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In Vitro Susceptibility of *Candida* spp. Isolates to Antifungals from Patients at Charles Mérieux Infectious Disease Centre (CICM) in Bamako, Mali

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Abstract: Background: The decision to treat infections due to *Candida* spp. should be based on the susceptibility of *Candida* isolates. The resistance of *Candida* spp. to antifungals is increasing with the use of empirical or repeated treatments. In Mali, there are few data on *Candida* species distribution and in vitro susceptibility to antifungals. We aim to describe the distribution and in vitro susceptibility of *Candida* isolates. **Methods:** A retrospective and prospective study was conducted from 1 January 2009 to 31 December 2019. A total of 1224 samples from inpatients and outpatients, including both males and females, were collected. The identification of the *Candida* species and in vitro antifungal susceptibility were performed using VITEK-2 (AST-YSO8 cards, bioMérieux). **Results:** In total, 1175 (95.9%) samples tested positive for *Candida* spp.; 54.33% were from community health care centers; 89% were female; 85% of the *Candida* spp. were isolated from vaginal discharge and 10.40% were isolated from pus; and the most common species were *Candida albicans* (68%), *Candida glabrata* (11%), and *Candida tropicalis* (6%). Fluconazole was the most potent antifungal, with 99.81% susceptibility to all *Candida* isolates. Susceptibility to flucytosine was 98% for *C. albicans*, 100% for *C. glabrata*, 97% for *C. tropicalis*, and 36.96% for *C. krusei*. Susceptibility to amphotericin B was 96% for *C. albicans*, 97% for *C. glabrata*, 100% for *C. tropicalis*, and 81% for *C. krusei*. **Conclusions:** *C. albicans* and *C. glabrata* were common and susceptible to the antifungals tested. *C. krusei* and *C. rugosa* were the most resistant. Systematic antifungal in vitro susceptibility tests before the treatment of candidiasis infections should be reinforced in health care facilities in Mali.

Keywords: *Candida* spp.; antifungals; susceptibility; VITEK-2; Bamako; Mali

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1. Introduction

Clinical presentations of *Candida* spp. infection in outpatients have shown a high variability in affected sites, with the most frequent site of candidiasis being the nails, followed by the skin and vagina. Most *Candida* spp. from outpatients were susceptible to fluconazole, followed by 5-flucytosine, voriconazole, itraconazole, and ketoconazole [1]. *C. albicans* has shown a good susceptibility to most antifungals used in topical applications [2]. *Candida* spp. polyresistance to antifungals has also been reported to have a higher resistance rate in *non-C. albicans* than in *C. albicans* species [3]. *Candida* spp. isolates from both patients with and without HIV infections have shown a preserved antifungal susceptibility, but Minimum Inhibitory Concentration (MIC) values were variable among isolates [4]. *Candida* species distribution varies according to hospital departments; *C. parapsilosis* was mostly identified in intensive care units (ICUs) and surgery services, whereas *C. krusei* was most frequently recovered from patients in the internal medicine department [5]. *Candida parapsilosis* isolates were the second most frequently isolated in pediatric patients [6]. Several methods are used to test in vitro antifungal susceptibility of *Candida* spp. isolates: microdilution EUCAST (the European Committee on Antimicrobial Susceptibility Testing) or CLSI (the Clinical and Laboratory Standards Institute) methods are standard references. The VITEK-2 system reduces the period required for identification and improves the rate of identification of *Candida* species isolates. VITEK-2 also appeared to be an alternative method for identification and antimicrobial susceptibility testing (AST) for the *Candida* species, with the aim of prescribing appropriate antifungals for the management of opportunistic infection among immunosuppressed patients [7].

Vulvovaginitis due to *Candida* is the most frequent clinical form [8]. Genital *Candida* infection is a public health problem and is reported in 22.71% of pregnant women; *C. albicans* was isolated in 40.39% of patients, and non-*C. albicans* species were isolated in 59.61% of patients [9]. In Côte d'Ivoire, the occurrence of recurrent vulvovaginal candidiasis is common, and the non-*Candida albicans* species play a major role in disease epidemiology [10]. *C. albicans* was the most common yeast species isolated in onychomycosis in Dakar [11].

