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Antifungal Susceptibility of *Candida* Strains Isolated from Patients with Vaginal Candidiasis at the Centre University Hospitalist of Brazzaville

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Abstract

Objective: To study the sensitivity to antifungal agents of **Candida** strains isolated from vaginal candidiasis at the Brazzaville University hospital.

Materials and methods: This was a descriptive cross-sectional study conducted from 8 July to 8 October 2019 in the parasitology mycology department of the Brazzaville University Hospital centre. *Candida* strains isolated from vaginal samples were subjected to an *in vitro* sensitivity study using the Sabouraud disc diffusion method. The sensitivity of *Candida* to antifungal agents and their activity on *Candida* strains were assessed.

Results: The median age was 32 years (q1: 24 and q3:40 years). The most common age group was (25-35 years): 30.9%. *C.* non-albicans was present in 64.7% (22/34) of cases. *Candida albicans* was found in 35.3% of cases (12/34). Sensitivity to antifungal agents varied according to species. Econazole had a sensitivity of 70.6%, miconazole 38.2% and ketoconazole 64.7%. Itraconazole and fluconazole were resistant to 79.4% and 58.8% respectively. All *Candida* strains were sensitive to econazole. They had good sensitivity to miconazole.

Conclusion: The sensitivity of *Candida* strains to antifungal agents was reduced overall. Econazole remains the antifungal still active on all *Candida* strains.

Keywords: Sensitivity; Antifungals; Antifungal chart; Vaginal candidiasis; Brazzaville University hospital centre

Introduction

Vaginal candidiasis is an infection caused by a fungus of the genus *Candida*. This genus includes more than 200 species, around ten of which are involved in a pathological process in humans.

The worldwide incidence of vaginal candidiasis has increased in recent years, with *Candida albicans* being the most common species. At the same time, there has been a growing involvement of non-albicans *Candida*, often isolated in recurrent candidiasis.

Vaginal candidiasis is second only to bacterial vaginosis. Worldwide, more than 75% of women develop *Candida* vaginitis during their period of genital activity.

A study carried out in Gabon showed that *Candida albicans* was the main causative agent of vaginal candidiasis in 68.2% of cases. Another study conducted in the Congo reported a predominance of the same species in 32.3% of cases of vaginal mycosis.

Treatment of candidiasis is essentially based on the use of antifungal agents. However, treatment failures are sometimes observed. This is most often due to a problem in identifying the *Candida* species involved, especially as the incidence of non-albicans *Candida* is increasing daily. In addition, resistance to antimicrobials, including antifungals, is implicated in these failures. Some *Candida* species have natural resistance. Primary resistance of *Candida krusei* to fluconazole and possible resistance of *Candida glabrata* to fluconazole *via* an efflux mechanism have been demonstrated. These resistances are not only natural, but are mainly due to selection pressure associated with certain therapeutic practices, such as the probabilistic administration of antifungal agents or self-medication.

In the Republic of Congo, *Candida* vaginitis is a frequent reason for consultations in health facilities. Antifungal tests are not routinely carried out. This explains the paucity of data on resistance to antifungal agents, even though antifungal treatment is usually prescribed. In order to improve patient care, it is necessary to identify *Candida* species and assess their

sensitivity profile. It was against this background that we conducted this study, the aim of which was to investigate the sensitivity to antifungal agents of *Candida* strains isolated during vaginal candidiasis at the Brazzaville University hospital [1].

Materials and Methods

This was a descriptive cross-sectional study that took place from 8 July to 30 October 2019 at the parasitology mycology laboratory of the Brazzaville University hospital center. All women seen at the laboratory for vaginal swab analysis with or without clinical signs, who had not received any antifungal treatment and who had given informed consent were included. Women with genital bleeding were excluded. For each patient included in the study, sociodemographic, clinical and mycological data were collected using a survey form. After inclusion, samples were taken by swabbing (using 2 sterile swabs) of the vaginal mucosa after the speculum had been removed.

