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Study of the Frequency of Superficial Candidiasis at the Fann National Hospital: Search for *Candida auris*

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Abstract

Background: Superficial candidiasis is a very frequent opportunistic disease caused by yeasts of the genus *Candida*. Among *Candida* types, some, such as *Candida auris*, have developed resistance to several antifungal agents. The objective of this study was to determine the hospital frequency of superficial candidiasis diagnosed at the CHU Fann and to investigate the presence of *C. auris* among the identified *Candida* strains. **Methods**: A cross-sectional study was conducted from February to June 2019. It involved all patients received at the Parasitology-Mycology laboratory of the CHU of Fann for suspected superficial candidiasis. Nails, skin, and vaginal specimens were subjected to direct examination and culture to identify yeasts of the genus *Candida*. The *Candida* strains were then tested by molecular biology targeting the specific *C. auris* ITS2 region. **Results**: A total of 1196 patients were examined. One thousand two hundred and five specimens (1205) were collected, including 1042 vaginal specimens, 92 nail specimens, and 71 skin specimens. Superficial candidiasis was diagnosed in 408 patients (37%). Women (34.52%) and patients under 30 years of age (39.60%) were the most affected. Yeasts of the genus *Candida* were found in 411 specimens (349 vaginal swabs, 36 nail fragments, and 26 skin flakes) by routine mycological techniques. The *Candida albicans* complex (*C. albicans*, *C. dubliniensis*, *and C. africana*) represented 75.91% of the *Candida* strains isolated. Molecular biology did not identify *C. auris*. **Conclusion**: Superficial candidiasis remains very common in hospitals in Senegal. *Candida auris* was not found in our study. Due to its rapid spread, surveillance is necessary to prevent epidemics in our hospitals.

Keywords

Superficial Candidiasis, *C. auris*, Frequency, Senegal

1. Introduction

Candidiasis is a fungal infection caused by yeasts of the genus *Candida*, which are commensal to the skin and mucous membranes and form part of the microbial flora [1]. They manifest as mucocutaneous lesions, fungemia, and sometimes a focal infection of various organs [2]. They are generally benign and are favored by immunosuppression, humidity, corticosteroids, etc. The most commonly isolated species in human pathology is *Candida albicans* [3]. Several factors, including chemotherapy and excessive antifungal chemoprophylaxis, have led to the emergence of multidrug-resistant species other than *C. albicans*, such as *C. glabrata* and *C. auris* [4]. The latter is an emerging fungal pathogen that was isolated and described in 2009 from a patient's ear canal discharge [5]., *C. auris* has been isolated from only six countries (South Africa, Kenya, Nigeria, Egypt, Algeria, and Sudan) on the African continent, with Algeria and Nigeria being the closest countries to Senegal [6]. It is one of the most worrying fungal pathogens due to the epidemics it causes, and it has very high hospital mortality rates worldwide [7]. Apart from its resistance to available antifungal agents and its ease of maintaining colonization in the human body and the environment, *C. auris* is frequently misidentified by the diagnostic platforms available in clinical laboratories [7]. Indeed, not only do the macroscopic and microscopic characteristics of the cultures not provide sufficient information to identify this pathogen, but biochemical methods (API 20 C AUX, VITEK) also easily confuse it with other species, such as *Candida haemulonii*, *Candida famata*, *Saccharomyces cerevisiae*, and *Rhodotorula glutinis* [8]. Early identification of this pathogen by innovative techniques, especially in low- and middle-income African countries, could improve management and avoid complications. Our study aimed to determine the frequency of superficial candidiasis and identify *C. auris* at Fann Hospital.

2. Methods

2.1. Study Area

This descriptive cross-sectional study was conducted from February to July 2019 in the Parasitology—Mycology laboratories of FANN Teaching Hospital and the Cheikh Anta Diop University of Dakar.

2.2. Study Population

The study population consisted of all patients who were examined at the parasitology-mycology laboratory of Fann Hospital for suspected superficial mycosis based on the clinical characteristics (intertrigo, peri-onyxis, pruritus in the folds,

leucorrhoea, erythema, dyspareunia) [9]. All patients in our study population in whom we identified yeasts of the genus *Candida* were included. Patients in whom we identified fungi other than *Candida* were declared negative for superficial candidiasis.

2.3. Sample Collection

Skin flakes, nail fragments, and vaginal swabs were collected depending on the type of lesions the patient had.

Each sample was taken separately before any antifungal treatment was applied. Patients were asked to wash the areas to be sampled thoroughly with neutral soap beforehand and to wear clean socks for skin and nail samples (mainly for lesions on the feet). For vaginal swabs, patients had to abstain from intimate hygiene, sexual intercourse since the previous day, antibiotic therapy, and ensure that they were not menstruating.