In West Africa, *Candida* spp. susceptibility to antifungals has exhibited varying patterns. In Ivory Coast, *C. albicans* has shown a low susceptibility to itraconazole and the best susceptibility to amphotericin B, 5-fluorocytosine, voriconazole, and fluconazole [12]. In Burkina Faso, the resistance rates of *Candida* spp. isolated in vulvovaginal and oral environments to fluconazole, itraconazole, ketoconazole, and amphotericin have been reported [13].

In Mali, recurrent vulvovaginal candidiasis is listed as the second most common fungal disease [14], and among cases of vulvovaginal candidiasis in a hospital setting, *Candida* species were identified in 60.42% of women, with *C. albicans* being the common species, followed by *C. famata*, *C. dubliniensis*, and *C. krusei* [15]. However, few studies have explored the distribution and susceptibility of *Candida* spp. to antifungals. What is the antifungal susceptibility rate of *Candida* species identified at the Rodolphe Mérieux laboratory (LRM) from Bamako patients' samples? We aimed to describe the distribution and antifungal susceptibility profile of *Candida* isolates from outpatient and inpatient samples routinely tested for fungal disease diagnosis at the Charles Mérieux Infectious Disease Centre (CICM) laboratory in Bamako.

2. Methods

2.1. Study Site

Sample testing for fungal infections was carried out in the Rodolphe Mérieux laboratory (LRM) of the Charles Mérieux Infectious Diseases Centre (CICM) in Bamako, which is a clinical biology laboratory with a routine lab diagnosis practice.

2.2. Study Design and Period

This is a retrospective cross-sectional study on data collected over ten years from 1 January 2009 to 31 December 2018, and a prospective cross-sectional study with data collected for one year, from 1 January 2019 to 31 December 2019.

2.3. Study Population

The study participants were symptomatic patients of all ages and genders who sought fungal disease diagnosis at the LRM. Samples were taken from superficial lesions (swabs, stools, semen, and dander) in LRM facilities. Samples from deep lesions, such as cerebrospinal fluid and bronchioalveolar fluid, were taken from inpatients at their respective hospital settings. Patients originated from the population of Bamako and sought care at public hospitals, private clinics, and community health care centers.

2.4. Selection Criteria

2.4.1. Inclusion Criteria

All samples received or taken in CICM facilities and tested in the LRM for mycological examination were included. Samples from deep lesions, such as cerebrospinal fluid and bronchioalveolar fluid, were collected at hospitals from hospitalized patients.

2.4.2. Non-Inclusion Criteria

Samples received for mycological examination and diagnosed with other non-*Candida* fungal agents were not included in the analysis.

2.5. Sample Collection and Processing

Samples were taken from lesions, at specific times after any antifungal treatment (15 days after for skin and 2 months after for nail lesions). In the case of inflammatory or suppurative lesions, a cotton swab was used. Before proceeding to vaginal discharge collection, women were advised to abstain from intercourse one day before vaginal swab sampling and were recommended to avoid personal hygiene before swabbing. Vaginal discharge was not taken during their menstrual periods. Two swabs were performed at the LRM: One of the swabs was dipped into a tube containing distilled water to release germs. A drop was placed between the slide and coverslip and examined with a microscope. The second swab was used for culturing.

2.5.1. Culture

For each sample, 10 microliters was inoculated on Sabouraud + chloramphenicol and Sabouraud + actidione + chloramphenicol for initial culturing (reference 51021, BioMérieux, Marcy d'Etoile, France)

on a medium plate and incubated at 20–25 °C for fungal growth until 48 h. If no growth was observed after 48 h, the sample was classified as negative for *Candida* spp.

2.5.2. *Candida* Species Identification and In Vitro AFST Using VITEK 2 COMPACT

(BioMérieux, Marcy d'Etoile, France).

