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Antifungal Resistance Profile for Clinical Isolates of Candida spp. at Institute Pasteur of Côte d'Ivoire

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Abstract

Background: The aim of this study was to identify Candida species isolated from clinical samples and detect their susceptibility patterns to antifungal agents.

Methodology: This study was conducted on clinical samples collected from patients with suspected candida infection referring to mycology laboratory of Institute Pasteur of Côte d'Ivoire for diagnosis. Candida species were isolated and identified using conventional and the innovative VITEK 2 microbial identification system. Antifungal susceptibility test for Amphotericin-B, 5-Flucytosine, Fluconazole, Itraconazole and Voriconazole were performed using ATB Fungus 3® of Biomerieux.

Results: Most of the isolates were obtained from vaginal swabs 806 (64.8%), followed by Nail scrapings 49 (5.67%). Overall *C. albicans* counting was 58.9% (732/1234) of the infections.

Sensitivity rates of C. albicans species from vaginal swab to 5-FC, AMB, FCZ, VCR and ITR were 97.26, 94.34, 94.89, 95.98 and 88.69 respectively. Resistance rates of *C. albicans* from vaginal swab were observed with AMB (5.66%) and ITR (5.29%). Sensitivity rate of *C. glabrata* was 100% with 5-FC, AMB and VCR. All C.krusei species were resistant to fluconazole. The sensitivity rate of *C. tropicalis* to the antifungal drugs tested varied between 91.66% with ITR to 100 with VCR.

Conclusion: *C. albicans* was the most common of the candida species isolated in this study and remains sensitive to the drugs tested. The study showed resistance of all *C. krusei* strains to fluconazole. Knowledge about etiologic agents and their susceptibility patterns is helpful for successful treatment of the patients.

Keywords: Antifungal Susceptibility Testing; Antifungal Agents; Antifungal Resistance; Candida species

Abbreviations: 5-FC: 5-fluorocytosine; SC: Sabouraud-Chloramphénicol; FCZ: Fluconazole; AMB: Amphotericin-B; SAC: Sabouraud-Actidione-Chloramphénicol; ITR: Itraconazole; VCR: Voriconazole.

Introduction

Candida species are ubiquitous yeasts that can cause a broad spectrum of human infections, known as candidiasis.

Candida albicans is by far the most common species. However, the increasing of non-albicans Candida (NAC) species have been recognized significantly during the last two decades [1]. The most NAC infections are caused by *C. glabrata*, *C. tropicalis, C. parapsilosis, C. dubliniensis, C. guilliermondii, C. krusei, and C. kefyr* [2-4]. Various antifungal drugs with different modes of action have been developed over the years. These include polyene antifungals (e.g. nystatin and amphotericin B), the imidazoles (e.g. miconazole, clotrimazole, econazole and ketoconazole), the echinocandins (e.g. anidulafungin, micafungin and caspofungin), the triazoles (including fluconazole, posaconazole, voriconazole and itraconazole) and the Fluoropyrimidines 5-fluorocytosine (5-FC). In the recent years, treatments of systemic candidiasis are a challenge due to resistant etiologic agents.

Resistance to available antifungal therapies is widespread probably due to the widespread and repeated use of these drugs. Different Candida species have varying resistance patterns, which appear to be geographically determined [5,6]. Antifungal resistance was generally uncommon; however, azole resistance occurs in several species, such as *C. glabrata, C. tropicalis, and Candida krusei* [7,8].

C. albicans is by far the most common species causing infections in humans in Côte d'Ivoire [9,10]. Meanwhile, non-albicans Candida spp. were found to be emergent [11]. In Previous studies conducted at Abidjan *C. albicans* strains exhibited varying levels of resistance to 5-fluorocytosine, fluconazole, itraconazole, and voriconazole and amphotericin B [10,12,13]. Antifungal resistance is a major concern in clinical practice in Côte d'Ivoire. Azole antifungals such as fluconazole are often preferred treatment for many Candida infections as they are inexpensive, exhibit limited toxicity, and are available for oral administration [14]. The treatment is often carried out entirely on pragmatic basis, because fungal culture is not routinely taken, and susceptibility testing is scarcely done. Rapid species identification and antifungal susceptibility testing are essential for the treatment of candida infection in particular, as successful treatment of infections requires adequate information of the specific causative agent(s) and the drugs to which they are susceptible.