2.4. Mycological Examinations

For each sample, we performed a direct examination in 30% potash or physiological water, culture on Sabouraud Chloramphenicol (SC) and Sabouraud Chloramphenicol Actidione (SCA) agar. We then incubated the cultures at 27˚C (for nail and skin specimens) and 37˚C (for vaginal specimens).

The identification of *Candida* yeasts was based on the macroscopic, microscopic, and physiological (Blastese test) characteristics of the colonies from the culture [9]. We retained the diagnosis of superficial candidiasis in front of a positive direct examination (presence of budding yeasts or mycelial filaments) and an abundant culture (more than 10 colonies for the vaginal specimens) [10].

All *Candida* strains were stored in 20% glycerol at −20˚C and −80˚C for molecular biology.

2.5. Molecular Tests

1) DNA extraction

The DNA that was contained in *Candida* strains isolated from the culture was extracted using the CTAB (Cetyltrimethylammonium Bromide 2%) technique described by Benbouza *et al*. [11], which we modified. Two hundred microliters (200 µL) of CTAB 2% was added to 100 µL of the sample and incubated at 65˚C for 5 minutes. Then, 200 µL of chloroform was added before vortexing the mixture briefly. After a 5-minute centrifugation at 12,000 revolutions per minute (rpm), the upper phase was collected in another Eppendorf tube containing 200 µL isopropanol. Another 15-minute centrifugation at 12,000 rpm was performed, and the isopropanol was drained before the addition of 200 µL ethanol. A final centrifugation was performed for 5 minutes. The resulting pellet was dried and then resuspended in 100 µL of pure water.

2) DNA extraction control

To validate our extraction, we performed conventional PCR targeting the ITS2

region of fungal rRNA genes [12]. Primers ITS3 and ITS4, which are panfungal primers targeting 5.8S and 28S rRNA genes, were used, and we followed the instructions of the manufacturer of Tempase Master Mix (Ampliqon, Denmark) for the reaction.

A mixture containing 12 µL of the Ampliqon Tempase Master Mix (ID: 5200400-1250), 8 µL of distilled water, 1 µL of ITS3 (5'-GCA-TCG-ATG-AAG-AAC-GCA-GC-3') and 1 µL of ITS4 (5'-TCC-TCC-GCT-TAT-TGA-TAT-GC-3') primers was prepared and dispensed into a 96-well PCR plate. Then, 3 µL of each extracted DNA was added to the wells containing the previously distributed mix, and the plate was placed in the Applied Biosystems 2720 thermal cycler (serial number: 2725705231) using the program below: initial denaturation at 96˚C for 1.30 minutes, denaturation at 96˚C for 30 seconds, hybridization at 58˚C for 30 seconds, extension at 68˚C for 2 minutes, and final extension at 68˚C for 5 minutes. The denaturation, hybridization, and elongation steps were repeated 35 times before the final extension.

The products of this amplification were migrated on a 1% agarose gel at 100 V for 25 minutes. We then looked for DNA bands between 320 and 500 bp [13] using a Bio-Rad Doc Gel EZ imager (serial number: 735BR0564) to confirm the presence of fungal DNA in our extracts.

3) Real-time PCR of the *C. auris* ITS2 region

Real-time amplification of our starting extracts in addition to a *C. auris* positive control (accession number: KX810325, obtained from IHU Méditerranée Infection, Marseille) was performed using primers and probes designed by Leach *et al*. [14]. For each sample, a 15 µL volume reaction mixture (according to the instructions provided by the manufacturer of the Qiagen Quantitect Master Mix) consisting of 10 µL of Qiagen Quantitect Probe Master Mix (ID: 21935311512), 0.5 µL of *Candida auris* FORW (5'-CAG-ACG-TGA-ATC-ATC-GAA-TCT-3); 0.5 µL of *Candida auris* REV (5'-TTT-CGT-GCA-AGC-TGT-AAT-TT-3), 0.5 µL of *Candida auris* PROB (6-FAM-AAT CTT CGC NGG TGG CGT TGC ATT CA-TAMRA) and 3.5 µL of distilled water was prepared. Then, 5 µL of the extracted sample was added to the mixture and placed in the thermal cycler following the program below: UDG at 50˚C for 2 minutes, initial denaturation at 95˚C for 15 minutes, denaturation at 95˚C for 30 seconds, and hybridization at 60˚C for 1 minute. The last two steps were repeated 40 times.

