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IDENTIFICATION, PREVALENCE AND SUSCEPTIBILITY PROFILE OF *CANDIDA* ISOLATES AT THE PASTEUR INSTITUTE IN CÔTE D'IVOIRE FROM 2017 TO 2019

Ama Valérie Bonouman Ira[1,2], Reine Mboh[2], Fréderic Roger[1], Donika Krasteva[1], Virginie Bellet[1], David Koffi[2], Julie Noell[1], Cyrille Pottier[1], Pascal Drakulovski[1], Hervé Menan[3], Offianan Toure[2], Mireille Dosso[2], Stéphane Ranque[4,5] and Sébastien Bertout[1].

[1]UMI TransVIHMI, Faculté de Pharmacie, Université de Montpellier-IRD, INSERM, U1175, Montpellier, FRANCE

[2]Institut Pasteur de Côte d'Ivoire, Abidjan, CÔTE D'IVOIRE

[3]Laboratoire de Parasitologie Mycologie, Diagnosis and Research Centre on AIDS and Other Infectious Diseases, Cèdres Abidjan, Côte d'Ivoire

[4]Aix-Marseille Université, AP-HM, IRD, SSA, VITROME, 13005 Marseille, France

[5]Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France

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ABSTRACT: *This study was conducted in Côte d'Ivoire to determine the respective prevalence of Candida species and to study their susceptibility profile to antifungal agents to identify the emergence of resistance.This retrospective study was conducted from 2017 to 2019 at the Institute Pasteur of Côte d'Ivoire on patients with fungal disease. The yeasts isolated and identified in Côte d'Ivoire were subcultured on a chromogenic medium and identified with MALDI-TOF-MS. Sensitivity tests were performed using the CLSI method and evaluated by the M27. In total, 227 Candida spp. were isolated from 1941 patients. Most of the samples were vaginal swabs (120/228). Using the conventional method, C. albicans was prevalent (52.6%). There were mixtures of 2 to 4 species in 36.8% of the samples. Thus MALDI-TOF-MS identified in 315 isolates 13 distinct species, C. tropicalis was prevalent at 35.2%, and the species in both C. albicans and C. parapsilosis complexes were differentiated. This study highlights the hurdle of correct yeast identification in developing countries. The transition from conventional to modern MALDI-TOF based identification of these life-threatening opportunistic pathogens is mandatory and should be emphasised as a public health priority.*

KEYWORDS: *Candida*, identification, MALDI-TOF, Côte d'Ivoire, resistance.

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INTRODUCTION

Yeasts of the *Candida* genus are the most frequently isolated in medical mycology **[11,30]**. These microorganisms are involved in superficial infections of the skin and mucous membrane (vulvovaginitis) and in deep systemic infections **[16,24]**. Invasive candidiasis is estimated to affect more than a quarter of a million people and is the cause of more than 50,000 deaths worldwide every year **[12]**.

Five species are responsible for over 92% of cases of candidiasis*: C. albicans, C. glabrata, C. tropicalis, C. parapsilosis and C. krusei* **[11,27,31]**. *Candida albicans* was demonstrated to be the most virulent species and is commonly isolated from clinical specimens **[16]**. It accounts for more than 80% of isolates of all forms of human candidiasis [**5]**. However, the number of infections due to non-*C. albicans* species (NCAC) has increased significantly [**11**]. While *C. albicans* remains the most commonly isolated *Candida* species, its proportion relative to other *Candida* species has decreased from 71% to 65% over time and, in some cases, the prevalence of non-*albicans* species has surpassed that of *C. albicans* **[9]**. Accurate identification at the species level is critical for successful treatment and appropriate patient care [**30]**. However, in recent years, the taxonomy of the most important *Candida* species, such as *C. albicans*, *C. parapsilosis*, and *C. glabrata,* has undergone significant changes due to the description of new closely related species; therefore, they are currently recognized as cryptic species complexes **[11,25]**. In low-income countries, the identification of these species largely relies upon both biochemical profiles and microscopic morphological characteristics. However, the identification of cryptic species by phenotypic techniques is almost impossible. Hence, high-resolution methods, including MALDI-TOF mass spectrometry profiling and nucleotide sequence analysis, are required for taxonomic differentiation among *Candida* species **[3].**

The adequate identification of cryptic species in a clinical setting is relevant from both epidemiological and medical points of view. The emergence of new *Candida* cryptic species represents a challenge for clinical laboratories in low-income countries, where the latest identification methods are unavailable. Conventional methods based on carbohydrate assimilation profiles or chromogenic media designed to identify the most common yeast species may misidentify cryptic species. The introduction of matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF-MS) has greatly improved the identification of fungi in the clinical laboratory **[11,14]**.

