infected by fungal spores [8, 11]. Consequently, concerns have been expressed that beach sand or similar materials could act as reservoirs or vectors of infection.

Studies in several countries in Africa, America, Asia and Europe have demonstrated the presence of fungi on beach sands confirming the possibility of contamination across beaches [11-15] . However, to our knowledge, no such studies have yet been conducted in Senegal. It is in this context that we proposed to carry out this study with the objective of determining the fungal biodiversity of the Fundamental Institute of Black Africa (IFAN) beach in Dakar (Senegal) which remains one of the most frequented beaches.

Methods

We conducted a descriptive cross-sectional study which was spread over eight (08) months from August 1st, 2017 to April 1st, 2018 at the IFAN range. This beach is located in the Corniche west Dakaroise in front of IFAN, located at the University Cheikh Anta DIOP (UCAD) of Dakar. It is also next to the Radisson Hotel and the Sea Plaza shopping center. It is a place where the practice of outdoor physical activities is of great importance. Indeed, this place seems to be a meeting place where all categories of the population come to practice their physical maintenance activities. It is at all hours of the day and especially in the afternoons that we meet sportsmen and women of all kinds in the traditional quest for fitness. Because it is a beach very frequented by many sportsmen and women, it could thus constitute a favorable place for contamination via the sand hosting infected keratin fragments and fungus spores.

Samples

Sand samples were taken monthly at the IFAN beach according to a method already described by **Chabasse et al**, (1985) [16] . On average 5 samples of about 70 g of sand were taken per month. For each sampling session, once arrived on site, a location was targeted, then a square of 4 m side was considered. Samples were taken from each vertex and the centre of the square. Each location was sampled only once and was not sampled again until the end of the study. Samples were taken at three levels (at the water contact, at the boundary between wet and dry sand and at the dry sand level). Each sample was collected from a surface layer with a depth not exceeding 2-5 cm. The samples were then placed in marked sterile bottles and transported directly to the laboratory.

Sample processing

Once in the laboratory, each sample was processed using two techniques:

a) Washing technique.

It was mainly used for the detection of yeasts and non-dermatophytic filamentous fungi. It consisted in suspending 20 g of sand in 20 ml of sterile distilled water. After centrifugation, the supernatant was inoculated by the quadrant technique on two culture media, namely Sabouraud Chloramphenicol (SC) and Sabouraud Chloramphenicol and Actidione (SCA), and then incubated at 25˚C. Cultures were monitored daily and in case of fungal growth, an identification was performed. In the absence of fungal growth, a minimum delay of two weeks was observed before considering the result negative.

b) Keratin trapping technique [11].

It has been used for the detection of dermatophytes. It consists of putting in contact in a sterile Petri dish, a tuft of hair sterilized by autoclaving at 121˚C for 15 to 20 minutes and a sample of dried sand. The boxes were incubated at 25˚C for one to two months. After two months of incubation, or upon the appearance of a fine greyish down sheath, the hair was cultured on Sabouraud Chloramphenicol and Actidione (SCA) media and incubated at 25˚C. After

5 days of incubation, the cultures were examined daily for keratinophilic fungal growth and the maximum incubation period was stopped at 4 weeks before concluding to a sterile culture.

Identification of samples

The identification of filamentous fungi has been based on a number of criteria, including growth rates, and especially on the macroscopic and microscopic morphological characteristics of the cultures [17]. On the other hand, for yeasts, in addition to the above-mentioned criteria, physiological (Actidione sensitivity, Blastesia test), biochemical (urease test) and antigenic (search for cryptococcal antigens) criteria have sometimes also been associated. As identification at the species level is very difficult and involves other means such as molecular biology, our study was generally limited to genus identification except for a few strains for which we were able to complete identification down to the species level.