Antifungal resistance in the country reinforces the need to further study these pathogens and investigate susceptibility to drugs commonly used for treatment. Therefore National Surveillance Program to monitor antifungal resistance among yeasts and other pathogenic fungi in Côte d'Ivoire is increasingly important. This study was undertaken to assess antifungal susceptibility pattern of Candida isolates for an effective antifungal drug therapy for Candida infection.

Methodology

Study Setting and Samples Collection

This cross-sectional study was carried out on patients referred to the Mycology Laboratory of Institute Pasteur of Côte d'Ivoire for diagnosis of suspected fungal infection from January 2017 to April 2021. Informed consent to participate in this study was signed by all patients. Clinical samples including, vaginal discharge, nails, Squama and pus were collected and processed as per the standard microbiological procedures. Two vaginal swabs per patient were collected by the clinician from the posterior fornix of the vagina with sterile Dacron cotton swab stick after dilation using a sterile speculum. The tube containing the swab was labelled with the patients study number, initials and date and then transported into the Stuart transport medium, to the laboratory.

Identification of Candida species

The samples underwent a direct examination by wet mount preparation and Gram stain, inoculated on Sabouraud-Chloramphénicol (SC) and Sabouraud-Actidione-Chloramphénicol (SAC) media. The inoculated media were incubated at 37°C for 24 to 48 h; if no growth was observed, the incubation was extended up to 72h. Colonies were identified on the basis of their colour on chromogenic agar (Chromatic™ Candida): C. albicans produces pale green colonies, C. tropicalis are blue-green, C. krusei are pink, and other species are white-pink.

Yeast Identification

All the yeast isolates were subjected to identification with the Vitek 2 compact system using Yeast card ID (VITEK 2 YST) as described by the manufacturer. Purity check on chromogenic medium was performed for all Vitek2 identifications. Quality control was achieved using *C. parapsilosis* ATCC 22019, *C. albicans* ATCC 611098, *C. glabrata* ATCC myA2950 and *C. tropicalis* ATCC 13803.

Susceptibility Testing

Antifungal susceptibility testing for Amphotericin-B (AMB), 5-Flucytosine (5-FC), Fluconazole (FCZ), Itraconazole (ITR) and Voriconazole (VCR) was performed using ATB Fungus 3® of Biomérieux as previously described [12,15]. Briefly ATB Fungus 3® of Biomérieux strip consists of 16 pairs of cupules including two growth control wells and five antifungal drugs at different concentrations: 5-Flucytosine (4, 16µg/ml), Amphotericin B (0.5 to 16µg/ml), Fluconazole (1 to 128µg/ml), Itraconazole (0.125 to 4µg/ml) and Voriconazole (0.06 to 8µg/ml). The inoculated strips were used in duplicate

(c and C) and were read visually after incubation at 37°C for 24h. For each antifungal agent, the reading of the strips was started with the lowest concentration. The growth score was recorded for each of the wells and compared with the control wells as follows: No reduction in growth (4), slight reduction in growth (3), distinct reduction in growth (2), very weak growth (1) and no growth (0).

For Amphotericin B, the minimum inhibitory concentration (MIC) of the Candida species corresponded to its lowest concentration, thus enabling complete growth inhibition. For Fluconazole, Itraconazole and Voriconazole, as the possibility of a trailing growth existed; the MIC corresponded to the lowest concentration of the anti-fungal agent, with which a score of 2, 1 or 0 was obtained. For Flucytosine, a growth was looked for and was quantified in both the wells and tested for two concentrations. The results obtained gave an MIC that helps to classify the strain insensitive, intermediate or resistant. The anti-fungal breakpoints used followed the CLSI guidelines (National Committee for Clinical Laboratory Standards, 1997). Quality control was ensured by testing the CLSI-recommended quality control strains C. parapsilosis ATCC 22019 and C. krusei ATCC 6528 for both CLSI BMD and ATB FUNGUS 3 [16,17].

