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Table of Content

Identification and antifungal susceptibility of <i>Candida</i> species isolated from vulvovaginal candidiasis in Dakar	1
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Mame Cheikh Seck, Pasca Elie Engo, Papa Aly Thiam Gueye,
Cheikh Faye, Moustapha Mbow, Khadim Diongue,
Mamadou Alpha Diallo, Mouhamadou Ndiaye,
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Full Length Research Paper

Identification and antifungal susceptibility of *Candida* species isolated from vulvovaginal candidiasis in Dakar

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In Senegal, studies regarding the distribution of *Candida* species involved in vulvovaginal candidiasis (VVC) and their antifungal susceptibility are scarce. Here we reported the frequency of *Candida* species isolated in VVC and their antifungal susceptibility in Dakar. A cross-sectional study included women referred to the laboratory of the Military Hospital of Ouakam for vaginal discharge was carried out. From each patient, two vaginal swabs and socio-demographic parameters were collected. The automated Vitek® 2 compact system determined species identification and antifungal susceptibility testing. Overall, 119 patients were enrolled. The mean age was 34.8 ± 10 years. The overall VVC prevalence was 31.93%, with the predominant *Candida albicans* (72%) followed by *Candida glabrata* (6.7%), *Candida tropicalis* (1.7%), and *Candida kefyr* (0.8%). *C. albicans* strains showed 100% susceptibility regarding the different antifungals except for flucytosine, in which two cases of resistance (8.34%) were noted. The observed susceptibility rates of *C. glabrata* to fluconazole, voriconazole, caspofungin, micafungin, amphotericin B, and flucytosine were 0, 100, 14.29, 100, 100 and 100%. *C. tropicalis* antifungal susceptibility was 100% for all tested drugs, while *C. kefyr* susceptibility was only noted for voriconazole and flucytosine. This study provides an overview of the distribution of *Candida* species involved in VVC in Dakar with *C. albicans* which showed a high susceptibility rate to the different antifungals.

Key words: Vulvovaginal candidiasis, antifungal susceptibility, Dakar.

INTRODUCTION

Vulvovaginal candidiasis (VVC) is a fungal infection caused by yeasts belonging to the genus *Candida* which includes around 200 species of non-pigmented, non-capsulated, multilateral budding yeasts that produce

filaments, except for *Candida glabrata* (Achkar and Fries, 2010). *Candida albicans*, the primary yeast involved in candidiasis, is a polymorphic fungus that can rapidly alternate between round cells (yeasts) and,

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pseudomycelian filaments or hyphae (Berman and Sudbery, 2002). Hyphae are resistant to phagocytosis more adherent to host surfaces, and can invade epithelial cell layers resulting in tissue damage (Nicholls et al., 2011). VVC is one of the most common gynecological disorders, with symptoms such as leukorrhea, pruritus, and burning frequently identified as causes of consultation (Barajas et al., 2019; Sobel, 2007). Approximately 70 to 75% of women have at least one episode of VVC during their lifetime (Sobel, 2007; Yano et al., 2019; Gonçalves et al., 2016). Although *C. albicans* is commonly reported to be the pathogenic agent, species known as non-*albicans* Candida (NAC), such as *C. glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei*, have recently emerged (Denning et al., 2018; Felix et al., 2019; Amouri et al., 2010). In Senegal, previous studies regarding VVC caused by *C. albicans* showed a prevalence range of 24 to 34% (Boye et al., 2022; Seck et al., 2015; Sylla, 2018). However, Sylla (2018) found in their study carried out at Fann Teaching Hospital of Dakar a prevalence rate of 27.96% of VVC due to NAC species. While both clinical and diagnosis of VVC are well-known and performed routinely, therapeutic management raises challenging concerns regarding the choice of appropriate antifungal drugs. Thus, despite the availability of effective antifungals, vulvovaginal candidiasis remains a complicated disorder to manage because of the drug resistance of some strains and the recurrent character of the diseases (Sobel, 2016). Also, antifungal susceptibility testing is not performed in most mycology laboratories in Africa. This study aimed to determine the frequency of *Candida* species involved in VVC and their antifungal susceptibility among patients referred to the microbiology laboratory of the Military Hospital of Ouakam, Dakar.

METHODOLOGY

Study site and population

This study was carried out from April to June 2022 at the microbiology laboratory of the Military Hospital of Ouakam. The study population included women referred to the laboratory for vaginal discharge during the study period. The Military Hospital of Ouakam is a level 3 public hospital located in Dakar, the capital city of Senegal.

