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Distribution of *Candida* Species and Their Susceptibility to Antifungal Drugs in Dakar, Senegal

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Abstract: The large spectrum of Candida species and their susceptibility to antifungal drugs has made the identification of Candida species and the detection of drug resistance necessary for the management of Candida infection. This study was carried out to determine the distribution of *Candida* species and to evaluate their susceptibility to antifungal drugs. A prospective observational and descriptive study was conducted from March to June 2016 in the laboratory of Parasitology-Mycology at Fann University Hospital in Dakar. Samples were analyzed by direct microscopy and culture. Identification of *Candida* species was based on filamentation test, chlamydosporulation formation, auxanogramme (AUXACOLOR™ Bio-Rad) and Candi-Select® 4 (Bio-Rad,). The susceptibility of *Candida* species to antifungal drugs was tested using Fungitest® (Bio-Rad) against 5-fluorocytosine, amphotericin B, miconazole, ketoconazole, itraconazole and fluconazole. A descriptive analysis was performed using Stata MP 14. Among 336 specimens received for mycological examination, 68 (20.2%) were positive for *Candida*. The most identified *Candida* species were *C. albicans* (58.8%), *C. glabrata* (16.2%), *C tropicalis* (7.4%), *C krusei* (7.4%), *C. parapsilosis* (4.4%), *C. dubliniensis* (4.4%) and *C. kefyr* (1.5%). The majority of isolates were susceptible to ketoconazole (94.3%), fluconazole (85.7%), amphotericin B and 5 fluoro-cytosine (88.6%). The susceptibility rates were lower for itraconazole (51.4%) and miconazole (68.6%). One strain of *C. albicans* was resistant to 5 fluoro-cytosine, one strain of *C. glabrata* and *C. tropicalis* were resistant to itraconazole. The results of this study provide useful information regarding the distribution of Candida species and the susceptibility to antifungal drug. Routine identification of *Candida* species and monitoring of resistance patterns are necessary to manage Candida infection and to control the spread of resistance in clinical isolates of Candida species.

Keywords: *Candida*, Identification, Antifungal Susceptibility, Fungitest, Senegal

1. Introduction

Fungal species within genus *Candida* are known to colonize skin, nails, gastro-intestinal and vaginal mucosa. 20 to 25% of vaginal tract infections in women are due to *Candida* species [1-2]. Regarding the onychomycosis, *C. albicans* is the most frequent species causing *Candida* onychomycosis [3]. Overall, fungal infections are constantly increasing particularly fungal invasive infections. Over the last 20 years, the incidence of fungal invasive infection is highly increased and *Candida* species are identified as the main causal agent. In person with immune system deficiency, invasive candidiasis is the most frequent fungal infection. The most commonly isolated specie is *Candida albicans* 56%. The mortality due to *Candida albicans is* about 37.9%. Among the non-albicans species,

C. glabrata, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and *C. dubliniensis* have been identified as main etiologic agent [4-8].

The increased incidence and mortality related to invasive *Candida* infections (candidaemia) can be influenced by several factors such as characteristics of the population at risk (population age, HIV-positive, diabetes mellitus, nail traumatism, pregnancy, immunosuppressive and antibiotic therapy), standard of the healthcare facilities available, distribution of Candida species and prevalence of resistance [7, 9].

Correct identification of Candida species is necessary to confirm the etiological diagnosis and to guide the antifungal treatment. Providing adequate antifungal treatment is an essential component in the management of invasive *Candida* infection. Generally, the susceptibility of *Candida albicans* to azoles is well known. The primary resistance of *Candida krusei* to fluconazole and the possible resistance of *Candida glabrata* to fluconazole by the efflux mechanism are also demonstrated [10]. To avoid the emergence of resistance, it's important to identify the non-albicans Candida species in order to prescribe adequate treatment.

In Senegal, fungal infections due to *Candida* (onychomycosis, vaginal infection, invasive infection) are an important public health problem [10]. Data related to antifungal resistance are rare while fungal treatment is always prescribed in health facilities. Fluconazole, Itraconazole, Ketoconazole and Amphotericin B are the main fungal treatment prescribed to patients. Ketoconazole is currently removed from the list authorized drugs because of hepatotoxicity. In order to analyze the changing trends in the distribution of Candida species and to better guide clinician for the antifungal treatment prescription, we carried out this study aimed to determine the distribution of different *Candida* species and their susceptibility to six antifungal drugs (fluconazole, itraconazole, ketoconazole, miconazole, amphotericin B and 5-fluorocytosine).