2.6. Statistical Analysis

Our data were entered into Excel 2016 and analyzed using Epi info version 7.2. Quantitative variables are presented as the means and standard deviations, and qualitative variables are presented as numbers and percentages.

2.7. Ethical Considerations

Our study was approved by the Ethics Committee of the Cheikh Anta Diop Univer-

sity (Reference: 0413/2019/CER/UCAD). All patient data were anonymized prior to analysis.

3. Results

We received a total of 1191 patients with suspected superficial mycoses during the collection period. The mean age was 33.62 years (standard deviation = 13.12), with a minimum and maximum of 6 months and 89 years, respectively. Patients between 30 and 60 years of age were the most common, with a frequency of 50.46% (601 patients), followed by patients under 30 years of age, with a frequency of 41.56% (495 patients). Patients over 60 years of age represented 5.04% (60) of the total population. Women were in the majority, with a frequency of 94.37% (1124). The majority of patients (35.35%, N = 421) were seen for assessment. Other clinical occurrences that prompted the request for a mycological examination were vulvovaginitis (14.53%), leucorrhoea (13.94%), onychomycosis (6.55%), intertrigo (2.77%) and keratoderma (1.34%). The number of specimens obtained in this study was 1205. They consisted of 1042 (87.49%) vaginal swabs, 92 (7.72%) nail fragments, and 71 (5.96%) skin flakes (Table 1).

Of the 1191 patients examined, 37% (408) had superficial candidiasis (Table 2). The mean age of the latter was 32.57 (±12.37) years, with a minimum and maximum of 2 and 89 years, respectively. The most affected age group was the under 30 years group (39.60%), followed by the over 60 years group (33.33%), and then the 30 - 60 years group (30.12%). A female predominance (94.37%) was also noted. Regarding the diagnosis, *Candida* was found much more frequently in patients with onychomycosis associated with epidermophytosis (100%). Other diagnoses were intertrigo (42.42%), sexual abuse (50%), and vulvovaginitis (40.46%) (Table 2).

Of the 92 nail specimens collected, 36% or 39.13% were positive for *Candida* (Table 2). The average age in this category was 42 years (±16.95). Among the latter, subjects between 30 and 60 years of age were the most affected with a proportion of 61.11%. The same was true for females, who represented 72.22% of the subjects with *Candida*-positive nail specimens. Among the skin specimens, 26% or 36.62% out of 71 were positive. The average age of these patients was 46.04 years (±19.62 years). The majority of these patients were between 30 and 60 years of age (50%) and were female (57.7%). Concerning the vaginal swabs, 349 were positive, *i.e.*, a frequency of 33.49%. The average age of these patients was 30.81 years (±10.12). Most of these positive specimens were from patients under 30 years of age (N = 185; 53.01%).

Patients between 30 and 60 years of age were less numerous (N = 148; 42.41%), followed by those over 60 years (N = 9; 2.58%). Candidiasis was also found in 2 subjects whose age was not reported for nail specimens, 2 for skin specimens, and 7 for vaginal specimens. Sex was also missing in 2 patients with positive nail specimens and in one patient with a positive skin specimen (Table 3).

Table 1. Distribution of the population according to sociodemographic and clinical characteristics.

	Number	%	Confidence Interval
AGE GROUP, Years (N = 1191)			
0 - 30	495	41.56	38.79 - 44.38
30-60	601	50.46	47.63 - 53.30
More than 60	60	5.46	3.93 - 6.43
NS	35	5.04	2.12 - 4.06 - 4.06
SEX (N = 1191)			
Male	63	2.94	4.16 - 6.7
Female	1124	5.29	92.9 - 95.55
NS	4	94.37	0.13 - 0.86
SAMPLE TYPE (N = 1205)			
Nail	92	7.72	6.34 - 9.38
Skin	71	5.96	4.75 - 7.45
Vaginal swabs	1042	87.49	85.49 - 89.25
DIAGNOSIS (N = 1191)			
Sexual abuse	2	0.17	0.05 - 0.61
abortion	11	0.92	0.52 - 1.65
Assessment	421	35.35	32.68 - 38.11
Pelvic pain	62	5.21	4.08 - 6.62
Vulvovaginitis	173	14.53	12.64 - 16.64
Infertility	72	6.05	4.83 - .7.55
Leucorrhoea	166	13.94	12.09 - 16.02
Keratoderma	16	1.34	0.83 - 2.17
Epidermophytosis	6	0.50	0.23 - 1.09
Onychomycosis	78	6.55	5.28 - 8.10
ITO	33	2.77	1.98 - 3.87
ITO + keratoderma	2	0.17	0.05 - 0.61
Keratoderma + onychomycosis	3	0.25	0.09 - 0.74
Onychomycosis + ITO	9	0.76	0.40 - 1.43
Onychomycosis + epidermophytosis	2	0.17	0.05 - 0.61
NS	135	11.34	9.66 - 13.26

NS: Not specified, ITO: Intertrigo.