Identification of *Candida* Species

A sterile swab or applicator stick was used to transfer pure culture colonies and to suspend them in 3 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5–7.0) in a 12 mm × 75 mm clear plastic (polystyrene) test tube. The turbidity was adjusted to a 1.8-2.2 McFarland turbidity range and measured using a DensiChek™ turbidity meter. Identification cards were inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension was placed into a special rack (cassette), and the identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The filled cassette was placed manually into a vacuum chamber station. After the vacuum was applied, the microorganism suspension was forced through the transfer tube into microchannels that filled all the test wells. Inoculated cards were sealed and incubated at 35.5 °C ± 1.0 °C. Each card was removed from the carousel incubator once every 15 min, transported to the optical system for reaction readings, and then returned to the incubator until the next reading time. Data were collected at 15 min intervals during the entire incubation period [16].

Antifungal Susceptibility Testing

The Clinical and Laboratory Standards Institute's (CLSI) susceptibility breakpoints for *Candida* isolates were used. The expected QC range for the CLSI Broth Microdilution at 24 h and the expected QC range for the FDA/CLSI Broth Microdilution at 48 h were used [Ref 420 739, VITEK 2™, technology AST -YS08 fungal susceptibility card, 9311888-P1EN1 - 2016/01]. The turbidity of the inoculum was adjusted to 2.0 McFarland (1.8–2.2; DensiCheck, BioMérieux) with 0.45% sterile NaCl as recommended by the manufacturer. The inocula were loaded into the VITEK 2™ AST YS08 fungal susceptibility card (BioMérieux), and the cards were placed into the instrument. The card contained the following antifungals with their calling ranges: amphotericin B (≤0.25—≥16 μg/mL), flucytosine (≤1—≥64 μg/mL), fluconazole (≤0.5—≥64 μg/mL), voriconazole (≤0.125—≥8 μg/mL), caspofungin (≤0.125—≥8 μg/mL), and micafungin (≤0.06—≥8 μg/mL). The fluconazole susceptibility breakpoints were used for *C. albicans*, *C. dubliniensis*, *C. parapsilosis*, *C. tropicalis*, *C. guillermondii*, and *C. lusitaniae*, namely, susceptible (S) ≤ 0.5 μg/mL; susceptible dose-dependent (SDD), 0.5 to 64 μg/mL; and resistant (R) ≥ 64 μg/mL. The flucytosine susceptibility breakpoints (calling ranges) used for *C. albicans*, *C. dubliniensis*, *C. parapsilosis*, *C. tropicalis*, *C. guillermondii*, and *C. lusitaniae* ranged from 1 to 64 μg/mL. The voriconazole susceptibility values for *C. albicans*, *C. parapsilosis*, *C. tropicalis C. albicans*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. guillermondii*, and *C. lusitaniae* were S ≤ 0.12 μg/mL; SDD, 0.25 to 8 μg/mL; and R ≥ 8 μg/mL [Ref 420 739, VITEK 2™, technology AST -YS08 fungal susceptibility card] as previously described [17–19].

2.6. Data Collection and Analysis

Data was collected using the SYSLAM 64™ software, which was used to list the cases of *Candida* spp. infections diagnosed at the LRM from 2009 to 2019. The parameters recorded for each patient were sample processing date, patient identifier number, age, gender, health care centers, residence of the patient, and in vitro susceptibility testing results. Data were entered into Microsoft Excel, Version 2016, Microsoft 2016, and analyzed using SPSS for Windows, Version 16.0. Chicago, 2007. The proportion

of *Candida* species identified and the proportion of *Candida* spp. isolates that were susceptible, intermediately susceptible, or resistant to antifungals were calculated.

2.7. Ethical Considerations

All data presented are from a routine diagnosis laboratory database and did not require approval from an ethical review committee. Data were anonymized, ensuring respect for patients' confidentiality. Data were processed in line with the principles of the Declaration of Helsinki. The CICM laboratory director granted permission to access, analyze, and publish the data.

3. Results

A total of 1224 positive samples were collected during the study period at the Rodolphe Mérieux laboratory of the CICM. All samples were found to be positive to fungi, with 1190/1224 (97.22%) positive to the genus *Candida*, 20/1224 (1.63%) to the genus *Aspergillus*, 5/1224 (0.41%) to the genus *Trichophyton*, 2/1224 (0.17%) to the genus *Trichoderma,* 2/1224 (0.17%) to the genus Stephanoascus, 1/1224 (0.08%) to the genus *Cryptococcus*, 1/1224 (0.08%) to the genus *Malassezia*, 1/1224 (0.08%) to the genus Mucor, 1/1224 (0.08%) to the genus *Penicillium*, and 1/1224 (0.08%) to the genus *Trichosporon*.