2. Materials and Methods

2.1. Study Design and Population

A prospective observational and descriptive study was conducted from March to June 2016 in the laboratory of Parasitology-Mycology at Fann University Hospital in Dakar which is a mycological diagnostic reference center. All patients attending to the laboratory for a mycological examination, were included in this study.

2.2. Laboratory Methods

Isolation and identification of Candida species

All specimens were analyzed by direct microscopic examination. For the culture, specimens were inoculated both on Sabouraud-Chloramphenicol and on Sabouraud-Chloramphenicol-Actidione medium. Incubation of these media was done at 37° for 24 to 48 hours. Identification of *Candida* species was based on macroscopic and microscopic examinations of cultures, filamentation test on serum, chlamydospore formation, AUXACOLOR® rapid identification system (Bio-Rad, France) and Candi-Select® 4 (Bio-Rad, France).

Antifungal susceptibility testing

The study of the antifungal susceptibility of Candida isolates was performed using Fungitest® (Bio-Rad, France). Six antifungal agents tested were: 5-fluorocytosine, amphotericin B, miconazole, ketoconazole, itraconazole and fluconazole.

Principle: Fungitest® is used to study the growth of yeasts in the presence of 6 antifungal agents at 2 different concentrations, in modified Roswell Park Memorial Institute, (RPMI) 1640 buffered medium, in the presence of a redox indicator. Growth assessment is based on reduction of the colored indicator which turns the medium from blue to pink. When growth is inhibited by the antifungal agent, the medium remains blue. This test, presented in the form of a 16 well microplate, consists of: (2 growth control wells), 12 wells containing the dehydrated antifungal agents (6 antifungal agents at 2 different concentrations) and (2 negative control wells). The breakpoints have been chosen following the study of the distribution of the antifungal agent's minimal inhibitory concentration (MIC) obtained with prototype microplates used with the same procedure as Fungitest® [11].

Reading and Interpretation of results: (i) Only examine the plate when the positive control (T+) wells are pink. (ii) Observe any color change in the wells containing the antifungal agent compared to the negative control wells (blue). (iii) Interpret according to the color of the 2 wells for each antifungal agent: Blue-Blue=no growth: strain inhibited by the antifungal agent *in vitro*, Pink-Blue=low growth: intermediate strain, Pink-Pink=growth: strain not inhibited by the antifungal agent *in vitro* [11].

2.3. Statistical Methods

After data collection, data were entered in Excel software and the analysis was performed using Stata software version MP 14. Quantitative variables were described in terms of means, standard deviation. For qualitative data, percentage was used to assess the frequency of each outcome with a 95% confidence interval (CI). Significance level of the different tests was 0.05 two-sided.

2.4. Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki. To respect the confidentiality, an identification code was assigned to each patient. This study was a hospital-based research conducted in routine conditions. The protocol was approved by the by the Research Ethic Committee (Comité d'Ethique et de Recherche: CER) of University Cheikh Anta Diop of Dakar (UCAD) (approval number: 48/2019/CER/UCAD).

3. Results

3.1. Mycological Data

During the study period, 336 patients were enrolled in this study. The mean age was 34.08±12.4 years. Study population was mainly constituted by patients aged between 25 to 35 years (44.1%) and women (95.6%) (Table 1).

Table 1. *General characteristics of study participants.*

	Frequency (n=68)	Percentage (%)	95% CI
Age group (years)			
14 - 25 years	18	26.5	15.6 – 41.8
25 - 35 years	30	44.1	29.7 – 62.9
> 35 years	20	29.4	17.9 – 45.4
Gender			
Male	3	4.4	0.9 – 12.8
Female	65	95.6	73.7 – 99.9
Origin of specimens			
Vaginal swab	54	79.4	59.6 – 99.9
Nails	8	11.8	5.1 – 23.2
Squama	5	7.4	2.4 – 17.2
Auricular specimen	1	1.5	00 – 8.2

Among total specimens received for mycological examination, 68 (20.2%) were found to be positive for *Candida*. The majority of *Candida* isolates came from a vaginal swab (79.4%), nails specimens (11.8%) and squama specimen (7.4%).