Sample and data collection

From each patient, two vaginal swabs (VS) and socio-demographic parameters such as age, symptoms, marital status, education level, and address were collected. One VS was used to perform a wet smear and a thin smear for Gram Stain. The second VS was used for inoculation of Sabouraud Dextrose Agar (SDA). The culture medium was incubated at 35 to 37°C during 18 to 24 h under aerobic conditions. Plates without growth after 24 h were re-incubated for a further 24 h. Preparation and performance evaluation of the culture media was performed according to the manufacturer's instructions.

Identification and antifungal susceptibility of *Candida* species

Species identification and antifungal susceptibility testing of yeasts were determined by the automated Vitek® 2 compact system (bioMérieux, France) using YST-21343 and AST-YS01 cards. The VITEK 2 compact is a computerized microbiology system using growth-based technology using colorimetric reagent cards that are automatically incubated and interpreted. The YST-21343 identification card is based on established biochemical methods and newly developed substrates. The AST-YS07 Vitek® 2 Antifungal Susceptibility Card is intended for use with Vitek® 2 systems in clinical laboratories as an *in vitro* test to determine the susceptibility of *Candida*. Briefly, isolated yeast colonies were selected on Sabouraud agar, then transferred aseptically 3.0 ml of sterile saline (0.45 to 0.50% aqueous NaCl, pH 4.5 to 7.0) into a clear plastic test tube (12 mm x 75 mm). Next, a sterile swab transfers a sufficient number of morphologically similar colonies into the saline tube. A homogeneous suspension of organisms with a density equivalent to a McFarland No. 1.80 to 2.20 using the Vitek® 2 DensiCHEK™ Plus was prepared. Finally, the suspension tube and the YST card were placed into the cassette. The filled cassette was inserted manually into Vitek® 2 compact reader-incubator module. The final identification of excellent, good, acceptable, or low discrimination was considered correct.

Statistical analysis

Statistical analyses were performed using R software. Continuous variables were described as mean (standard deviation). Normally distributed variables were compared with a t-test. Categorical variables were presented as percent, and Fisher exact or Chi-squared tests were used for proportional assessments. Univariate logistic regression analysis assessed the association between the related risk factors and positivity. Four age groups were defined for analysis, that is, 17 to 25 years; 26 to 35 years; 36 to 45 years, and over 45 years. For all statistical tests, we accepted that a two-sided level of significance was set at $p \leq 0.05$.

Ethical considerations

This study was hospital-based research conducted in normal conditions under the Declaration of Helsinki. Ethical permission was obtained from the hospital authorities, and the patient's consent was also received before specimen collection. Information collected during the study was analyzed using the participant's identification code to ensure confidentiality.

RESULTS

Socio-demographic characteristics of the study population

Over the course of the study period, 119 patients were enrolled. The mean age was 34.8 years (Standard Deviation +/- 10 years). The age group 26 - 35 years was most represented [44.54% (95% CI: 40 - 59)]. According to marital status, 87.4% (n =104) were married. By educational level, patients who completed their secondary school level were most represented with 43.70% (n = 52), followed by university and primary level with 29.41 and 11.76%, respectively. The patient's most common symptoms were grouped as follows: discharge

Table 1. Characteristics of the study population.

Characteristics	N = 119, n (%)	95% CI
Age group		
17 - 25	18 (15.1)	9.5 - 23
26 - 35	59 (49.6)	40 - 59
36 - 45	25 (21.0)	14 - 30
>45	17 (14.3)	8.8 - 22
Marital status		
Married	104 (87.4)	80 - 93
Single	15 (12.6)	7.5 - 20
Education level		
Illiterate	18 (15.1)	9.5 - 23
Primary	14 (11.8)	6.8 - 19
Secondary	52 (43.7)	35 - 53
University	35 (29.4)	22 - 39
Symptoms		
Burning sensation only	6 (5.0)	2.1 - 11
Discharge & irritation	44 (37.0)	28 - 46
Discharge & irritation & burning sensation	21 (17.6)	11 - 26
Discharge only	34 (28.6)	21 - 38
Irritation only	2 (1.7)	0.29 - 6.5
No symptom	12 (10.1)	5.6 - 17
Culture		
Positive	38 (31.9)	24 - 41
Negative	81 (68.1)	59 - 76
Species		
<i>C. albicans</i>	27 (22.7)	16 - 31
<i>C. glabrata</i>	8 (6.7)	3.2 - 13
<i>C. kefyr</i>	1 (0.8)	0.04 - 5.3
<i>C. tropicalis</i>	2 (1.7)	0.29 - 6.5

Source: Authors

and irritation [37% (95% CI: 28 - 46)], discharge only [28.6% (95% CI: 21 - 38)], discharge and irritation and burning sensation [17.6% (95% CI: 11 - 26)]. Detailed characteristics of the study population are presented in Table 1.