It is important to better understand the evolution of antifungal resistance **[11].** Various antifungal drugs are used to treat candidiasis. These include polyene antifungals (e.g., nystatin and amphotericin B); imidazoles (e.g., miconazole, clotrimazole, econazole and ketoconazole); echinocandins (e.g., anidulafungin, micafungin and caspofungin); and triazoles (including fluconazole, posaconazole, voriconazole and itraconazole) [**1]**. The use of antifungal agents may lead to the development of drug-resistant strains. The development of antifungal resistance among yeasts has been linked to misuse and inappropriate prescription of antifungal agents **[28]**. Different *Candida* species have varying resistance patterns, which appear to be geographically determined **[1]**. In the African health care setting, amphotericin B and fluconazole are routinely used to treat *Candida* infections. The other antifungals are unavailable. The epidemiology of *Candida* species changes in parallel with the emergence of antifungal drug-resistant species **[22]**. Several international surveys have tracked the incidence of *Candida* infections and the rates of drug resistance over the past decades **[27,34]**. The gap in antifungal drug resistance surveillance in Africa has been pointed out recently **[1]**. This study

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aimed to determine the distribution of yeast species and to assess their antifungal susceptibility profile to document a potential antifungal resistance emergence in Côte d'Ivoire, a resource-limited sub-Saharan African country.

MATERIALS AND METHODS

Sample Collection

In this study, we analysed 227 clinical yeast isolates, sampled from various sites, including vaginal swabs (120), pus (17), nails (14), semen (11), stool (7), skin flakes (5), urine (5), pharyngeal swabs (5), gastric fluid (1), respiratory samples (1), ascites (1), alveolar liquid (1, and CSF samples (1). The origin of 38 samples could not be determined. These clinical samples were collected over a 2 year period (2017–2019) from 1,941 out patients at the Pasteur Institute of Côte d'Ivoire. The positivity rate ranged from 12 to 50%, depending on the year. We selected for this study all the isolates that were associated with minimal clinical information. Some samples collected during the period had been lost due to storage difficulties. The 1,941 patients studied had 40% positive samples, yet only 227 (11.7%) correctly labeled and uncontaminated samples were used in this study.

PHENOTYPIC ANALYSIS

First Identification: Macroscopic Identification, India Ink Test and *Candida* Chromatic

The strains were identified in Abidjan by phenotypic methods routinely used at the Pasteur Institute of Côte d'Ivoire. After isolation of samples on Sabouraud medium with added chloramphenicol (home-made) in a Petri dish, the colonies suspected of being *Candida* were seeded on chromogenic medium, *Candida* Chromatic© (LIOFILCHEM, Roseto degli Abruzzi, Italy), and stored at 37 °C for 24–48 hours. In agreement with the manufacturers' recommendations, *Candida* Chromatic© is a chromogenic medium for the isolation and differentiation of *C. albicans*, *C. tropicalis* and *C. krusei*. *C. albicans* colonies appear light green, *C. tropicalis* colonies appear blue-green and *C. krusei* colonies appear pink. The colonies of the other *Candida* species appeared white. Identification of colonies of the genus *Trichosporon spp* was done by macroscopic identification on cultures of Sabouraud Chloramphenicol medium (home-made). The colonies had a cerebriform appearance (brain shape). The search for *Cryptococcus spp* in case of suspected neuromeningeal cryptococcosis was done by direct microscopic analysis of the CSF using Indian ink staining. Cryptococcosis diagnosis was based on the detection of budding yeast cells exhibiting a translucent capsule contrasting with the ink. The strains were preserved in saline and stored in Eppendorf tubes at +4 °C before being sent to France (Parasitology Mycology Laboratory, Faculté de Pharmacie Montpellier, France) after signing a Material Transport Agreement between Abidjan and Montpellier.