Table 2. Frequency of superficial candidiasis according to sociodemographic and clinical characteristics.

	Number	%
SEX (n = 408)		
Female	388	34.52
Male	17	26.98
NS	3	75
AGE GROUP, YEARS (n = 408)		
0 - 30	196	39.60
30 - 60	181	30.12
More than 60	20	33.33
NS	11	31.43
SAMPLE TYPE (n = 411)		
Nail	36	39.13
Skin	26	36.62
Vaginal swabs	349	33.49
DIAGNOSIS (n = 408)		
Sexual abuse	1	50
Abortion	4	36.36
Assessment	140	33.25
Pelvic pain	13	20.97
Vulvovaginitis	70	40.46
Infertility	22	30.56
Leucorrhoea	66	39.76
Intertrigo	14	42.42
Keratoderma	6	37.50
Onychomycosis	30	38.46
Keratoderma + Onychomycosis	1	33.33
Onychomycosis + Intertrigo	6	66.67
Onychomycosis + Epidermophytosis	2	100
NS	33	24.44

NS: Not specified.

Three hundred and twelve (312) *Candida* or 75.91% identified by routine mycological techniques (direct examination, culture, and Blastese test) belonged to the *C. albicans* complex (*Candida albicans*, *Candida dubliniensis*, *and Candida africana* [15] [16]) and were distributed as follows: 26 in the nail specimens, 16 in the skin specimens and 270 in the vaginal specimens. Ninety-nine (99) or 24.08% were *Candida sp*. (10 nail specimens, 10 skin specimens, and 79 vaginal specimens) (Table 4). In molecular biology, all of these *Candida* strains were

Table 3. Frequency of skin, nail, and vaginal candidiasis by age group and sex.

	Nails N = 36		Skin N = 26		Vaginal swabs N = 349	
	Number	%	Number	%	Number	%
AGE GROUP (Year)						
0 - 30	6	16.67	5	19.23	185	53.01
30 - 60	22	61.11	13	50	148	42.41
More than 60	6	16.67	6	23.07	9	2.58
Not specified	2	5.55	2	7.7	7	2
SEX						
Female	26	72.22	15	57.7	349	100
Male	8	22.22	10	38.46	-	
Not specified	2	5.56	1	3.84	-	

Table 4. Distribution of *Candida* species identified by routine mycological techniques.

	Nails (%)	Skin (%)	Vaginal swabs (%)	Total (%)
Candida albicans Complex (*Candida albicans*, *Candida dubliniensis* and *Candida africana*)	26 (72.22)	16 (61.54)	270 (77.36)	312 (75.91)
Candida sp.	10 (27.78)	10 (38.46)	79 (22.64)	99 (24.08)
Total	36 (100)	26 (100)	349 (100)	411 (100)

positive for the ITS2 region, which allowed us to validate our extraction method. *C. auris* was not found.

4. Discussion

In recent decades, the incidence of candidiasis has increased dramatically with the emergence of non-albicans species [17] [18]. *C. auris*, in this case, has been associated with epidemics in several countries of the five continents [19], making it a major public health problem. This study was conducted to determine the hospital frequency of superficial candidiasis diagnosed at Fann University Hospital and to investigate the species *C. auris*. Superficial candidiasis was confirmed in 408 patients, which corresponds to a hospital frequency of 37%. Tayibi in Morocco reported a frequency of 82.88% (unpublished data). Superficial candidiasis is often a chronic disease that requires restrictive treatments due to its topical administration, short- or long-term tolerance, and long-term use. All these factors could reduce patient compliance and explain the differences in frequency

from one population to another.

Women were the most affected, which is logical given that over 80% of our study population were women. According to diagnosis, the association onychomycosis + epidermophytosis predominated, followed by the association onychomycosis + intertrigo.