Prevalence of VVC and distribution of *Candida* spp.

The overall VVC prevalence was 31.93% (95% CI: 24 - 41). Four *Candida* spp. were isolated with the predominant *C. albicans* [72% (n=27)] followed by *C. glabrata* [6.7% (n=8)], *C. tropicalis* [1.7% (n=2)], and *C. kefyr* [0.8% (n=1)]. According to age group, the highest prevalence was recorded in patients aged between 36 and 45 years with 36.0% (95% CI: 19 - 57). Those over 45 years were less affected, with 11.11% (95% CI: 2.1 - 18). By marital status, single patients were more concerned with 40.0% (95% CI: 17 - 67) than married ones with no

significant difference ($p = 0.55$). The distribution of VVC prevalence by education level showed that the highest rate was recorded [48.57% (95% CI 32- 66)] in patients with university level and the lower one in the illiterate group [16.7% (95% CI: 4.4 - 42)] with no significant difference ($p = 0.079$). According to symptomatologic status, the highest prevalence rate [47.6% (95% CI: 26 - 70)] was noted in patients with discharge, irritation, and burning sensation, followed by women with discharge and irritation [34.1% (95% CI: 21 - 50)] and discharge only with 26.5% (95% CI: 14 - 45). Any VVC was recorded in patients who presented irritation only as a symptom. The other details regarding VVC prevalence are shown in Table 2.

Antifungal susceptibility

Antifungal susceptibility was tested for six drugs:

Table 2. Vulvovaginal prevalence regarding socio-demographic parameters and symptoms.

Characteristics	Positive (N = 38) n (%)	95% CI	Overall, N =119	p
Age group				
17 - 25	7 (38.9)	18 - 64	18 (100)	0.3
26 - 35	20 (33.9)	22 - 47	59 (100)	
36 - 45	9 (36.0)	19 - 57	25 (100)	
46 - 68	2 (11.8)	2.1 - 18	17 (100)	
Marital status				
Married	32 (30.8)	22 - 41	104 (100)	0.6
Single	6 (40.0)	17 - 67	15 (100)	
Education level				
Illiterate	3 (16.7)	4.4 - 42	18 (100)	0.08
Primary	4 (28.6)	9.6 - 58	14 (100)	
Secondary	14 (26.9)	16 - 41	52 (100)	
University	17 (48.6)	32 - 66	35 (100)	
Symptoms				
Burning sensation only	1 (16.7)	0.88 - 64	6 (100)	0.5
Discharge & irritation	15 (34.1)	21 - 50	44 (100)	
Discharge & irritation & burning sensation	10 (47.6)	26 - 70	21 (100)	
Discharge only	9 (26.5)	14 - 45	34 (100)	
Irritation only	0 (0.0)	0.00 - 80	2 (100)	
No symptom	3 (25.0)	6.7 - 57	12 (100)	

Source: Authors

Amphotericin B (AMB), Flucytosine (FCT), Fluconazole (FLU), Caspofungin (CAS), Micafungin (MCF), and Voriconazole (VRC)). From the 38 *Candida* strains isolated, 34 antifungal susceptibilities were successfully performed as follows: *C. albicans* (n=24), *C. glabrata* (n=7), *C. tropicalis* (n=2), and *C. kefyr* (n=1). *C. albicans* strains showed 100% susceptibility regarding the different antifungals except for Flucytosine, in which two cases of resistance (8.34%) were noted. The observed susceptibility rates of *C. glabrata* to FLU, VRC, CAS, MCF, AMB, and FCT were 0, 100, 14.29, 100, 100, and 100%. *C. tropicalis* antifungal susceptibility was 100% for all tested drugs, while *C. kefyr* susceptibility was only noted for Voriconazole and Flucytosine (Figure 1).

DISCUSSION

This study aimed to determine the frequency of *Candida* spp involved in vulvovaginal candidiasis and their antifungal susceptibility among patients referred to the microbiology laboratory of the Military Hospital of Ouakam using the automated Vitek® system.