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Culture on CHROMagar™*Candida* before MALDI TOF Identification

All yeast strains were subcultured onto chromogenic medium, CHROMagar™*Candida* (Becton Dickinson GmbH, Heidelberg, Germany) to detect potentially mixed cultures and then identified by MALDI-TOF-MS. CHROMagar™*Candida* is a chromogenic medium for isolating fungi. As chromogenic substrates are incorporated into the medium, the colonies of *C. albicans*, *C. tropicalis* and *C. krusei* produce distinct colours that allow these yeast species to be detected directly on the isolation plate. Strains were plated on CHROMagar™*Candida* and kept at 37 °C for at least 48 hours.

MALDI TOF Identification

The isolates subcultured on CHROMagar™*Candida* were identified by MALDI-TOF-MS in France (IHU Méditerranée Infection, Marseille, France) as previously described by Cassagne et *al.* **[6]**. Positive control was *Candida krusei* ATCC 6258. MALDI-TOF-MS acquisition was performed using Biotyper RTC software. If the MALDI Biotyper identification score was below 1.9, ITS PCR and nucleotide sequence analysis were carried out **[6]**.

PCR and Nucleotide Sequence-based Identification

A standard PCR mix was prepared with 1 µl of DNA prepared from the strain tested and 29 µl of the following master mix: 19.1 µl water, 1.8 µl MgCl2, 0.6 µl dNTP, 0.6 µl of each ITS primer (ITS1 (5'TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'TCTTTTCCTCCGCTTATTGATATG-3′), and 0.3 µl GoTaq Polymerase. The PCR program consisted of 1 cycle of 94 °C for 1 min, 35 cycles of 30 s at 94 °C and 30 s at 60 °C and a final cycle at 60 °C for 3 min. A reference strain was used as a control. Sequencing services were provided by Genewiz. The sequences were edited with Geneious 9.1.7 software and deposited into NCBI GenBank.

Antifungal Susceptibility Testing

In vitro antifungal susceptibility assays were performed using the CLSI reference (M57 and M59) method of microdilution broth for all isolates of the species. Three hundred and fifteen species from the 227 starting isolates were tested. Indeed, after culture on chromogenic medium (CHROMagar™*Candida*, 144 (64.9%) isolates contained a single species, and we found 2 to 4 species in the rest (83/227) of the mixtures. In the end, the number of strains isolated after culture on CHROMAGAR (chromogenic medium) was 315. The *Candida* MIC for all antifungal drugs was read after 24 and 48 hours incubation. The antifungals used were amphotericin B (Sigma-Aldrich, St Louis, USA), caspofungin (Sigma-Aldrich, St Louis, USA), fluconazole (Sigma-Aldrich, St Louis, USA), flucytosine (Sigma-Aldrich, St Louis, USA), and ketoconazole (Sigma-Aldrich, St Louis, USA). Quality control was performed following the M27-A3 and M60 CLSI documents, using *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019, for quality control.

For amphotericin B and fluconazole, the epidemiological cut-off values (ECVs) for in vitro susceptibility testing of various *Candida* spp. have been defined by M60 ED1 and M59 ED2 (CLSI, Wayne, USA). According to the M57 document: "the ECV is the highest MIC/MEC value within the wild-type-minimal concentration/minimal effective concentration (WT-MIC/MEC) population that presumptively separates WT from NWT isolates." No interpretation criteria for posaconazole, ketoconazole, or amphotericin B are available in the

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CLSI M27-S4 document. Since there were neither official breakpoint nor ECV value for ketoconazole **[11]**, we used MIC breakpoints recommended in **[36]:** susceptible dose-dependent was defined by MICs from 0.25 µg/mL to 0.5 µg/mL for ketoconazole; resistance was defined by MICs ≥ 2 µg/mL for amphotericin B and ≥ 1 µg/mL for ketoconazole.