The distribution of *Candida* species was as follows: *C. albicans* 58.8% (n=40); *C. glabrata* 16.2% (n=11); *C tropicalis* 7.4% (n=5); *C krusei* 7.4% (n=5); *C. parapsilosis* 4.4% (n=3); *C. dubliniensis* 4.4% (n=3) and *C. kefyr* 1.5% (n=1) (Figure 1).

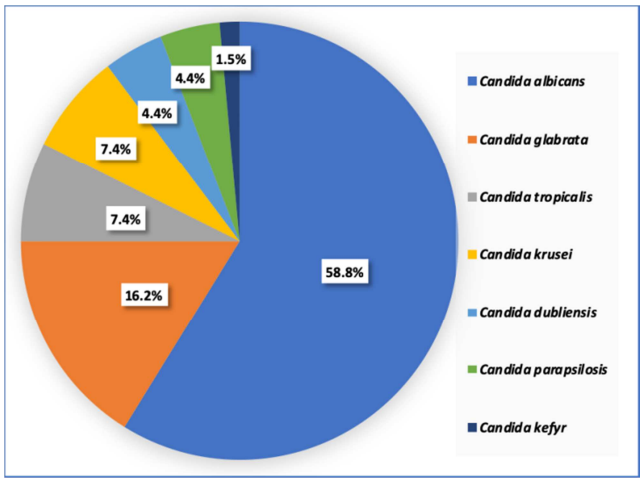

Figure 1. *Distribution of Candida species.*

3.2. Antifungal Susceptibility

Among 68 *Candida* strains isolated, 35 were tested for antifungal drugs: *C. albicans (n*=20), *C. glabrata* (n=6), *C. tropicalis* (n=5), *C. parapsilosis* (n=2) and *C. krusei* (n=2).

The majority of isolates were susceptible to ketoconazole (94.3%), fluconazole (85.7%), amphotericin B (88.6%) and 5 fluoro-cytosine (88.6%). The susceptibility rate was lower for itraconazole (51.4%) and miconazole (68.6%). Intermediate susceptibility was also described, and it was higher for itraconazole (42.9%) and miconazole (28.6%). 2.9% of resistance was observed for fluconazole, ketoconazole, miconazole and 5 fluoro-cytosine. It was 5.7% for itraconazole and amphotericin B (Table 2).

Table 2. *Percentage of susceptibility of antifungal agents by Fungitest (Bio-Rad) (%).*

	Fluconazole	Itraconazole	Ketoconazole	Miconazole	Amphotericin B	5 Fluoro-cytosine
Sensible	85.7	51.4	94.3	68.6	88.6	88.6
Intermediate	11.4	42.9	5.7	28.6	5.7	8.6
Resistant	2.9	5.7	2.9	2.9	5.7	2.9

C. albicans was susceptible to all antifungal drugs: 100% for fluconazole, ketoconazole, and amphotericin B, 80% for itraconazole and 90% for miconazole. Only one strain (5%) was resistant to 5 fluoro-cytosine. The susceptibility of *Candida glabrata* was 100% for 5 fluoro-cytosine, 83.3% for fluconazole, ketoconazole, miconazole, amphotericin B. One strain of *C. glabrata* (16.6%) was resistant to itraconazole and 4 strains (66.6%). The majority of *C. tropicalis* (80%) was susceptible to fluconazole, ketoconazole, amphotericin B and 5 fluoro-cytosine. One strain of *C. tropicalis* was resistant to all the azole and amphotericin B

C. parapsilosis was susceptible to ketoconazole and 5 fluoro-cytosine but all strains had intermediate susceptibility (100%) to itraconazole and miconazole. For *all C. krusei*, an intermediate susceptibility to fluconazole, itraconazole, miconazole and 5 fluoro-cytosine was noted. One *C. krusei* strains was resistant to amphotericin B (Table 3).