Frequency of vulvovaginal candidiasis

In our study, the frequency of vulvovaginal candidiasis

was 31.93%. This finding is in line with those previously reported in the same region of Dakar by Sylla (2018) who found a prevalence rate of 32.6%. Comparable prevalence rates were reported in Ghana and Iran by Waikhom and Matehkolaei with 30.7 and 28.3%, respectively (Waikhom et al., 2020; Rezaei-Matehkolaei et al., 2016). However, in Lebanon, Ghaddar et al. (2020) registered a higher frequency rate of 39%. Similarly, a prevalence of VVC of 51.3% was reported in Vietnam by Anh et al. (2021). These findings show that the frequency of vulvovaginal candidiasis is relatively high and differs from country to country.

In our series, patients aged between 26 and 35 years were the most represented. Still, the highest VVC prevalence was recorded in patients aged between 36 and 45 years, with 36.0%, indicating that this age group is most concerned by vaginal disorders in Dakar. Contrary to the findings of Sylla (2018), VVC was more prevalent among women aged 20-35 years. Likewise, in Ghana, Waikhon et al. (2020) noted that the 20-29 age group was the most affected by VVC. In another study carried out in Iran and involving 120 patients, Rezaei-Matehkolaei et al. (2016) reported that the 41-50 age group was the most affected.

Also, in our study, patients with university degrees were the most affected by VVC (48.57%), while illiterate patients were the least likely to be involved. Our finding is in line with the conclusion reached by Rathod et al.

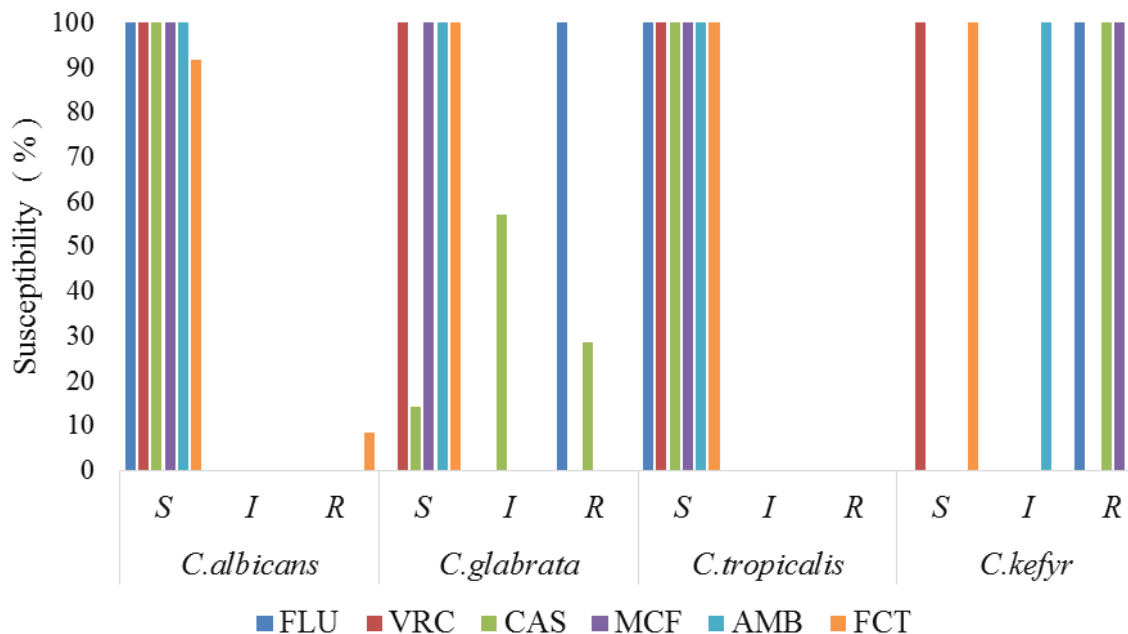


Figure 1. Antifungal susceptibility of isolated *Candida* species. FLU: Fluconazole, VRC: Voriconazole, CAS: Caspofungin, MCF: Micafungin, AMB: Amphotericin B, FCT: Flucytosine. S: Susceptible, I: Intermediate (susceptible dose-dependent), R: Resistant.
Source: Authors

(2012) but in contradiction with the results of Yadav and Prakash (2016) and Bitew and Abebaw (2018), who reported that illiterate women were more affected than those patients with primary school education and above. These findings suggest that the hypothesis that improved personal hygiene resulting from schooling can reduce the risk of vaginal infection may not always be verified. According to marital status, singles were more affected than married patients in our study, but the association was not statistically significant. The same trend was previously observed by Sylla (2018) in Dakar. Similarly, in Ethiopia, Bitew and Abebaw (2018) reported that vulvovaginal candidiasis was higher among divorced study subjects (52.6%) compared to unmarried (41.5%) or married (37.4%) study subjects. Contrary to the report of Waikhom et al. (2020) in Accra, most of the VVC were noted as married subjects.