FKS Genes Sequencing

Although *C. glabrata* has three FKS genes, the mutations that confer echinocandin resistance have been found in the hot spot 1 (HS1) in both FKS1 and FKS2 genes **[9]**. Thus, HS1 from both genes were amplified by PCR using the following oligonucleotides: for HS1 from FKS1, CGFKS1-HS1-fw (TTCTCAGTCATGCCATTGG) and CGFKS1-HS1-rv (ACAAT GTACCACAGATAGG); for HS1 from FKS2, CGFKS2-HS1-fw (AAGATATGTTGCTTCTCAGAC) and CGFKS2-HS1-rv (AAG GAGTTAAGATGGAAATACC). PCR conditions for amplifying the HS1 from FKS1 were 95°C for 10 min, 30 amplification cycles (95°C for 15 s, 50°C for 15 s, and 72°C for 30 s), and 5 min at 72°C. To amplify the HS1 from FKS2, PCR cycles were the same, but the annealing temperature was 53°C. Sequencing services were provided by Genewiz. The nucleotide sequences of FKS1 and FKS2 were aligned and compared with the reference wildtype (WT) sequences of *C. glabrata* ATCC 90030 FKS1 (GenBank accession numbers: HM366440.1) and FKS2 (GenBank accession numbers: HM366442.1), respectively **[15]**.

Table 1: Epidemiological cut-off values (ECV) for in vitro susceptibility testing of various *Candida* spp. (Referential M59, 2nd ed.)

Antifungal agent	Species	ECV (µg/ml)
Fluconazole	*C. albicans*	0,5
	C. dubliniensis	0.5
	C. glabrata	8
	C. parapsilosis	1
	C. tropicalis	1
	C. guilliermondii	8
	C. lusitaniae	1
Amphotericin B	*C. albicans*	2
	C. krusei	2
	C. glabrata	2
	C. parapsilosis	2
	C. tropicalis	2

Ethics Statement

This study was approved by the Ethics Committee of Health Sciences and Life of Côte d'Ivoire under the reference 020-19/MSHP/CNESV-km.

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RESULTS

1- Patient Characteristics

For the 227 patients whose samples were selected for this study, the age of the study population ranged from 6 months to 60 years. Most patients (50.3%) were young (between 16 and 35 years). The mean age was 45.6 years. In this population, 89.56% (103/115) were women, and the sex ratio was 5.3. Isolates were obtained from twenty different types of clinical samples. More than half of the samples collected were vaginal swabs (52.6%); 6.1% were ears and nails, and 4.8% were sperm samples. There were few deep-seated samples, such as CSF, alveolar liquid, and gastric liquid (0.4% for each type).

2- Identification Results

The initial conventional phenotypic identification of the 227 isolates on *Candida* Chromatic© agar in Abidjan yielded three *Candida* species: 120 were *C. albicans*, 15 were *C. tropicalis* and 14 were *C. krusei*. The predominant species was *C. albicans* 120/227 (52.8%). Identification of *Trichosporon* sp. was based on the direct examination of the culture and that of *Cryptococcus* was based on the Indian ink test characteristics.

In Marseille, these 227 initial yeast isolates were subcultured on CHROMagar^{TM}*Candida* plates and subsequently identified by MALDI-TOF-MS. This procedure identified 315 distinct isolates because many initial isolates were mixed cultures. MALDI-TOF-MS identified 13 species (Figure 1). One-third (33.3%) of all isolates remained identified as *Candida* sp., but in the remaining, MALDI-TOF-MS identification yielded divergent results for the same isolates (Figure 1). Finally, the MALDI-TOF based-identification pointed out that *C. tropicalis* (35.5%) was the most prevalent species, whereas only 19% of the isolates were identified as *C. albicans*. Table 2 summarises the proportion of misidentifications and the correct identification after comparison of the results of the species recovered after confirmation by the molecular technique. Concerning the species identified as *C. albicans* by chromogenic medium (120), some were rather *tropicalis* strains (58 among the 120 *C. albicans*); others were *C. glabrata* strains (25/120), *C. parapsilosis* (6), *Lodderomyces* (4), and *Kodamaea* (2). Finally, among the three strains initially identified as *C. albicans*, one was *C. famata*, one was *Cyberlindnera fabianii*, and the last was *Wickerhamomyces anomalus*.