Ethical Considerations

The study was conducted within the ethical standards and approved by the Comite National des Sciences de la Vie et de la Santé of Côte d'Ivoire. Written informed consent was obtained before any assessment was performed. If the patient was unable to read and write, then a witnessed consent was used. Patients < 18 years old, who were capable of providing assent, provided assent in addition to parental/ legal guardian consent.

Statistical Analysis

All statistical analyses were performed using IBM SPSS software (version 22.0; IBM SPSS Inc., New York, USA). Categorical variables were compared using the $\chi 2$ or Fisher's exact test, and continuous variables by the Mann–Whitney U test. A P value of 0.05 was considered significant.

Results

Yeast Isolated

Most of the isolates were obtained from vaginal swabs 806 (64.8%), followed by Nail scrapings 49 (5.67%). The other swabs (224) were taken fromsperm, oropharyngeal, sputum and stools. Overall *C. albicans* counting was 58.9% (732/1234) of the infections. *C. glabrata, C. krusei* and *C. tropicalis* were observed in a proportion of 1.20%, 6.4% and 4.8% respectively. Among the 806 vaginal yeast isolates, *C. albicans* was the most common species and identified in 548 (68%) isolates, followed byC. krusei (4.8%) (Table 1).

Yeast Isolate	Number (%) of Yeast species Isolated from Sample Type					Total	p-values
	VS	Squama	Nail scrapings	Pus	Others		
C. albicans	548 (67,99)	8 (18,60)	28 (31,82)	29 (35,36)	119 (53,12)	732 (58,89)	<0,0001
C. krusei	39 (4,84)	10 (23,25)	12 (13,63)	3 (3,66)	16 (7,14)	80 (6,43)	<0,0001
C. tropicalis	24 (2,97)	7 (16,28)	6 (6,82)	5 (6,09)	17 (7,59)	59 (4,75)	0,0001
C. glabrata	13 (1,61)	0	0	0	2 (0,89)	15 (1,20)	0,42
C. sp	182 (22,58)	18 (41,86)	42 (47,72)	45 (54,88)	70 (31,25)	357 (28,72)	<0,0001
Total	806 (100)	43 (100)	88 (100)	82 (100)	224 (100)	1243 (100)	-
p-values	<0,0001	<0,0001	<0,0001	<0,0001	0,2	-	-

Table 1: Yeast species isolated from clinical sample tested.

Antifungal Susceptibility Testing of Candida spp. Isolated from Vaginal Swab

Susceptibility patterns of the common Candida species isolates from vaginal swab are shown in Table 2. Overall the sensitivity rates of Candida species from vaginal swab to 5-FC, AMB, FCZ, VCR and ITR were 95.04, 93.30, 89.83, 87.96 and 86.72.

Yeast species Isolated	No of Isolates	5-FC			AMB			FCZ			VCR			ITR		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C. albicans	548	533 (97,26)	8 (1,46)	7 (1,28)	517 (94,34)	0	31 (5,66)	520 (94,89)	9 (1,64)	19 (3,47)	526 (95,98)	12 (2,19)	10 (1,8)	486 (88,69)	33 (6,02)	29 (5,29)
C. glabrata	13	13 (100)	0	0	13 (100)	0	0	9 (69,23)	0	4 (30,77)	13 (100)	0	0	12 (92,3)	1 (7,69)	0
C. tropicalis	24	23 (95,83)	1 (4,17)	0	22 (91,67)	0	2 (8,33)	23 (95,83)	0	1 (4,17)	24 (100)	0	0	22 (91,66)	1 (4,17)	1 (4,17)
C. krusei	39	17 (43,59)	21 (53,85)	1 (2,56)	28 (71,80)	0	11 (28,20)	0	0	39 (100)	35 (89,74)	1 (4,17)	3 (7,69)	8 (20,51)	16 (41,02)	15 (38,46)
C. sp	182	180 (98,90)	2 (1,10)	0	172 (94,50)	0	10 (5,5)	172 (94,50)	6 (3,3)	4 (2,20)	111 (60,99)	44 (24,17)	27 (14,84)	171 (93,96)	5 (2,75)	6 (3,29)
Total	806	766	32	8	752	0	54	724	15	67	709	57	40	699	56	51
% Susceptibility		95,04	3,97	0,99	93,30	0	6,7	89,83	1,86	8,31	87,96	7,07	4,97	86,72	6,95	6,33
p-value		<0,00001	<0,00001	<0,00001	<0,00001	-	<0,00001	<0,00001	<0,00001	<0,00001	<0,00001	<0,00001	<0,00001	<0,00001	<0,00001	<0,00001