Prevalence of different isolated *Candida* spp.

In this report, cultures were positive for 38 strains (31.93%), with four *Candida* species identified: *C. albicans* (72%), *C. glabrata* (19%), *C. tropicalis* (6%) and *C. kefyr* (3%). The most frequently isolated species was *C. albicans*. The non-albicans species (NAS) were observed in 28%. These findings align with those reported by Sylla (2018) who estimated the prevalence of *C. albicans* at 71.51% and that of NAS at 27.96%. Also,

the predominance of *C. albicans* was noted in the series of Shi et al. (2020) 76.9% and in Ethiopia by Bitew and Abebaw (2018) at 58.6%. However, in Accra, *C. glabrata* was identified as the main species causing vulvovaginal candidiasis with 57.4% (Waikhom et al., 2020), while this species ranked in the second position in our series with 19%. In Ivory Coast (Kpongbo et al., 2018) and Iran (Rezaei-Matehkolaei et al., 2016), *C. glabrata* ranks second in species causing vaginal candidiasis with 19.2 and 8.8%, respectively. In Ethiopia, Bitew and Abebaw (2018) reported that *C. krusei* was the most isolated species after *C. albicans* in vaginal candidiasis. Regarding the frequency of *C. tropicalis* (6%) and *C. kefyr* (3%), our results are in line with those found in Iran and Vietnam by Anh et al. (2021) and Nejat et al. (2018) who reported a prevalence of 4.31 and 5.7%, respectively.

Antifungal susceptibility

In our study, antifungal susceptibility was assessed for Fluconazole, Voriconazole, Caspofungin, Micafungin, Amphotericin B, and Flucytosine.

Regarding the Azoles antifungal group (Fluconazole and Voriconazole), all isolated *Candida* strains were 100% susceptible to Voriconazole. *C. kefyr* and *C. glabrata* were not tested with Fluconazole. On the other hand, no resistance to Fluconazole was found for *C.*

albicans and *C. kefyr* strains, that is, 100% susceptibility of both species to Fluconazole. The same trend of antifungal susceptibility of azoles was reported by Bitew and Abebaw (2018). However, Sharifynia et al. (2017) suggested a high resistance of *C. albicans* to Fluconazole than in non-*albicans* *Candida* spp.

The antifungal susceptibility test to the Echinocandins group (Caspofungin and Micafungin) showed 100% susceptibility of all isolated *Candida* spp., except *C. glabrata* which showed 28.57% of resistance to Caspofungin and 53.14% susceptible dose-dependent (intermediate) to the same drug. Regarding Micafungin, our results are supported by Anh et al. (2021) and Bitew and Abebaw (2018) who reported 100% susceptibility of 133 *Candida* spp. involved in VVC to Micafungin. However, regarding Caspofungin susceptibility, a resistance rate of 4.7% was reported for *C. albicans* by Rezaei-Matehkolaei et al. (2016).

Regarding Amphotericin B and Flucytosine susceptibility tests, all isolated *Candida* strains were 100% susceptible to amphotericin B. This finding agrees with Abidjan's findings by Kpongbo et al. (2018) who showed that all *Candida* strains isolated in his series were susceptible to amphotericin B.

Also, in our series, all *Candida* spp. were sensitive to Flucytosine, except for two *C. albicans* strains suggesting a resistance of 8.34%. Previously in Senegal, Mane et al. (unpublished data) reported a resistance rate of *C. albicans* to flucytosine of 4%. Similarly, a low resistance rate of 2% of *C. albicans* to Flucytosine was found by Bitew and Abebaw (2018).

Conclusion

This study regarding the frequency of species involved in vulvovaginal candidiasis and their antifungal susceptibility in Dakar reveals a prevalence rate comparable with those previously reported in Dakar. Furthermore, it overviews the distribution of *Candida* spp. in Dakar. Concerning the antifungal susceptibility, *C. albicans*, the main species involved in VVC, showed a high susceptibility rate to the different antifungals tested. However, antifungal susceptibility monitoring for early resistance detection should be continued.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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