The identification of filamentous fungi was based on a number of criteria, including growth rates, and especially on the macroscopic and microscopic characteristics of the colonies [17]. In contrast, for yeasts, in addition to the above criteria, we used the Blastése test (filamentation on serum) to identify the *Candida albicans* / *Candida dubliniensis* species complex and the urease test and the cryptococcal antigen test with the Biosynex® CryptoPS immunochromatographic test for the identification of *Cryptococcus neoformans/gattii.*

We have limited ourselves to the genus for the other strains for which identification to species is very difficult and requires other means such as molecular biology.

Results

A total of 45 samples were collected over an eight (08) month period: five (05) samples per month from nine (09) different locations across the Fundamental Institute of Black Africa (IFAN) range. Of these 45 sand samples collected, all contained at least one fungal species, representing a 100% crop positivity rate. A total of 53 strains of fungi were isolated for a corrected fungal index of 118% (53/45). Twelve (12) micomycetes belonging to nine (09) different genera were isolated. The genus *Aspergillus* was the most isolated with a frequency of 41.51% (22/53) of which 16.98% (9/53) were unidentified species, and the rest were *A. versicolor*, *A. niger* and *A. nidulans*. The genus *Cladosporium* was found in second position with 26.42% (14/53) followed by the genus *Penicillium* with 15.09% (8/53) **(Table 1)**.

The average number of strains isolated per month was six (06) strains with a minimum of 5 isolates observed during the months of August, September, and October 2017, and a maximum of 7 isolates observed during the months of January and April 2018.

Aspergillus spp. and *Cladosporium spp.*, which are more represented, were found throughout the study period with maximum frequencies exceeding 60.00% for the genus *Aspergillus* in February and 42.86% (3/7) for *Cladosporium* in April. The minimum frequencies for these two genera were 14.29% for each observed in January and April for *Aspergillus* and *Cladosporium* respectively. In contrast, the genus *Penicillium*, which ranks third in terms of frequency, was only found in some months with a maximum frequency of 40.00% in September and a minimum frequency of 16.67% in December. As for the species *Phaeoacromonium parasiticum*, it was only found in December and January with frequencies of 16.67% (1/6) and 14.29% (1/7) respectively. Finally for the rest of the species they were isolated only in one month with proportions of 33.33% (2/6) for *Syncephalastrum racemosum* in November, 28.57% (2/7) for *Rhodotorula* spp. in April and 16.67% (1/6) for *Paecilomyces* spp. in November, *Cryptococcus neoformans* and *Candida albicans* in March **(Figure 1)**.

Fungi with a non-negligible pathogenicity potential were the most found, i.e. nearly 94.34%, followed by low or non-pathogenic fungi with 3.77% and pathogenic fungi represented only 1.89%. Potentially pathogenic fungi were represented by the species *Candida albicans*. Among those with non-negligible pathogenic potential, *Cryptococcus neoformans* was the most important species and *Phaeoacromonium parasiticum* was the species with little or no pathogenicity in humans **(Table 2)**.

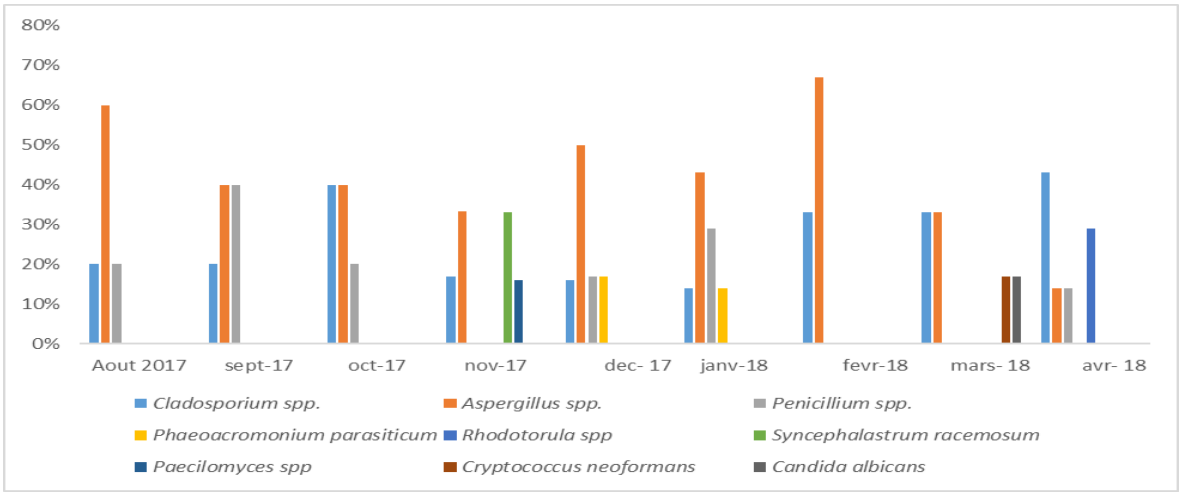
Table 1. Frequency of isolated fungi.