The susceptible dose-dependent (SDD) or intermediate antifungal susceptibility was high in *Candida* isolated from vaginal discharge; the resistance to antifungals, although rare, was also observed in *Candida* isolated in pus (1.4%) and vaginal discharge (1.04%, $p = 0.204$). Resistance to antifungals showed a trend of being increasingly detected in samples from community health centers (46%).

The majority of the samples, 54.33% (665/1224), were collected from patients in community health care centers; 10.95% (134/1224) of the samples were from hospital patients, and 34.72% (425/1224) were from unknown sources (sites).

Furthermore, 11% (135/1224) of samples were isolated from male patients, and 89% (1089/1224) were isolated from female patients; the sex ratio (M:F) was 0.12. In addition, 51% (624/1224) of fungal infections were detected in patients aged from 25 to 36 years (Table 1).

Table 1: Socio-demographic parameters.

Parameters	Frequency (n = 1224)	Percentage (%)
Health care structures		
Community health care centers	665	54.3
Unknown sources	425	34.7
Hospitals	134	10.9
Gender		
Female	1089	89.0
Male	135	11.0
Age groups (years)		
0–12	49	4.0
13–24	233	19.0
25–36	624	51.0
37–48	220	18.0
49–60	61	5.0
≥61	37	3.0

The most frequent samples were taken from vaginal discharge, with 85% (1038/1224) (Table 2).

Table 2: Type of samples.

Samples	Frequency (n)	Percentage (%)
Expectoration	26	2.1
Blood culture	2	0.2
Body liquids	4	0.3
Broncho-alveolar lavage liquid	1	0.1
Urethral samples	1	0.1
Pus	127	10.4
Vaginal swabs	1038	84.8
Stool	1	0.1
Semen	4	0.3
Dander	11	0.9
Urine	9	0.7
Total	1224	100

Susceptibility to antifungals has not been tested for other fungal genera that have been detected, such as *Aspergillus* spp., *Cryptococcus* spp., *Malassezia* spp., *Mucor* spp., *Penicillium* spp., *Stephanoascus* spp., *Trichoderma* spp., *Trichophyton* spp., and *Trichosporon* spp.

Eighteen *Candida* species have been identified in 1175 samples tested, with *C. albicans* being the commonest species (68%), followed by *C. glabrata* (11%) (Table 3).

Table 3: Frequencies of *Candida* isolates.

***Candida* Isolates**	**Frequency (n)**	**Percentage (%)**
Candida albicans	805	68.5
Candida glabrata	132	11.2
Candida tropicalis	71	6.04
Candida krusei	52	4.4
Candida parapsilosis	33	2.8
Candida famata	22	1.8
Candida dubliniensis	18	1.5
Candida kefyr	9	0.7
Candida lusitaniae	8	0.6
Candida pelliculosa	6	0.5
Candida rugosa	5	0.4
Candida sphaerica	*5*	*0.4*
* Other species	6	0.5
Candida species not identified	3	0.2
Total	1175	100

* *C. ciferrii*, *C. colliculosa*, *C. globosa*, *C. guilliermondii*, *C. utilis*, and *C. sake* were identified only once.

We observed a variable susceptibility to flucytosine, with *C. glabrata* (100%) and *C. parapsilosis* (100%) being the most susceptible, followed by *C. albicans* (98.02%), *C. tropicalis* (96.78%), *C. dubliniensis* (77.78%), and *C. krusei* (45.65%). The higher SDD rate was in *Candida Lusitaniae*, and the higher R rate (36.96%) was in *C. krusei*. Two isolates of *C. tropicalis* were resistant to flucytosine (Table 4).

Table 4: In vitro susceptibility of *Candida* spp. to flucytosine.