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Table 2: Comparison of initial conventional (Chromatic *Candida*, Indian ink) and confirmatory (MALDI-TOF-MS after ChromAgar medium subculture) identification results

MALDI-TOF MS-based identification results	Initial conventional identification results (*Candida* Chromatic and India ink) (n= 227) *						Adequate initial Identification/total (%)
	C. albicans (120)	*C. tropicalis* (15)	*C. krusei* (14)	*Cryptococcus sp* (1)	*Trichosporon sp.* (2)	*Candida sp.* (75)	
C. albicans	48	4	1	0	0	4	48/57 (84.2)
C. africana	4	0	0	0	0	0	0/4 (0)
C. tropicalis	58	7	5	0	0	41	7/111 (6.3)
C. glabrata	26	2	5	1	2	30	0/66 (0)
C. krusei	12	5	9	1	0	10	12/37 (32.4)
C. parapsilosis	6	1	1	0	0	11	0/19 (0)
C. guilliermondii	0	0	0	0	1	1	0/2 (0)
C. lusitaniae	0	0	0	0	0	1	0/1 (0)
C. haemulonii	0	0	0	0	0	1	0/1 (0)
C. famata	1	0	0	0	0	0	0/1 (0)
Kodamaea ohmeri	2	0	0	0	0	4	0/6 (0)
Lodderomyces elongisporus	4	1	1	0	0	1	0/7 (0)
Cyberlindnera fabianii	1	0	0	0	0	1	0/2 (0)
Wickerhamomyces anomalus	1	0	0	0	0	0	0/1 (0)
Total	**163**	**20**	**22**	**2**	**3**	**105**	**(n= 315) ***

* The discrepancy between the initial conventional identification and the MALDI-TOF identification results are linked to the fact conventional identification missed mixed cultures that were subsequently identified by MALDI-TOF MS-based identification procedure.

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Table 3: Yeast species distribution according to the sampling site

Samples Species	Vaginal swabs	Semen	Nails	Pus	Skin flakes	Stool	Pharyngeal Swabs	Urine	Ascites	Respiratory	Gastric liquid	CSF	Unknown	Total
C. albicans	31	1	1	3		2	3			1			20	**61**
C. tropicalis	52	9	7	10	3	3	1	2	1				23	**111**
C. glabrata	37	5	5	5		2		1				1	10	**66**
C. krusei	17	3	1	2	1	2	1	1				1	8	**37**
C. parapsilosis	9		3	3	2						1		0	**19**
C. guilliermondii			1										1	**2**
C. lusitaniae	1												0	**1**
C. famata							1						1	**1**
C. haemuloni i													1	**1**
Cyberlindnera fabianii	1												1	**2**
Lodderomyces	4	1		1							1		0	**7**
Kodamaea ohmeri	3		1	1	1								0	**6**
Wicherhamomyces anomalus	1												0	**1**
Total	**156**	**19**	**19**	**25**	**6**	**9**	**6**	**4**	**1**	**1**	**2**	**2**	**65**	**315**

Table 3 details the distribution of each species according to the sampling site from where they were isolated. Mixtures of species were identified in several samples that were initially identified as pure cultures.

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3- Susceptibility Testing

Antifungal susceptibility testing was performed on *Candida* spp. isolates. The minimal inhibitory concentrations (MICs) were determined using the CLSI broth microdilution method (referential M57 and M59) (Table 1).

Table 4 presents a summary of non wild-type (NWT) strains depending on the species for which ECVs exist (fluconazole (FCZ) or amphotericin B (AMB). In the case of caspofungin, threshold values are present within the reference framework.