Table 3. *Antifungal susceptibility of Candida species using Fungitest (Bio-Rad) (n, %).*

		C. albicans (n=20)	*C. glabrata* (n=6)	*C. tropicalis* (n=5)	*C. krusei* (n=2)	*C. parapsilosis* (n=2)
Fluconazole	S	20 (100%)	5 (83.3%)	4 (80%)	1 (50%)	0
	I	0	1 (16.7%)	0	1 (50%)	2 (100%)
	R	0	0	1 (20%)	0	0
Itraconazole	S	16 (80%)	1 (16.7%)	1 (20%)	0	0
	I	4 (20%)	4 (66.6%)	3 (60%)	2 (100%)	2 (100%)
	R	0	1 (16.7%)	1 (20%)	0	0
Kétoconazole	S	20 (100%)	5 (83.3%)	4 (80%)	1 (50%)	2 (100%)
	I	0	1 (16.7%)	0	1 (50%)	0

		***C. albicans* (n=20)**	***C. glabrata* (n=6)**	***C. tropicalis* (n=5)**	***C. krusei* (n=2)**	***C. parapsilosis* (n=2)**
	R	0	0	1 (20%)	0	0
	S	18 (90%)	5 (83.3%)	1 (20%)	0	0
Miconazole	I	2 (10%)	1 (16.7%)	3 (60%)	2 (100%)	2 (100%)
	R	0	0	1 (20%)	0	0
	S	20 (100%)	5 (83.3%)	4 (80%)	1 (50%)	1 (50%)
Amphotericin B	I	0	1 (16.7%)	0	0	1 (50%)
	R	0	0	1 (20%)	1 (50%)	0
	S	19 (95%)	6 (100%)	4 (80%)	0	2 (100%)
5 Fluoro-cytosine	I	0	0	1 (20%)	2 (100%)	0
	R	1 (5%)	0	0	0	0

Sensible: S; Intermediate: I; Resistant: T.

4. Discussion

The prevalence of fungal infection is highly increasing worldwide, particularly invasive fungal infection. *Candida albicans* is described as the most frequent etiological agent of candidemia but other non-albicans species have been reported as emerging causal agents.

The management of *Candida* infection required correct identification of *Candida* species in order to establish definitive etiological diagnosis and to guide the prescription of antifungal drugs. The objective of this study was to determine the spectrum of different *Candida* species, and their susceptibility to antifungal drugs.

The prevalence of *Candida* infection was 20.2%. The main source of *Candida* was vulvovaginal infection (70.5%) and onychomycosis (12.6%). The frequency of *Candida* in vaginal swab and nail specimen was described by other authors. *Candida* species were found to be the principal source of vaginal infection (33.3% prevalence) in women attending to the laboratory of Mycology in Fann university hospital [12]. Seck et al when studying the epidemiological profile of onychomycosis found *Candida* species as the main etiological agent [13].

In our study, *C. albicans* was the main specie (58.8%) followed by *C. glabrata* (16.2%); *C tropicalis* (7.4%); *C krusei* (7.4%); *C. parapsilosis* (4.4%) and *C. dubliniensis* (4.4%). Similar results were previously described in the same department. Dieng et al when assessing the distribution of *Candida* species found *C. albicans* (52.75%), *C. tropicalis* (4.4%), *C. glabrata* (4.4%), *C. dubliniensis* (1.1%) [14]. Sow et al, when using MALDITOF Mass Spectrometry for *Candida* species identification found similar trends: *C. albicans* (n=128), *C. glabrata* (n=27), *C. tropicalis* (n=24), *C. krusei* (n=5), *C. parapsilosis* (n=1) [15].

A strain of *C. kefyr* was found in our result. This was previously described by other authors in the same laboratory [14-15].

C. albicans (72.6%), *C. glabrata* (14.5%) and *C. tropicalis* (9.7%) were the main *Candida* species isolated in Abidjan in a study conducted by Djohan et al in 2012 [16]. Similar results were also described in Cameroon by Kamga et al in 2012 [17]. Another study conducted in Abidjan have found *C. glabrata* as main specie followed by *C. albicans* and *C. tropicalis* [18]. *Candida dubliensis* was also described in strains from Abidjan and Cameroon [17-18].

The distribution of *Candida* species found in our study is similar to what was found in Morocco by Uwingabiye et al in 2012 [19]. It was also similar to the distribution in Iran and Kuwait [20-21].

The evaluation of the susceptibility of *Candida* strains using Fungitest® show that the majority of *Candida* species were susceptible to ketoconazole, fluconazole, amphotericin B and 5 fluoro-cytosine (88.6%).