Table 2: Antifungal Susceptibility of isolates from Vaginal Swabs.

This sensitivity rates with *C. albicans* species to 5-FC, AMB, FCZ, VCR and ITR were 97.26, 94.34, 94.89, 95.98 and 88.69 respectively. High resistance rates of C. albicans were observed with AMB (5.66%) and ITR (5.29%). Sensitivity rate of *C. glabrata* was 100% with 5-FC, AMB and VCR. All *C. krusei* species from vaginal swab were resistant to fluconazole. The resistance rate of C. krusei to 5-FC, AMB, FCZ, and ITR was 2.56, 28.20, 7.69 and 38.46 respectively.

The sensitivity rate of C. tropicalis to the antifungal drugs tested varied between 91.66% with ITR to 100 with VCR.

Antifungal Susceptibility Testing of Candida spp. Isolated from other Samples

Overall the sensitivity rates of Candida species from other samples to 5-FC, AMB, FCZ, VCR and ITR 98.02%, 92.08%, 78.22%, 96.04% and 83.17%.

Antifungal susceptibility of Candida species isolates from others samples are shown in Table 3.

Yeast species Isolated	No of Isolates	5-FC			AMB			FCZ			VCR			ITR		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C. albicans	63	61 (96,82)	1 (1,59)	1 (1,59)	61 96,83)	0 (0)	2 (3,17)	61 (96,83)	2 3,17)	0 (0)	62 (98,41)	0 (0)	1 (1,59)	55 (87,30)	6 (9,52)	2 (3,17)
C. parapsilosis	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
C. tropicalis	18	18 (100)	0 (0)	0 (0)	14 (77,78)	0 (0)	4 (22,22)	17 (94,45)	1 5,55)	0 (0)	17 (94,45)	0 (0)	1 (5,55)	14 (77,78)	2 (11,11)	2 (11,11)
C. krusei	19	19 (100)	0 (0)	0 (0)	17 (89,47)	0 (0)	2 (10,53)	0 (0)	0 (0)	19 (100)	17 (89,47)	2 (10,53)	0 (0)	14 (73,68)	2 10,53)	3 (15,79)
Total		99	1	1	93	0	8	79	3	19	97	2	2	84	10	7
% Susceptibility		98, 02	0,99	0,99	92,08	0	7,92	78,22	2,97	18,81	96,04	1,98	1,98	83,17	9,90	6,93
p-value		<0,00001	<0,39	<0,39	<0,00001	-	0,26	<0,00001	0,29	<0,00001	<0,00001	0,11	0,57	<0,00001	0,05	0,43

Table 3: ATF Susceptibility Others samples.

The sensitivity rates with C. albicans species to 5-FC, AMB, FCZ, VCR and ITR were respectively 96.82%, 96.83%, 96.83%, 98.41% and 87.30%. *C. albicans* resistance rate was 3.17% with both AMB and ITR. All *C. krusei* species were resistant to FCZ while all were sensible to 5-FC.