Table 4: Fluconazole, amphotericin B, and caspofungin resistance profile of the *Candida* spp. isolates based on the CLSI broth microdilution method MICs

SPECIES	Susceptibility	MIC(µg/ml) fluconazole	amphotericin B	caspofungin
***Candida tropicalis* (111)**	< ECV	108	111	NA[a]
	> ECV	**3**	0	
	S	NA	NA	111
	R			**0**
Candida glabrata (66)	< ECV	65	66	NA
	> ECV	**1**	0	
	S			60
	R			**6**[c]
Candida albicans (61)	< ECV	56	61	NA
	> ECV	**5**	0	
	S			61
	R			0
Candida krusei (37)	< ECV	NA[b]	35	NA
	> ECV		**2**	
	S			34
	R			**3**
Candida parapsilosis (19)	< ECV	15	18	NA
	> ECV	**4**	**1**	
	S			20
	R			0
Candida guilliermondii (2)	< ECV	2	NA	NA
	> ECV	0		
	S	2		2
	R	0		0
Candida lusitaniae (1)	< ECV	1	NA	NA
	> ECV	0		
	S	1		
	R	0		

a. NA: not applicable

b. Natural resistance of *C. krusei* to fluconazole

c. Caspofungin resistance breakpoints for *C. glabrata*: MIC ≥ 0.5 mg/l.

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FKS Genes Study

In three resistant strains (18079G, 18479G and 18574G) among the six caspofungin-resistant *C. glabrata* isolates, we found a T to C substitution at position 1875 in the *FKS1* gene HS1. Three substitutions in the *FKS2* gene HS1: (G to A) at nucleotide 1956, (T to A) at nucleotide 2010 and (C to T) at nucleotide 2025. However, these mutations were not associated with amino acid change.

DISCUSSION

Correct Identification and Misidentification

Identification of yeast at the species level is a challenge for clinical microbiologists in low-income countries due to the lack of appropriate technologies. Conventional methods, based on morphological or biochemical characteristics, sometimes provide an inaccurate identification of the aetiological infection agent **[11,23]**. This study's findings highlight the limitation of conventional tests for adequate identification of *Candida* species compared to mass spectrometry or DNA sequence-based methods, which are currently scarcely accessible in developing countries. In line with our findings, a study in China showed that a biochemical test (API 32C auxanogram) misidentified the strains that were further identified by nucleotide sequence analysis. The correct identification rates of *C. albicans, C. glabrata, C. parapsilosis, and C. tropicalis* were 98.51% (2708/2748 isolates), 84.59% (439/451 isolates), 80.26% (61/76 isolates), and 65.57% (40/61 isolates), respectively **[30]**. Correct identification for these species in this study using biochemical test is much better than that found in our study (84.2% for *C. albicans* and 6.3% for *C. tropicalis*, two other species not being identifiable by using chromogenic medium). Two hundred fifty-one isolates (83.7%) were correctly identified, and there were no unidentified species using API 20C AUX. Additionally, Azike *et al*. in Nigeria highlighted the gap between identification results using conventional methods or using nucleotide sequence-based identification **[3]**. In this study, conventional phenotypic methods identified 33 (45.8%) *Candida albicans* strains and 36 (50%) non *albicans* species, and 3 strains (4.2%) were not identified. This is in line with our study, in which the conventional method based on chromogenic medium identified only *C. albicans*, *C. tropicalis*, and *C. krusei.* The other species were identified as *Candida* spp. More than 80% (49/61) of the *C. albicans* isolates were correctly identified by the phenotypic methods.

Species Distribution

Reporting the identification of emerging *Candida* species is clinically relevant because these species can differ both in virulence and in antifungal susceptibility profiles **[11]**. When precise identification and antifungal susceptibility profile are lacking, physicians have no other choice than to treat life-threatening mycoses empirically with broad-spectrum antifungals. This might negatively impact the patient's outcome and worsen antifungal resistance issues **[11]**. In this study, MALDI-TOF-MS precisely identified *Candida* spp. and rare yeast species.

Regarding the 61 strains from the *C. albicans* complex species studied, 4 strains were identified as *C. africana*. They were isolated from vaginal swabs, and one was isolated from a lung sample. Previous studies on cryptic species in Latin America (2/66), Cameroun **[24]**, Senegal **[7]**, and Nigeria (2/84) **[25]** have found some strains of *C. africana* among *C. albicans* strains.