***Candida* Isolates**	**Sensitive n (%)**	**SDD n (%)**	**Resistance n (%)**	**Total**
Candida albicans	689 (98.0)	4 (0.5)	10 (1.42)	703
Candida glabrata	128 (100)	0 (0.0)	0 (0.0)	128
Candida tropicalis	60 (96.8)	0 (0.00)	2 (3.2)	62
Candida krusei	21 (45.6)	8 (17.4)	17 (37.0)	46
Candida parapsilosis	33 (100)	0 (0.0)	0 (0.0)	33
Candida famata	22 (100)	0 (0.0)	0 (0.0)	22
Candida dubliniensis	14 (77.8)	4 (22.2)	0 (0.0)	18
Candida kefyr	9 (100)	0 (0.0)	0 (0.0)	9
Candida lusitaniae	5 (62.5)	2 (25.0)	1 (12.5)	8
Candida pelliculosa	6 (100)	0 (0.0)	0 (0.0)	6
Candida rugosa	5 (100)	0 (0.0)	0 (0.0)	5
Candida sphaerica	5 (100)	0 (0.0)	0 (0.0)	5
* Other species	6 (100)	0 (0.0)	0 (0.0)	6
Candida species not identified	2 (100)	0 (0.0)	0 (0.0)	2
Total	1005 (95.5)	18 (1.7)	30 (2.9)	1053

** C. ciferrii*, *C. colliculosa*, *C. globosa*, *C. guilliermondii*, *C. utilis*, and *C. sake* were identified only once.

Candida clinical isolates showed lower resistance rates to caspofungin, micafungin, and voriconazole.

Among *C. albicans* isolates, one was resistant to voriconazole, and one was resistant to micafungin (Table 5).

Table 5: In vitro resistance of *Candida* spp.

***Candida* Species**	**Voriconazole R n (%)**	**Itraconazole R n (%)**	**Micafungin R n (%)**	**Fluconazole R n (%)**	**Total n/N**
C. albicans	1 (0.1)	0 (0.0)	1 (0.1)	0 (0.0)	2/703
C. glabrata	0 (0.0)	2 (1.5)	1 (0.8)	0 (0.0)	3/128
C. tropicalis	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	1/62
C. krusei	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0/46
C. parapsilosis	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	1/33
C. famata	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0/22
C. dubliniensis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0/18
C. kefyr	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0/9
C. lusitaniae	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0/8
C. pelliculosa	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0/6
C. rugosa	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0/5
C. sphaerica	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0/5
* Other species	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0/6
Candida species not identified	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0/2
Total	1 (0.1)	2 (0.2)	2 (0.2)	2 (0.1)	7/1053

** C. ciferrii*, *C. colliculosa*, *C. globosa*, *C. guilliermondii*, *C. utilis*, and *C. sake* were identified only once. R: resistance of *Candida* spp.

One *Candida* isolate of *Candida parapsilosis* and *Candida tropicalis* was resistant to fluconazole (Table 6).

Table 6: In vitro susceptibility of *Candida* spp. to fluconazole.

***Candida* Species**	**Sensitive n (%)**	**SDD n (%)**	**Resistant n (%)**	**Total**
Candida albicans	703 (100)	0 (0.0)	0 (0.0)	703
Candida glabrata	128 (100)	0 (0.0)	0 (0.0)	128
Candida tropicalis	61 (98.4)	0 (0.0)	1 (1.6)	62
Candida krusei	46 (100)	0 (0.0)	0 (0.0)	46
Candida parapsilosis	32 (96,9)	0 (0.0)	1 (3.1)	33
Candida famata	22 (100)	0 (0.0)	0 (0.0)	22
Candida dubliniensis	18 (100)	0 (0.0)	0 (0.0)	18
Candida kefyr	9 (100)	0 (0.0)	0 (0.0)	9
Candida lusitaniae	8 (100)	0 (0.0)	0 (0.0)	8
Candida pelliculosa	6 (100)	0 (0.0)	0 (0.0)	6
Candida rugosa	5 (100)	0 (0.0)	0 (0.0)	5
Candida sphaerica	5 (100)	0 (0.0)	0 (0.0)	5
* Other species	6 (100)	0 (0.0)	0 (0.0)	6
Candida species not identified	3 (100)	0 (0.0)	0 (0.0)	3
Total	1051 (99.8)	0 (0.0)	2 (0.2)	1053

* *C. ciferrii*, *C. colliculosa*, *C. globosa*, *C. guilliermondii*, *C. utilis*, *C. sake*, and *Candida* spp. were identified only once.