Genus/species	Number of isolation	Frequencies
Aspergillus	22	41.51%
A. versicolor	6	11.32%
A. niger	4	7.55%
A. nidulans	3	5.66%
Aspergillus sp.	9	16.98%
Cladosporium	14	26.42%
Penicillium	8	15.09%
Phaeoacromonium parasiticum	2	3.77%
Syncephalastrum racemosum	2	3.77%
Rhodotorula sp.	2	3.77%
Cryptococcus neoformans/gattii	1	1.89%
Candida albicans	1	1.89%
Paecilomyces sp.	1	1. 89%

Table 2. Species found, their pathogenicity potential and frequency.

Categories of mushrooms	**Species found**	**Frequencies**
Potentially pathogenic	*Candida albicans*	1.89%
Significant potential for pathogenicity	*Cryptococcus neoformans/gattii* *Aspergillus sp.* *Rhodotorula sp.* *Cladosporium sp.* *Penicillium sp.* *Syncephalastrum racemosum* *Paecilomyces sp.*	94.34%
Little or no pathogens	*Phaeoacromonium parasiticum*	3.77%

Figure 1. Distribution of the different fungi isolated according to months.



Discussion

The microbiological quality of sand has a direct impact on health [14,18] . Some studies have raised the question of the role of beach sand in the origin of superficial fungal infections [11,19,20] . Since the transmission of keratinophilic fungi is favored essentially by humidity and maceration, beach sand could indeed constitute a favourable environment for contamination by these microorganisms [11,21]. Thus, different authors have been interested in the study of the fungal flora of beach sand [4, 12, 15] .

The results of the present study show that the Fundamental Institute of Black Africa (IFAN) beach sand contains fungi incriminated in human pathology with a high diversity of isolated fungal species. Six genera of filamentous fungi and three genera of yeast fungi were found with a predominance of the Aspergillus genus (41.4%). The same observation was made in different coastal ecosystems such as in Morocco (54.3%) in 2004 and (54%) in 2019, in Brazil (30.4%) in 1996 and in Israel (28.7%) in 2020 [8,15,22,23] . However, in Algeria, Benmessaoud showed that the genus *Penicillium* was predominant with 41.4%, followed by *Aspergillus* with only 13.3% [24]. According to our results, the second position was occupied by the genus *Cladosporium* with 26.40%. Its constant presence in beach sands has been reported in several studies, notably in Algeria in 2010, Morocco in 2004 and Brazil in 1996, but with smaller proportions than the one we found, which were 9.01%, 2.9% and 3.1% respectively [8, 23, 24]. The genus *Penicillium* was found in third place with a proportion of 15.10%. Similar proportions were found in Morocco in 2004 and in Brazil in 1996 with 18.57% and 16.20% respectively [8, 23] but also a higher proportion was found in Algeria in 2010 with 55.18% [24].