For each sample, the vaginal pH was assessed using a pH strip. A direct examination and culture, on Sabouraud-Chloramphenicol and Sabouraud-Chloramphenicol-Actidione media, was performed and incubated at 37°C for 24 hours. All positive cultures were subjected to a filamentation test using a yeast suspension mixed with 1 ml of fresh human serum. The preparation was incubated at 37°C for 3 hours. *Candida* species were identified using Biomerieux's API *Candida* gallery.

All the *Candida* species identified were subjected to an *in vitro* antifungal susceptibility study using the Sabouraud agar disc diffusion method based on an inoculum of 0.3 Mac Farlan. The antifungal discs tested were Econazole (10 µg), Fluconazole (100 µg), Ketoconazole (10 µg), Itraconazole (50µg) and Miconazole (10 µg). The diameter of inhibition formed around the disc was used to determine the Sensitive (S), Intermediate (I) or Resistant (R) characteristics of each antifungal agent, taking into account the interpretation criteria given by the manufacturer [2].

The data collected were analyzed using EPi-info7.2.2.6 software and calculations were made using frequencies for qualitative variables and central tendency and dispersion parameters for quantitative variables.

Results

Gynecological and obstetric sociodemographic characteristics

A total of 152 patients were selected. The median age of the patients was 32 years, with q1: 24 years to q3:40 years.

Species isolated from vaginal swabs

Non-albicans *Candida* species were the most commonly isolated. *C. glabrata* represented for 36.4% of non-albicans *Candida* species. It was followed by *Candida albicans*.

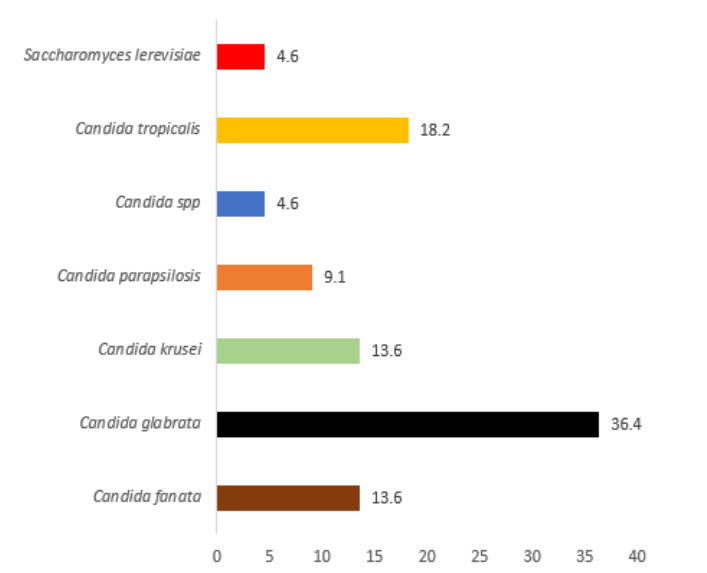

Figure 1: Distribution of different non-albicans *Candida* species.

Sensitivity to antifungal agents

The overall sensitivity of the antifungal agents tested was 100% for Econazole, 94.1% for miconazole, 91.2% for ketoconazole, 41.2% for fluconazole and 17.8% for itraconazole. Table 1 shows the detailed sensitivity of each antifungal agent to *Candida* strains isolated from vaginal swabs. Econazole showed a decrease in sensitivity to *Candida* strains, with an intermediate sensitivity of 29.4% and a sensitivity of 76.6%. No *Candida* strains were found to be resistant to econazole. On the other hand, for itraconazole, there was a decrease in the sensitivity of *Candida* strains to this compound, with an intermediate sensitivity rate of 17.6% and a higher resistance rate than for all the other compounds (79.4%) [3-6].

Table 1: Interpretation of antifungal inhibition zones.