Candida isolates were susceptible to amphotericin B, with *C. dubliniensis* (100%), *C. tropicalis* (100%), and *C. parapsilosis* (100%) showing a higher susceptibility rate, followed by *C. glabrata* (96.77%), *C. albicans* (95.89%), and *C. krusei* (81.25%), and *C. rugosa* was more resistant to amphotericin B (20%), followed by *C. krusei* (4.17%) (Table 7).

Table 7: In vitro susceptibility of *Candida* spp. to amphotericin B (AMB).

***Candida* Spp.**	**Sensitive n (%)**	**SDD n (%)**	**Resistance n (%)**	**Total**
Candida albicans	748 (95.8)	26 (3.3)	6 (0.7)	780 (68.3)
Candida glabrata	120 (96.7)	3 (2.4)	1 (0.8)	124 (10.8)
Candida tropicalis	65 (100)	0 (0.0)	0 (0.0)	65 (5.6)
Candida krusei	39 (81.2)	7 (14.5)	2 (4.1)	48 (4.2)
Candida parapsilosis	32 (100)	0 (0.0)	0 (0.0)	32 (2.8)
Candida famata	22 (100)	0 (0.0)	0 (0.0)	22 (1.7)
Candida dubliniensis	16 (100)	0 (0.0)	0 (0.0)	16 (1.4)
Candida kefyr	9 (100)	0 (0.0)	0 (0.0)	9 (0.7)
Candida lusitaniae	7 (87.5)	1 (12.5)	0 (0.0)	8 (0.7)
Candida pelliculosa	5 (100)	0 (0.0)	0 (0.0)	5 (0.4)
Candida rugosa	3 (60,0)	1 (20,0)	1 (20,0)	5 (0.4)
Candida sphaerica	4 (100)	0 (0.0)	0 (0.0)	4 (0.3)
Candida globosa	2 (100)	0 (0.0)	0 (0.0)	2 (0.1)
* Other species	4 (100)	0 (0.0)	0 (0.0)	4 (0.3)
Candida species not identified	3 (100)	0 (0.0)	0 (0.0)	3 (0.2)
Total	1079 (95.7)	38 (3.3)	10 (0.8)	1127 (100)

* *C. colliculosa*, *C. guilliermondii*, *C. sake*, *and C. ciferii* were identified only once.

We did not observe an association between the resistance of *Candida* spp. isolates and HIV status in study participants ($p > 0.05$) (Table 8).

Table 8: Distribution of antifungal susceptibility according to participants' HIV status.

HIV Status	Sensitive n (%)	Intermediate n (%)	Resistant n (%)	Total
Negative	275 (99.2)	1 (0.3)	1 (0.3)	277
Positive	19 (100)	0 (0.0)	0 (0.0)	19
Total	294 (99.3)	1 (0.3)	1 (0.3)	296

Candida isolates resistant to antifungals were frequent in patients with high blood sugar levels (1.11%) ($p > 0.05$) (Table 9).

Table 9: Distribution of resistant strains according to the glycemic status of participants.

Blood Sugar Level	Sensitive n (%)	Intermediate n (%)	Resistant n (%)	Total
Normal	506 (97.8)	6 (1.1)	5 (0.9)	517
High	88 (97.7)	1 (1.1)	1 (1.1)	90
Total	594 (97.8)	7 (1.1)	6 (0.98)	607

4. Discussion

This study was a pilot evaluation of *Candida* spp.'s in vitro susceptibility using VITEK 2 in Mali. The majority of participants were young women, aged between 25 and 36 years, who were sexually active [20]. This explains the high number of vulvovaginitis cases. *Candida* spp. are the most common species involved in vulvovaginitis. Other fungal species were identified by VITEK- 2 during the study but were not considered because they did not meet the inclusion criteria.

In our study, both male and female were susceptible to *Candida* infection, but females were predominantly infected (89%). This finding is similar to that reported by Razzaghi-Abyaneh et al. [1]. This probably results from a selection bias towards vulvovaginal samples in our study.