We performed 1042 vaginal swabs during our study period, of which 349 were positive, representing a hospital frequency of 33.49%. This frequency is similar to the 32% reported by Sylla *et al.* in 2015 in Senegal [20] and the 32.87% reported by Mtibaa *et al.* in Tunisia [21]. Other frequencies reported by different authors are disparate: This is the case for 38.9% reported by Ogouyémi-hounto in Benin [22]; 23.5% reported by Djohan V *et al.* in Ivory Coast [23]; and 36.39% reported by Anane *et al.* in Tunisia [24]. On the one hand, these differences could be related to cultural factors specific to each country. On the other hand, they could be related to the differences in sample sizes and in the diagnostic methods used in each study. The mean age of our patients (30.81 years) is close to the mean age reported in the majority of studies concerning vulvovaginal candidiasis: 29.83 years and 31.82 years, respectively, by Ogouyémi-hounto in Benin [22] and Benchellal *et al.* in Morocco [25]. These observations are reasonable because these ages correspond to the period of genital activities, which favors sexual transmission of the pathogens.

Of the 92 nail specimens, 36 were positive for *Candida*, giving a hospital frequency of 39.13%. Much lower frequencies have been reported by Sav *et al.* in Turkey [26] and Otašević *et al.* in Serbia [27]. These differences could be explained by the climatic conditions prevailing in Africa, which favor the proliferation of fungi. A predominance of candidiasis was noted in women, with a frequency of 47.27%. This observation, in agreement with some studies [28] [29], could be explained by the abusive use of chemical products (dermocorticoids, intimate hygiene products), the performance of household chores (regular contact of the hands with water), and nail microtrauma related to aggressive and repeated manicures. A significant number of our patients with nail candidiasis were between 30 and 60 years of age, similar to the findings of Nzenze Afène *et al.* [30], who reported a predominance of nail candidiasis in patients between 20 and 60 years of age. Sylla *et al.*, and Halim *et al.* also reported the same trend in their studies [28] [31]. This is because children are less susceptible to onychomycosis due to their rapid nail growth and they are less likely to suffer trauma [32]. In addition, with increasing age, certain underlying diseases, such as obesity, diabetes, and drug interactions, are present.

The hospital incidence of cutaneous candidiasis reported in this study was 36.62%. In the same country, Diongue *et al.* reported 25.3% of epidermomycosis due to *Candida* species between 2008 and 2015 in their study on cutaneous-ungual candidiasis [33]. Salim Z, in 2009 in Senegal, reported a frequency of 24% candidiasis (unpublished data). These findings, despite the availability and accessibility of antifungals to the Senegalese population, could be the result of

self-medication, a climate conducive to fungal proliferation, the use of bleaching agents on the skin, frequent recurrences, etc. Indeed, Diongue *et al.* also reported in the same study that 76.3% of his study population had recurrent cutaneous-ungual candidiasis [33]. Cutaneous candidiasis was more common in women over 60 years of age which confirms what was said above about the appearance of some underlying diseases (diabetes, obesity, etc…) with increasing age.

The *Candida albicans* complex was the isolated species group in the majority of cases and simultaneously in all three sample types (skin, nail, and vaginal samples). This is probably due to their wide distribution despite the emergence of non-albicans species in recent decades. *C. auris*, one of these species, attracted our interest in this study because of its rapid spread and multidrug resistance to various antifungal agents. So far, *C. auris* has been isolated from only six countries (South Africa, Kenya, Nigeria, Egypt, Algeria, and Sudan) on the African continent, with Algeria and Nigeria being the closest countries to Senegal [6]. This study was conducted considering the propensity of *C. auris* to colonize the human body for a long time (mainly the skin) [7] and the fact that patients hospitalized in intensive care units could potentially contaminate their companions. It was not found in our study for two main reasons. First, nearly 80% of the clinical specimens were vaginal swab specimens and the vagina is not a major anatomical site for colonization by *C. auris*, even though it has previously been detected in vaginal swab specimens in a study [34]. *C. auris* usually prefers the axilla, groin, and nares, for colonization. Another reason is that *Candida auris* has most likely not yet been introduced into Senegal, likely due to limited travel by *C. auris*-colonized subjects from countries considered hot spots for *C. auris* (India, Pakistan, South Korea, Japan, South Africa, United Kingdom, Spain, etc.) [7] [35].

It is necessary to monitor this "super germ" [36], using innovative techniques, because of the numerous epidemics it has caused and the high mortality (30% - 72%) associated with its infections [19] [37] [38] [39] [40].

However, this study has its limitations because it was carried out in a laboratory (most of the patients were not hospitalized) and because of the nature of the samples used for screening.

5. Conclusion

Superficial candidiasis is still prevalent in our hospitals. The *Candida albicans* complex remains the most common group species. However, *C. auris* was not found in this series after screening with molecular techniques. Further studies are needed to better describe the epidemiology of this species, especially in invasive candidiasis.

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Competing Interests

The authors declare no competing interests regarding the publication of this paper.

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