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C. africana has not been found in any previous study conducted in Côte d'Ivoire **[2,8,17]**. In Europe, *C. africana* has been isolated from German, Italian, Spanish, and British patients **[11]**. In Turkey, vaginal *C. albicans* isolates have also been re-identified as *C. africana* and *C. dubliniensis* **[13]**. Cryptic species, such as *C. dubliniensis* and *C. africana*, are routinely misidentified or unidentified **[11]**. We did not isolate *C. dubliniensis* strains. This is probably due to the low prevalence of oral samples. This finding is in line with a previous study in Cote d'Ivoire that did not find this cryptic species **[21]**.

C. tropicalis was prevalent from vaginal samples in our study, even if many samples were of vaginal origin (52.6%). *C. tropicalis* is a species generally found in deep samples. It has been found to be the main species involved in fungemia in Algeria for example **[20]**. Similarly, it was the predominant species isolated in two hospitals in Malaysia from 2004 to 2009 **[19]**. *C. tropicalis* is commonly isolated from various samples in India, South America, and Asia **[29]**. No previous study in Africa or in West Africa has documented a predominance of *C. tropicalis*.

Antifungal Susceptibility Profiles

We reported the presence of fluconazole resistance (8%; 5 NWT in 61 *C. albicans* isolates) in Côte d'Ivoire. By using ATB fungus, 2 previous studies in Cote d'Ivoire reported 2 (3.6%) resistant *C. albicans* isolates out of 56 tested **[7]**; a second study found even higher fluconazole resistance rate (26.3% (18/68 resistant isolates) **[8]**. In Burkina Faso, Zida *et al.* **[37]** reported higher resistance levels (66.5%) among 206 *C. albicans* isolates. The 2.9% fluconazole resistance level assessed by Sylla among 20 *C. albicans* strains using the Fungitest^{TM} in Senegal **[32]** was lower than that observed in our study.

Feglo and Narkwa in Nigeria found no resistant *C. albicans* strain **[10]** and only one resistant strain (4.5%) in all yeast isolates tested (67). Additionally, in Ethiopia, no resistance was documented among 104 *C. albicans* isolates tested using the YST-21343 on the automated VITEK 2 compact system (bioMérieux, France) **[29]**. Fluconazole resistance levels seemed higher (6.1%) in our study than in the rest of Africa, ranging from 2 to 10% for all *Candida* species tested. However, this comparison is hampered by the heterogeneous antifungal susceptibility assays used, which need to be standardized.

In Europe, a 6-year prospective candidemia survey from the fungal infection network of Switzerland **[26]** of 675 *C. albicans* strains non-susceptibility was assessed in 13 (1.6%) *vs*. 3 (0.4%) of all *C. albicans* isolates when applying EUCAST/new CLSI breakpoints *vs*. the old CLSI breakpoint. In summary, fluconazole resistance is relatively higher in our study than in the rest of the world. Only 1.2% resistant strains were reported in France **[18]**, and Whaley et *al.* found only 3.5% resistant strains among 5,265 **[35]**.

Resistance to amphotericin B and to all polyenes is rare or low in most West African countries. In our study, 2 strains were found to be non-wild type (NWT). Other studies have not reported resistance to amphotericin B in Côte d'Ivoire by using the ATB Fungus method **[7,8]**. In contrast, in other West African countries, like Senegal or Nigeria, resistance to AMB was found in 5.7% **[32]** or 22.4% of the strains, respectively **[26,37].** One study described amphotericin B as the most effective drug in Ethiopia **[4]**. In contrast, a relatively high resistance rate (7.7%) was found in China by Shi et *al.* **[30]** in 1,844 samples, including 1,272 *C. albicans* isolates. Worldwide reported polyene resistance rates are in agreement with our findings.

However, echinocandins are not marketed in Côte d'Ivoire, like most other African countries.

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We evaluated in vitro resistance for epidemiological purposes only. Our results showed the presence of six NWT *C. glabrata* strains and 3 NWT *C. krusei* strains. A study conducted in Ethiopia found no caspofungin-resistant isolate among the 87 tested **[33]**.