Out of 1224 samples, 665 (54%) were from patients in community health centers. Most *Candida* isolates were from vaginal discharge (85%), followed by pus (10%) and sputum (2%). The predominance of female patients in whom genital *Candida* infection is more common than in men [1] could explain such a difference. Bonouman-Ira et al. reported similar findings in the study on the resistance profile of non-*albicans Candida* in Abidjan in 2011 [21].

4.1. *Candida* Species Frequencies

The most common species identified were *C. albicans* (67.65%), *C. glabrata* (11.09%), *Candida tropicalis* (5.97%), and *Candida krusei* (4.37%). These results confirm those reported in Côte d'Ivoire by Konate et al. [8], who reported *C. albicans* (82.5%) and *C. glabrata* (10.5%) in vulvovaginal candidiasis. Djohan et al. also reported a similar predominance of *C. albicans* (72.6%) and *C. glabrata* (14.5%) in the study of the in vitro susceptibility of vaginal *C. albicans* to antifungal drugs in Abidjan [12]. Doumbo et al., in a hospital setting in Bamako, observed a different distribution of *Candida* spp., with *C. famata* isolated as the second species after *C. albicans* [15]. This species was identified in the samples analyzed during our study, but with a lower frequency. Dieng et al. reported similar *Candida* isolate distributions in Dakar from oropharyngeal and vaginal swabs at the Fann University Hospital in Dakar using the phenotypic method [22].

4.2. Antifungal Susceptibility

This study showed that *Candida parapsilosis* and *Candida tropicalis* were resistant to fluconazole; however, *C. rugosa* and *C. krusei* were resistant to amphotericin B.

We reported a rate of *Candida* spp. resistant to antifungals, which is different from what Ing-Moi Hii et al. [23] found in Taiwan, where 2.8% of *C. glabrata* were resistant to micafungin, compared to our observed rate of 0.75% resistance to micafungin.

We found that all *C. krusei* isolates were susceptible to fluconazole, which is not consistent with what has been previously reported for *C. krusei* resistance to fluconazole [24–26]. This difference could be explained by *C. krusei* intrinsic factors and host factors, such as the age [27] and sources of *C. krusei* isolates. Fluconazole-resistant *C. krusei* isolates have been commonly found among inpatients with severe and chronic diseases [24,25]. The good susceptibility of our *C. krusei* isolates could be due to the possible underestimation of the resistance of *C. krusei* to fluconazole by the VITEK-2 system, as previously reported for *C. tropicalis*, *C. krusei*, and *C. auris* [26,27], or by the status of the current study participants, most of whom were young outpatients who were likely to have mild infections. We observed that one (0.75%) of the *C. parapsilosis* isolates and one (0.75%) of the *C. tropicalis* isolates were resistant to fluconazole, a finding similar to 0.8% for *C. parapsilosis* reported by Ing-Moi Hii et al. [23], but lower than the 12.4% resistance of *C. tropicalis* to fluconazole in the same study. In Burkina Faso, a higher rate of resistance was observed for fluconazole (66.5%) [13].

We observed the resistance of 48 (4.17%) isolates for *C. krusei* and 1 (0.81%) isolate for *C. glabrata* to amphotericin. Alimehr et al., at the Milad hospital (Iran) in 2015, reported more resistance, with 50% of *C. krusei* being resistant to amphotericin B and 9.4% for *C. glabrata* [28].

C. albicans has shown 6 (0.77%) isolates being resistant to amphotericin B in our study; this proportion is less than that (8%) reported by Bouchekoua et al. in 2017 at an intensive care unit in Tunis [29]. Furthermore, a 32.0% resistance to amphotericin for *Candida* isolates was reported by Zida et al. [13] in vulvovaginal and oral environments using the API-*Candida* test. The methods used to determine antifungal susceptibility and the environments of *Candida* isolates could explain the difference between Zida et al.'s [13] findings and ours.

A higher resistance rateof *C. krusei* to flucytosine (36.96%) in our study may not be surprising. *C. krusei* has shown a similar resistance profile to flucytosine and other antimycotics such as amphotericin B and fluconazole in vulvovaginitis in pregnant women [30].

These heterogeneities could be explained by the patient's disease severity and health care center characteristics. Our samples originated mainly from community health care centers, where the resistant *Candida* species may be rarer than in intensive care units.

