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Journal of  
**Yeast and  
Fungal Research**



October 2018  
ISSN 2141-2413  
DOI: 10.5897/JYFR  
[www.academicjournals.org](http://www.academicjournals.org)



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# Journal of Yeast and Fungal Research

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## ARTICLE

### **Susceptibility of *Candida* species isolated from recurrent vulvovaginal candidiasis to antifungal agents among women at Institut Pasteur of Côte d'Ivoire**

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*Full Length Research Paper*

## **Susceptibility of *Candida* species isolated from recurrent vulvovaginal candidiasis to antifungal agents among women at Institut Pasteur of Côte d'Ivoire**

**Kpongbo Etienne Angora<sup>1,2\*</sup>, Vincent Djohan<sup>1</sup>, Valérie A. Ira-Bonouman<sup>2</sup>, Henriette A. Vanga-Bosson<sup>1,2</sup>, Kondo Fulgence Kassi<sup>1,3</sup>, Abibatou Konaté<sup>1</sup>, Valérie A. Bedia-Tanoh<sup>1</sup>, Sébastien A. Miezan<sup>1</sup>, Christiane P. Kiki-Barro<sup>1</sup>, Hervé Menan<sup>1,3</sup>, André Offianan Touré<sup>2</sup> and William Yavo<sup>1</sup>**

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Received 11 August, 2018; Accepted 2 October, 2018

Recurrent vulvo-vaginal candidiasis is a major issue in pubescent females because of the fact that it is a source of health discomfort. For a better medical treatment, an analysis of susceptibility of *Candida* species to antifungal agents is fundamental. Therefore, this study aims to determine the susceptibility of *Candida* spp. to antifungal agents isolated in recurrent vulvo-vaginal candidiasis. This cross-sectional study was carried out in 2014 among 400 women with vaginal discharge. The recurrent vulvovaginitis was defined by the occurrence four times at least in a year. Vaginal swabs were cultured on Sabouraud-chloramphenicol medium, and *Candida* spp. were identified using a chromogenic technique. Susceptibility testing was performed using a microdilution method. *In vitro* susceptibility to antifungal agents test was performed using amphotericin B, 5-fluorocytosine, fluconazole, itraconazole and voriconazole against isolated *Candida* spp. Of 94 women positive from *Candida* spp., *Candida albicans* (59.6%) was the most prevalent with 59.6%. *C. albicans* did not show any resistance to amphotericin B and voriconazole. The susceptibility *Candida glabrata* to itraconazole was 50% and this species was relatively susceptible to fluconazole (77.8%) and voriconazole (83.3%). The medical treatment of recurrent vulvovaginal candidiasis requires a precise diagnosis based on a mycological analysis and the susceptibility to antifungal drugs.

**Key words:** Recurrent vulvovaginal candidiasis, susceptibility, antifungal, *Candida* species.

### **INTRODUCTION**

Vulvovaginal candidiasis is a frequent mycotic infection in women caused by a commensal yeast called *Candida* lying in the vaginal mucous membrane. *Candida* to produce either superficial or systemic infections depends

on the host immune system, risk factors (Arfiputri et al., 2018), sexual behaviour (Grinceviciene et al., 2018) and a loss of the vaginal equilibrium (Arendrup, 2013). Of sexually active women, 75% have at least once

experienced symptomatic of vulvovaginal candidiasis linked to vaginal discharge (Brandolt et al., 2017). Moreover, the recurrent vulvovaginal candidiasis is characterized by the fact that it occurs four times at least in a year (Sobel, 2015). Among the *Candida* species, *Candida albicans* is considered the most prevalent and 25 to 34 years age group has the highest prevalence at 9% (Denning et al., 2018). Hence, an efficient treatment generally requires a local and oral route administration of antifungal agents (Fan et al., 2015). Recurrent vulvovaginal candidiasis is a common cause of morbidity in women because of the fact that it is a source of health discomfort. It is also due to the difficulties in the treatment according to the clinical practitioners and fluconazole remains the most effective treatment option (Crouss et al., 2018).

Studies about candidiasis in Côte d'Ivoire showed the antifungal susceptibility pattern of the *Candida* spp. from vulvovaginal candidiasis (Djohan et al., 2012; Konaté et al., 2014). But there is no data available about recurrent vulvovaginal candidiasis, hence the importance of this study which aims to determine the antifungal susceptibility of *Candida* spp. isolated from recurrent vulvovaginal candidiasis in Côte d'Ivoire.

## MATERIALS AND METHODS

### Patient selection

This cross-sectional study was carried out at Institut Pasteur of Côte d'Ivoire from May to July 2014 among 400 women. Vaginal swabs were collected from women in "Centre Hospitalier Universitaire (CHU) Cocody" standing for Teaching Hospital of Cocody, and in "Unité d'Accueil et de Réception des Prélèvements" (URAP) standing for reception and sampling unit, Institut Pasteur de Côte d'Ivoire. *Candida* species identification and antifungal susceptibility testing were performed in the Mycological Laboratory in Institut Pasteur de Côte d'Ivoire. Target patients were women with vaginal discharge associated or not to other symptoms. The recurrent of vulvovaginitis was defined by the fact that it occurs four times at least in a year from a woman (Ringdahl, 2000). An informed consent form was obtained from the participants after the contents of the form were clearly explained.