Antifungals	Results interpreters	Diameter in mm	Diamaters thresholds	Speciality
Econazole	R	<10	10-20	Pevaryl
	S	>20		
	I	10-20		

Fluconazole	R	<10	10-20	Trifucan
	S	>20		
	I	10-20		
Itraconazole	R	<10	10-20	Sporanox
	S	>20		
	I	10-20		
Ketaconazole	R	<10	10-20	Nizoral
	S	>20		
	I	10-20		
Miconazole	R	<10	10-20	Daktarin
	S	>20		
	I	10-20		
Note: I: Intermediate; R: Resistance; S: Sensitive				

For the ketoconazole and miconazole molecules, the strains were more sensitive to antifungal agents than resistant. However, for fluconazole, the resistance rate (58.8%) was slightly higher than the sensitivity rate (41.2%) (Table 2) [7-10].

Table 2: Sensitivity of different antifungal agents to *Candida* strains.

Variables	Number (n)	Percentage (%)
Econazole		
Sensitive	24	70.6
Intermediate	10	29.4
Resistant	0	0.0
Fluconazole		
Sensitive	5	14.7
Intermediate	9	26.5
Resistant	20	58.8
Ketoconazole		
Sensitive	22	64.7
Intermediate	9	26.5
Resistant	3	8.8
Itraconazole		

Sensitive	1	0.2
Intermediate	6	17.6
Resistant	27	79.4
Miconazole		
Sensitive	13	38.2
Intermediate	19	55.9
Resistant	2	5.9

Table 3 shows the percentage sensitivity of *Candida* species to the antifungal agents tested *in vitro*. All strains of *C. albicans* and *C.* non-albicans were sensitive to Econazole. However, strains of *C. glabrata, C. krusei* and *C. tropicalis* showed total resistance (100%) to itraconazole. For the ketoconazole molecule, all non-albicans *C.* strains except *C. tropicalis* were fully susceptible (100%) [11-15].

Table 3: Susceptibility of *Candida* species to the different antifungal agents tested.

	Econazole (%)	**Fluconazole (%)**	**Ketoconazole (%)**	**Itraconazole (%)**	**Miconazole (%)**
C. albicans					
Sensitivity	100.0	41.7	83.3	25.0	91.7
Resistance	0	58.3	16.7	75.0	8.3
C. glabrata					
Sensitivity	100	50.00	100	0	100
Resistance	0	50.00	0	100	0
C. krusei					
Sensitivity	100	50.00	100	0	100
Resistance	0	50.00	0	100	0
C. famata					
Sensitivity	100	33.33	100	66.67	100
Resistance	0	66.67	0	33.33	0
C. parapsilosis					
Sensitivity	100	50.00	100	50.00	100
Resistance	0	50.00	0	50.00	0
C. tropicalis					
Sensitivity	100	0	33.33	0	66.67
Resistance	0	100	66.67	100	33.33

Discussion

Prevalence of vaginal candidiasis

The prevalence of vaginal candidiasis in our study was 22.4%. Our results are similar to those found by some authors in Ouagadougou, Bob Dioulasso, Punjab and Casablanca. Others, on the other hand, have obtained results higher than ours for some and lower for others. We note a wide variability in the prevalence of vaginal candidiasis in the literature. This variability can be explained by the susceptibility of each woman to different exposure to predisposing factors. These include factors associated with behaviour, contraception, sexual contamination. Candida virulence and anti-candida immunity. This anti-candida immunity is based on cell-mediated. humoral or innate immunity [16-18]. Some authors link this to genetic predisposition in certain populations.

Different *Candida* species isolated from vaginal candidiasis

The mycological study of our patients' samples showed a predominance of non-albicans *Candida* species over *Candida albicans*. This trend has also been reported by some authors. In general, a predominance of non-albicans species is observed in recurrent candidiasis. However, in addition to recurrent vaginal candidiasis, disruption of the vaginal ecosystem by the use of certain antiseptics or the practice of certain hygiene habits may also be responsible for this increase in non-albicans *Candida* species. These results are in contrast to those obtained by Mbou, et al., in the same town, who showed a predominance of *Candida albicans*. This is also what most of the literature reports [19-21].