Characterization of the Echinocandin-resistant Clinical Isolate

In this study, the 18079G, 18479G and 18574G clinical strains of caspofungin MICs were 0.5 µg/ml. According to literature, HS1 is a highly conserved region among the *FKS* family; hence, the amino acid changes in this region implicate a modification of the echinocandin target and a reduced susceptibility phenotype **[15]**. We found no previously described FKS mutation or amino acid substitution in our isolates. *C. glabrata* FKS genes mutations have been associated with echinocandin-resistance emergence under caspofungin treatment **[15]**. However, in our study, no isolate had been exposed to caspofungin; the high in vitro MICs may be associated with alternative drug resistance mechanisms.

CONCLUSION

This study highlights the hindrance of precise yeast identification in the developing countries' clinical laboratories, which hampers adequate patient management. The transition from conventional identification to MALDI-TOF-MS or nucleotide sequence-based identification methods must be encouraged to enhance fungal identification standards. This transition should start at least in African reference clinical laboratories. These fungal species identification issues should mandatorily be settled before launching an antifungal resistance surveillance program in the West African region.

Conflict of Interest

The authors report no conflict of interest.

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APPENDIX

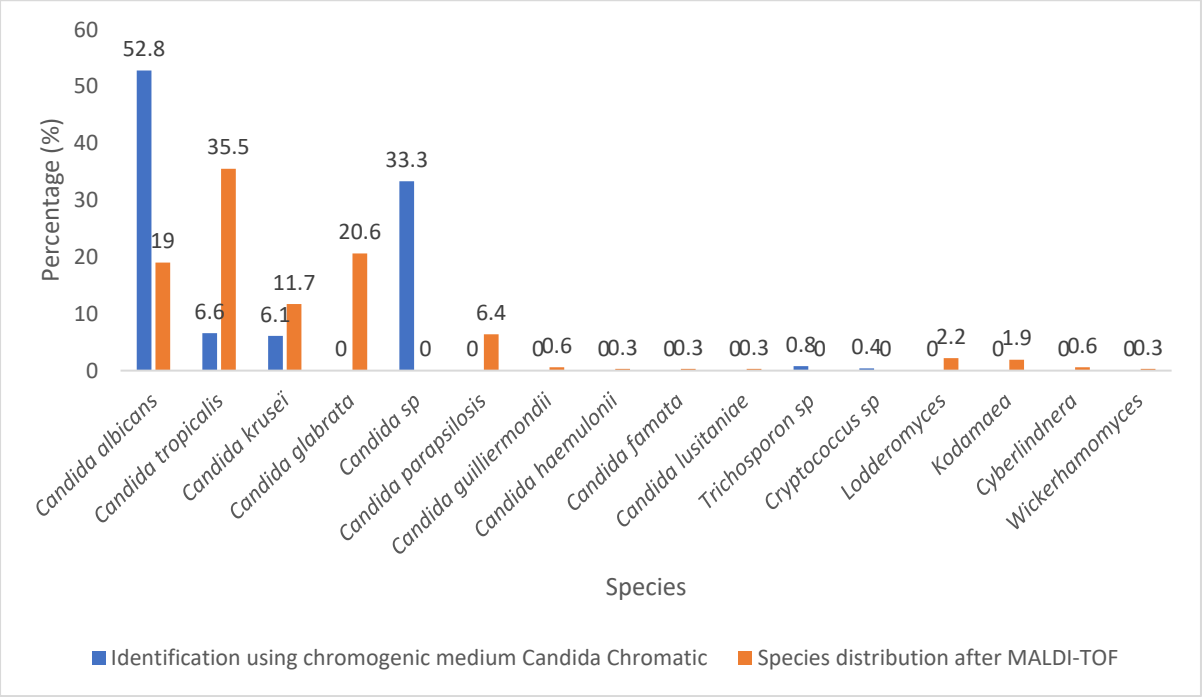

FIG 1: Comparison of species distribution between the initial conventional (Chromatic *Candida* and Indian ink) and secondary (Chromagar *Candida* + MALDI-TOF MS) identification results.

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