Discussion

Candida identification to species level is rarely made, and patients are treated empirically based on their clinical symptoms. The introduction of antifungal susceptibility testing before treatment initiation can be relatively expensive, but is certainly a long-term cost effective solution in preventing the progression of drug resistance. Increasing resistance to antifungal agents has been described and contributes to the difficulty in treating these infections. Hence, rapid yeast identification and susceptibility testing methods are required to optimize patient management. Most of the isolates in this study were obtained from vaginal swabs 806 (64.8%) and *C. albicans* was the most Candida species isolated in this study following by *C. krusei* and *C. tropicalis*. Previous studies conducted in the country demonstrated also the predominance of C. albicans among candida species [9,12,13]. Similar reports have been reported in previous studies [18-20]. The data in different parts of the world have recorded higher rates of *C. albicans* [21-24], while lower rates were also reported [25-28].

Among non-albicans Candida; *C. krusei* was the most common species isolated in our study followed by *C. tropicalis*. The predominance of *C. albicans* could be explained by its considerable ability to adhere to host constituents, as well as by its ability to modify its behavior according to the environment and the secretion of lytic enzymes, which involves specific ligand/receptor interactions with mannoproteins of the yeast wall [29,30].

Our results showed that *C. albicans* species isolated from vaginal swab was susceptible to 5-FC, AMB, FCZ, VCR and ITR. Similar results were obtained with *C. albicans* from others samples. In this study resistance of *C. albicans* to fluconazole, voriconazole and itraconazole was respectively 3.47%, 1.8% and 5.29%. These results are in contrast with results from others studies conducted in the country. According to a study carried out in Abidjan in 2017, *C. albicans* accounted for 64.8% of isolated strains of vaginal origin with rates of resistance of 26.3% for fluconazole, 27.7% for voriconazole and 39.7% for itraconazole [13]. Djohan V, et al. [9] found in their study conducted in 2008 in Abidjan, a *C. albicans* resistance rate of 2.2%, 11.1% and 22.2% respectively to fluconazole, voriconazole and itraconazole.

It seems that the strains of *C. albicans* have become more sensitive to these fluconazole, voriconazole and Itraconazole during the last years in view of our results. This could be due to a drop in drug pressure as a result of better use of these drugs.

The most commonly prescribed antifungal used for most *C. albicans* infections is fluconazole, a member of the azole class of antifungals [31]. Fluconazole is widely used in public health settings in the country and is used empirically in the treatment candida infection, as it is less toxic and regarded as more effective than imidazole antifungals, such as ketoconazole or amphotericin B [32]. In this study resistance rate of *C. albicans* isolate from vaginal swab to fluconazole was 3.47% less than those observed in others studies. Resistance to azole antifungals in Candida has been most extensively studied in *C.albicans*. One mechanism of resistance identified in this species is the Presence of point mutations in ERG11 [33].

In our study 5.66% of *C. albicans* strains from vaginal swab were resistant to AMB (5.66%) while no resistance was seen in others studies from Ivory Coast [9,13] and elsewhere [34]. *C. albicans* resistance to amphotericin B has also been reported in Africa [32,35].

All *C. krusei* species from vaginal swab and others samples were resistant to fluconazole and resistance to others drugs was high. *C. krusei* resistance has been reported from elsewhere [32,36,37].

C. krusei possesses intrinsic resistance to fluconazole while also rapidly developing acquired resistance to other antifungal drugs. The mechanisms of antifungal resistance of this yeast involve the alteration and over expression of drug target, reduction in intracellular drug concentration and development of a bypass pathway [14].

The study has some limitations. Firstly, risk factors for candida infection such as pregnancy, antibiotic therapy, uncontrolled diabetes mellitus, immunosuppression due to HIV and others were not assessed. Secondly, the study was limited to a single Laboratory reference and thus, the results may not be applicable to other settings. Despite the limitations, the study provided baseline information on the identification of Candida species and their antifungal susceptibility in a reference laboratory in Côte d'Ivoire. The study demonstrated the fact that most antifungals continue to be active against Candida strains from Côte d'Ivoire.

Conclusion

C. albicans was the most common species associated with candida infections followed by *C. krusei* and C. albicans was susceptible to antifungal drugs tested in this study and all *C. krusei* strains were resistant to fluconanazole. Knowledge

of Candida species distribution and antifungal resistance pattern of them plays an important role in appropriate therapy.

Statements & Declarations

Not applicable

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Competing Interests

The authors declare no competing interests.

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