C. albicans is susceptible to all antifungal drugs but one isolate (5%) is resistant to 5 fluoro-cytosine. Khosravi et al when assessing the in vitro susceptibility of *Candida* species to antifungal drugs found that *Candida albicans* was susceptible to amphotericin B, itraconazole, fluconazole and ketoconazole but only one strain was resistant de flucytosine [21]. Resistance of *C. albicans* to flucytosine was previously described in Senegal by Dieng et al in 2001 who showed 11.1% of resistance [22].

Our results regarding the resistance of *C. albicans* to flucytosine are not in line with what found in Abidjan by Djohan et al and Alfouzan et al in Kuwait who didn't show resistance of *C. albicans* to flucytosine [16, 21].

The susceptibility of *C. glabrata* to fluconazole, ketoconazole, miconazole and amphotericin B was similar (83.3%). It was 100% for 5 Fluoro-cytosine. One strain of *C. glabrata* was resistant to itraconazole (16.7%). Our result regarding the susceptibility of *C. glabrata* to fluconazole, amphotericin and 5 fluoro-cytosine was similar with what was noted by Alfouzan et al in Kuwait [20]. The findings of this study regarding the susceptibility of *C. glabrata* to 5 Fluoro-cytosine are not in line with what was found by Khosravi et al who found total resistant of all *C. glabrata* (4 strains) to flucytosine [21]. The resistant of *C. glabrata* to itraconazole was previously described in Spain by Miranda et al [23]. The primary resistance of *C. glabrata* to fluconazole described previously [10, 24-25], was not found in our study.

C. tropicalis was susceptible to fluconazole, ketoconazole, amphotericin B and 5 fluoro-cytosine. These were demonstrated by Bonouman et al in Abidjan and Khosravi et al in Iran but *C. tropicalis* was resistant to flucytosine (66.7%) in Iran [18, 21]. The susceptibility of *C. tropicalis* to fluconazole, amphotericin B and flucytosine was also described by Ozer et al in Turkey [26]. In previous study conducted in Senegal by Dieng et al, *C. tropicalis* had intermediate susceptibility to miconazole and one strain was resistant to ketoconazole [22]. A strain of *C. tropicalis* was

resistant to itraconazole and miconazole. This was not previously described but intermediate susceptibility to miconazole was observed for *C. tropicalis* [21, 27]. Similar results concerning the susceptibility of *C. parapsilosis* to ketoconazole, 5 fluoro-cytosine, fluconazole, itraconazole and miconazole results were demonstrated by other authors [28-30].

A strain of *C. krusei* and *C. tropicalis* was resistant to amphotericin B. This result was not in line with what found by other authors who described a susceptibility of *C. krusei* to amphotericin B [26-27]. The primary resistance of *C. krusei* to fluconazole was not observed in our study [28].

This study is preliminary study on antifungal susceptibility testing in Senegal. Results from this study give an idea regarding the prevalence of resistance and will allow to implement effective strategies for the prophylaxis and treatment of humans with Candida infections. Based on these results, a surveillance system could be implemented to monitor the emergence of resistance. One of the weaknesses of the study is non-use of molecular methods (PCR) which will allow to make the differentiation between *C. albicans* and the closely related species like *C. dubliniensis*.

5. Conclusions

Overall, the results of this study provide useful information regarding the distribution of Candida species and their susceptibility to antifungal drugs even if these findings may not allow to give sufficient conclusion regarding the susceptibility profile of Candida strains. However, surveillance is needed in order to identify any change in the species distribution and the emergence of resistance. To better describe the distribution of Candida species and their susceptibility to antifungal drugs, other studies using molecular methods for identification and conventional antifungal testing methods (CLSI or EUCAST) is required.

Abbreviations

RPMI: Roswell Park Memorial Institute, MIC: Minimal Inhibitory Concentration; CI: Confidence interval, UCAD; University Cheikh Anta of Diop, CER: Comité d'Ethique et de Recherche.

Declarations

Ethics Approval and Consent to Participate

The protocol was approved by the by the Research Ethic Committee of University Cheikh Anta Diop od Dakar (approval number: 48/2019/CER/UCAD).

Consent to Publish

The funder has no role to play in the manuscript writing, editing, and decision to publish.

Availability of Data and Materials

Data of this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

KS conceived and designed the study. LAN and MD monitored the data collection. LAN collected data in the site. KS analyzed the data. KS wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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