The most common non-albicans *Candida* species were *Candida glabatra, Candida tropicalis*, *Candida famata* and *Candida krusei*. *Saccharomyces cerevisiae* was also identified. In the literature, we note variability in the prevalence of non-albicans *Candida* species. Some authors have found *C. glabrata*, *C. Kefir*, *C. tropicalis*, *C. glabatra*, *C. parapsilosis*, *C. tropicalis*, *S. cerevisiae*, *C. famata*, *C. rugosa*, *C. parapsilosis*, *C. glabatra*, *C. tropicalis*, *C. parapsilosis*, *C. glabatra*, *C. tropicalis*, *C. famata*.

Despite this diversity of prevalence, it appears that *C. glabrata* is the non-albicans *Candida* species most frequently found in several studies. On the one hand, this could be explained by the fact that *C. glabrata* is one of the species most frequently found among the commensal *Candida* species of the digestive tract and genitourinary tract. Secondly, its incidence has increased due to the selection pressure exerted by the increasing use of azoles in the treatment of vaginal candidiasis.

The increase in the number of non-albicans *Candida* species should raise fears that recurrent vaginal candidiasis is becoming increasingly common. This is because these species are becoming less and less resistant to antifungal agents, especially in certain areas.

Sensitivity o f isolated *Candida* strains to antifungal agents

The antifungal agents we tested in this study were mainly azoles, which are the molecules most commonly used in the treatment of vaginal candidiasis. Overall, there was a reduction in sensitivity for all the azoles we tested. But only econazole did not show any development of resistance. Econazole was active on all the *Candida* strains we isolated. This azole molecule can therefore be used in all cases of vaginal candidiasis without the need for an antifungigram. However, prescribing it as a first line treatment can lead to the development of resistance, especially if the bacteria have not been identified beforehand. Our results differ from those found in Ahvaz, where strains isolated from vaginal candidiasis showed resistance to econazole. This difference in results clearly shows that resistance is not only region dependent but also depends on the possibility of genetic transfer of resistance from one strain to another.

Fluconazole had a resistance rate of around 60% in our study. This resistance rate is well above that reported by several authors, who reported fluconazole sensitivity of over 60%. In contrast, in Cameroon, 82% resistance was observed in strains isolated from vaginal candidiasis. Although resistance to fluconazole is increasingly being observed, there are still areas, such as Addis Ababa, where fluconazole remains active against all strains of Candida. This development of resistance to fluconazole may be due to the fact that, as this compound is more of a fungi static than a fungicide, its increased prescription has led to the emergence of resistance mechanisms in *Candida* strains. This phenomenon is all the more dangerous as fluconazole has become the drug of first choice in the treatment of certain fungal infections.

Ketoconazole is a compound that remains active on *Candida* isolated from vaginal candidiasis. However, our study shows an increase in intermediate sensitivity with a low rate of resistance, elements which should attract attention and increase vigilance in the use of this molecule. The resistance rate we obtained is lower than that reported in Cameroon, where resistance was 72% and in Ahvaz, Iran, where it was 37.3%. On the other hand, 100% sensitivity was reported in Abidjan.

Itraconazole reached equally worrying proportions in our study. This was not the case in China, where the rate of resistance to itraconazole was 2.5% or in Ahvaz, Iran, where it was 11.9%. As for miconazole, its use requires maximum doses to ensure optimal therapeutic efficacy, as it showed an intermediate sensitivity of almost 60%, whereas it has the lowest degree of resistance of all the molecules in our study. Its low resistance rate has been noted by several authors.

The *Candida* strains we isolated showed a different profile depending on the antifungal agent tested. All the *Candida* strains in our study were totally sensitive to econazole, whereas sensitivity to the other molecules varied according to species. This disparate sensitivity profile of *Candida* strains is an argument in favour of routine antifungal testing in our environment.

Conclusion

Vaginal candidiasis is highly prevalent at Brazzaville University hospital. The fungal agents responsible are dominated by non-albicans *Candida*. Several molecules are currently showing a loss of sensitivity and even total resistance to certain azole antifungals. However, Econazole is still active on all the *Candida* strains that have been identified.

Therefore, the fact that antifungals are not systematically performed in cases of vaginal candidiasis at Brazzaville University Hospital is an attitude that encourages the emergence of antifungal resistance. Antifungal grams should therefore be encouraged.

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