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. dublinsiensis 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 traumaism, 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 of 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°C 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 Fungitext® (Bio-Rad, France). Six antifungal agents tested were: 5-fluorocytosine, amphotericin B, miconazole, ketoconazole, itraconazole and fluconazole.

Principle: Fungitext® 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 Fungitext® [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=low 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).

| 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 specimen (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.](image)

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).

|            | 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, and 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 theazole 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).

|            | *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%)               |
| Itraconazole | S 16 (80%)          | 1 (16.7%)           | 1 (20%)              | 0                  | 0                      |
|            | I 4 (20%)            | 4 (66.6%)           | 3 (60%)              | 2 (100%)          | 2 (100%)               |
| Ketoconazole | S 20 (100%)        | 5 (83.3%)           | 4 (80%)              | 1 (50%)           | 2 (100%)               |
|            | I 0                  | 1 (16.7%)           | 0                    | 1 (50%)           | 0                      |
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 MALTIDO 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 dubliniensis* 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. Khozravi 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 fluconazole [21]. Resistance of *C. albicans* to fluconazole 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 fluconazole are not in line with what found in Abidjan by Djohan et al and Alfoouzan et al in Kuwait who didn’t show resistance of *C. albicans* to fluconazole [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 Alfoouzan et al in Kuwait [20]. The findings of this study regarding the susceptibility of *C. glabrata* to fluconazole, amphotericin and 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 fluconazole [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 fluconazole (66.7%) in Iran [18, 21]. The susceptibility of *C. tropicalis* to fluconazole, amphotericin B and fluconazole 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 \textit{C. tropicalis} [21, 27]. Similar results concerning the susceptibility of \textit{C. parapsilosis} to ketoconazole, 5 fluoro-cytosine, fluconazole, itraconazole and miconazole results were demonstrated by other authors [28-30].

A strain of \textit{C. krusei} and \textit{C. tropicalis} was resistant to amphotericin B. This result was not in line with what found by other authors who described a susceptibility of \textit{C. krusei} to amphotericin B [26-27]. The primary resistance of \textit{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 \textit{C. albicans} and the closely related species like \textit{C. dubliniensis}.

\section{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.

\textbf{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.

\textbf{Declarations}

\textit{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).

\textit{Consent to Publish}

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

\textit{Availability of Data and Materials}

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

\textbf{Competing Interests}

The authors declare that they have no competing interests.

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\textbf{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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