No dermatophytes were found in our study. This finding is in line with the results obtained in Morocco and Israel in 2020 [15, 23] . This absence of dermatophytes in our results could be explained by the fact that the sand did not contain dermatophyte spores or even in the application of the Vanbreusghem technique. In fact, this technique recommends using sterile impubescent children's hair and moistening the incubated sand containing the hair whenever necessary, whereas in our study, unable to find them, we used hair from adult subjects that we sterilized by autoclaving. However, it should be noted that the presence of dermatophytes has only been reported very rarely in the literature [14, 22, 25] and could be associated with specific ecological conditions, such as those found in the Madeira archipelago and on the five Moroccan beaches [14,22] reported a large survey along the Portuguese coast, in which they found dermatophytes in only a small proportion of samples [25].

Finally, in our study only one *Candida albicans* isolate and 3 yeast isolates other than *Candida albicans* (1 *Cryptococcus and 2 Rhodotorula*) were listed as yeasts.

The absence or low incidence of *C. albicans* was also reported elsewhere in Africa in a prospective study of the fungal flora of five beaches in Morocco in 2020 that isolated only three strains of *Candida parapsilosis* as yeasts [22]. Similarly, a single strain of *C. albicans* was isolated by studying the fungal biodiversity of sand on four beaches (Beau Séjour, Eden, Les Andalouses and Madagh) of the Algerian west coast [24]. However, other studies have shown significant proportions of *Candida, Rhodotorula and Cryptococcus*. This is the case, for example, of the one carried out in Korea in 2019 on the beaches of Gyungpodae and Lake Gyungpo, which isolated fifty-five species of yeast, including the genus *Candida*, which was the most frequently found, followed by the genus *Cryptococcus* [26]. Similarly, the study on the isolation and detection of fungi from Mediterranean beaches in Israel in 2020 revealed 32 yeast species, half of which were *Candida* and the rest *Cryptococcus and Rhodotorula* [14]. Some authors suggest that the isolation of yeasts, especially *C. albicans*, from beach sand could have an impact in medical pathology, mainly in the occurrence of cutaneous mucosal mycoses [14].

Our results also showed variations in isolation frequencies depending on the month. Similar studies had found the same, although quantitative and qualitative differences in the fungi isolated could be noted [4, 21, 27] . These observed differences could be explained by environmental conditions (temperature, climate, organic matter content of the sand, healthiness of the beaches, etc.) that differ from one locality to another. Contrary to other studies that were conducted during a specific period or season of the year [8,14] , our study confirmed the permanent existence of a reservoir of fungi that is minimal during the months of August, September and October and maximal during the months of January and April.

In this work, we were able to isolate a potentially pathogenic fungus, namely *Candida albicans.* The latter is the main yeast implicated in human pathology. It is responsible for half of all candidemia and is found in 70% of yeast isolations and nearly 50% to 60% of invasive candidiasis. *Candida albicans* is characterized by its ability to colonize all segments from the oral cavity to the anus. This digestive colonization is also at the origin of deep infections occurring in the hospital environment [28, 29] . Other species with significant pathogenic potential have also been isolated. These are species of the genus *Aspergillus, Cryptococcus neoformans, Penicillium, Rhodotorula, Cladosporium, Syncephalastrum,* and *Paecilomyces*. *Aspergillus* spp. are saprophytic and sometimes pathogenic fungi with A. fumigatus being the most pathogenic. It is an agent of an inflammatory and destructive disease of the bronchi and lungs called "pulmonary aspergillosis" [30-32]

This study reveals a diversity of fungal agents isolated from the Fundamental Institute of Black Africa (IFAN) beach sand, including fungi with significant pathogenic potential and potentially pathogenic fungi that may be responsible for human or animal pathologies. To confirm these data, further investigations on a larger scale are necessary, in particular to propose some preventive measures to reduce the risk of contamination of the population.

Conflict of interest

The authors declare that there is no conflict of interest.

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Competing interests

All authors participating in this study declare that they have no financial interests

Authors' contributions

All authors contributed to the design of the study. Material preparation, data collection and analysis were carried out by [Abdoulaye DIOP], [Khadim DIONGUE] and [Mouhamadou NDIAYE]. The first draft of the manuscript was written by [Abdoulaye DIOP] and all authors commented on earlier versions of the manuscript. All authors have read and approved the final manuscript.

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