C. albicans clinical isolates have shown good susceptibility to voriconazole, with one (0.12%) isolate being resistant. A similar susceptibility of 86.7% to voriconazole was reported by Djohan et al. [12]. In Iran, the overall resistance rates to fluconazole and voriconazole were, respectively, 2.4% and 0.8% [31].

Our findings are representative of the *Candida* distribution and the susceptibility to fungal infections in Mali, because the VITEK 2 system is fast and gives reliable results [32], but some errors may happen in the determination of in vitro antifungal susceptibility using the automated VITEK system [33]. Sow D. et al. showed that MALDI-TOF is less time-consuming and is accurate with minor discrepancies compared to phenotypic methods [34]. Both methods are currently available in Mali, and their use on a large scale could improve Candidiasis treatment in the health infrastructures in Mali.

During the study period, we observed more resistance to antifungals in women than in men. This result could be explained by the possible repeated and increased use of antifungals in women, as well as the recruitment bias given the higher number vaginal samples collected from women.

Candida spp. infection occurred frequently in immunocompromised patients or diabetic patients, and the susceptibility to antifungals may decrease according to patients' backgrounds. In the current study, the resistance to antifungals was high in hyperglycemic participants. Hedayati et al. observed the resistance of *C. albicans* to amphotericin B, itraconazole, ketoconazole, posaconazole, and

fluconazole in diabetic patients [35]; although diabetes is known as a risk factor of fungal infections, no differences in antifungal susceptibility of these six antifungals tested were observed between *Candida* isolates from diabetic and non-diabetic subjects.

However, the differences were observed between two geographically different diabetes mellitus populations. Oral yeast isolates from diabetes mellitus patients in the UK more often displayed resistance or intermediate resistance to fluconazole (p = 0.02), miconazole (p < 0.0001), and ketoconazole (p = 0.01) than did isolates from diabetes mellitus patients in Italy [36].

We did not detect any resistance of *Candida* isolates in patients with HIV in our study, which is likely due to the low number of HIV-positive cases in our study population; a similar finding was reported in Senegal, where *Candida* isolates from patients living with HIV were susceptible to antifungals [4]. HIV infection may favor the emergence of antifungal resistance in patients with HIV infection who are exposed to long-term azole treatments, which may induce the selection of fluconazole-resistant *C. albicans*. Yet, such resistance is rare and transient in patients on intermittent short-term antifungal treatments [37].

In a previous study, *C. albicans*, *C. tropicalis*, and *C. parapsilosis* were the common isolated species in blood cultures [38]. In our study, fewer blood cultures were performed, and *Candida* species showed a good susceptibility profile to the antifungals used. In contrast, Gorgun et al. observed a 26% antifungal resistance rate in *Candida* spp. isolated from blood cultures. Regarding *C. albicans*, three (11.5%) were resistant to fluconazole, and two (7.7%) were resistant to voriconazole; for *C. parapsilosis*, two (15.4%) were resistant to fluconazole, and two (15.4%) were resistant to voriconazole [39].

We did not compare the VITEK-2 to other techniques during the study. Although VITEK-2 is widely used for *Candida* spp. identification and antifungal susceptibility testing in many routine laboratory studies, several studies revealed its limitations with discrepant results in estimating the antifungal susceptibility of *Candida* spp. [40,41]. Azoles are the first-line treatment for vulvovaginitis candidiasis or recurrent vulvovaginal candidiasis, with fluconazole as the most common drug used. In the current study, *C. albicans* showed a good susceptibility profile to it; however, recently, a molecular analysis of *C. albicans* from vulvovaginitis demonstrated resistance to fluconazole with the occurrence of the point mutation ERG11 [42,43].

5. Conclusions

The automated VITEK 2 system shows a good in vitro susceptibility profile of clinical *Candida* isolates to the tested antifungals. Clinical *Candida* isolates from women were resistant at a lower level. Most *Candida* isolates were susceptible to fluconazole. *C. krusei* was mostly resistant to flucytosine, and *C. rugosa* was mostly resistant to amphotericin. Regular monitoring of the in vitro susceptibility of *Candida* isolates to antifungals should be reinforced before treatment.

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