### Questionnaire administration

Upon a clear consent, a questionnaire was submitted to patients for information about each individual's sociodemographic and clinical living factor. The questionnaire listed among others, points the age, the medico-surgical history, the factors that lead to the infection, the symptoms of the disease, some knowledge about the treatment used and about the recurrence factor.

### Sample collection and processing

Before the insertion of the swab stick, the vulva was cleansed.

Then, the swab was obtained from the cul-de-sac with the aid of a speculum. An immediate analysis was performed with each sample, which was streaked on Sabouraud-chloramphenicol medium and then on Sabouraud-chloramphenicol-Actidione medium. Yeasts were identified via chromogenic method (CandiSelect®4 Biorad) which shows *C. albicans* (purple colonies), *Candida tropicalis* (dark green colonies), *Candida glabrata* (light green colonies), and *Candida krusei* (blue-turquoise colonies). The Auxacolor® gallery 2 (Biorad) was used according to manufacturer's procedures in case of diagnosis difficulty.

The isolated *Candida* spp. were tested to determine their antifungal susceptibility, the micro-dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS) norms using the ATB® Fungus 3 gallery (Biomérieux, France). Five antifungal agents were tested in accordance with the manufacturing recommendation; there are amphotericin B, 5-fluorocytosin, fluconazole, itraconazole and voriconazole. Briefly, fungal suspension with turbidity of McFarland 2 was obtained with young colonies in an API NaCl 0.85% Medium tube. Then 20 mm of this suspension was poured into ATB F2 Medium with aid of a micropipette to homogenize. After that, 135 mm of the ATB F2 Medium content were divided up into cupules and incubated for 24 h at 37°C. The growth and the quantification factors of the yeasts were identified through visual reading by setting the gallery on a dark background to help along. A growth score for each antifungal agent was determined and noted in each cupule in comparison with the model cupules, beginning by the weakest growth.

### Data statistical analysis

Data analysis was performed using SPSS 21 software (Statistical Package for Social Science, IBM SPSS Statistics). All variables were classified in a group and the Fisher exact test was used to compare proportions. P-value < 0.05 was considered significant.

## RESULTS

### Sociodemographic and clinical data

In total, 400 women with a recurrent vaginal discharge were included in the study. The average age of the admitted patients was 29.2 years (standard deviation = 7.2 years) with extremes between 14 and 69 years. The patients between 14 and 19 years old were the most affected by the recurrent vulvovaginal candidiasis in a proportion of 31.6%. However, no statistically significant ratio between age and outbreak of recurrent vulvovaginal candidiasis was noticed. As far as marital status is concerned, women that are single or concubine were more exposed to recurrent vulvovaginal candidiasis ( $p=0.004$ ). The women with primary school education level represented the most vulnerable.

Clinically, apart from vaginal discharge, vaginal pruritus was the most common clinical manifestation. Table 1 shows the socio-demographic and clinical characteristics of the population studied.

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**Table 1.** Sociodemographic and clinical characteristics of the patient.

Variable	Total	N- (%)	N+ (%)	p-value
<b>Age (years)</b>				
14-19	38	26 (68.4)	12 (31.6)	0.273
20-29	191	146 (76.4)	45 (23.6)	
30-39	132	100 (75.8)	32 (24.2)	
40 and above	39	34 (87.2)	5 (12.8)	
<b>Marital status</b>				
Single/Concubine	168	117 (69.6)	51 (30.4)	0.004*
Married	232	189 (81.5)	43 (18.5)	
<b>Educational level</b>				
Unschooling	30	28 (93.3)	2 (6.7)	0.023*
Primary	36	22 (61.1)	14 (38.9)	
Secondary	121	92 (76.0)	29 (24.0)	
University	213	164 (77.0)	49 (23.0)	
<b>Symptoms</b>				
Dyspareunia	111	91 (82.0)	20 (18.0)	0.116
Vaginal burn	49	38 (77.6)	11 (22.4)	0.986
Vaginal pruritus	295	210 (71.2)	85 (28.8)	0.001*

N+: Infected women; N- : Non-infected women; %: percentage.

## Mycological data

From 400 included women with a recurrent vaginal discharge, 94 were positive to *Candida* spp., a prevalence rate of 23.5% (95% CI: 19.49-28.02).

Five *Candida* spp. were isolated and *C. albicans* was the most prevalent at 59.6%. No case of mixed infection was seen among women. Figure 1 shows the distribution *Candida* spp. isolated from women with recurrent vulvovaginal candidiasis infection.

## Antifungal susceptibility

The analysis on antifungal susceptibility focused on the three major species observed in the survey, which were *C. albicans* (56 strains), *C. glabrata* (18 strains) and *C. tropicalis* (15 strains). *C. albicans* did not show resistance to amphotericin B and voriconazole. Susceptibility rate varied from 89.3 to 96.4% with the other forms of antifungal agents. However, only a half of *C. glabrata* was found susceptible to itraconazole. This species was relatively susceptible to fluconazole (77.8%) and to voriconazole (83.3%). For *C. tropicalis*, the susceptibility rate to antifungal agents varied from 86.7 to 100% (Table 2).

## DISCUSSION

Vulvovaginal candidiasis represents one the most frequent causes of vaginal discharge and infection,

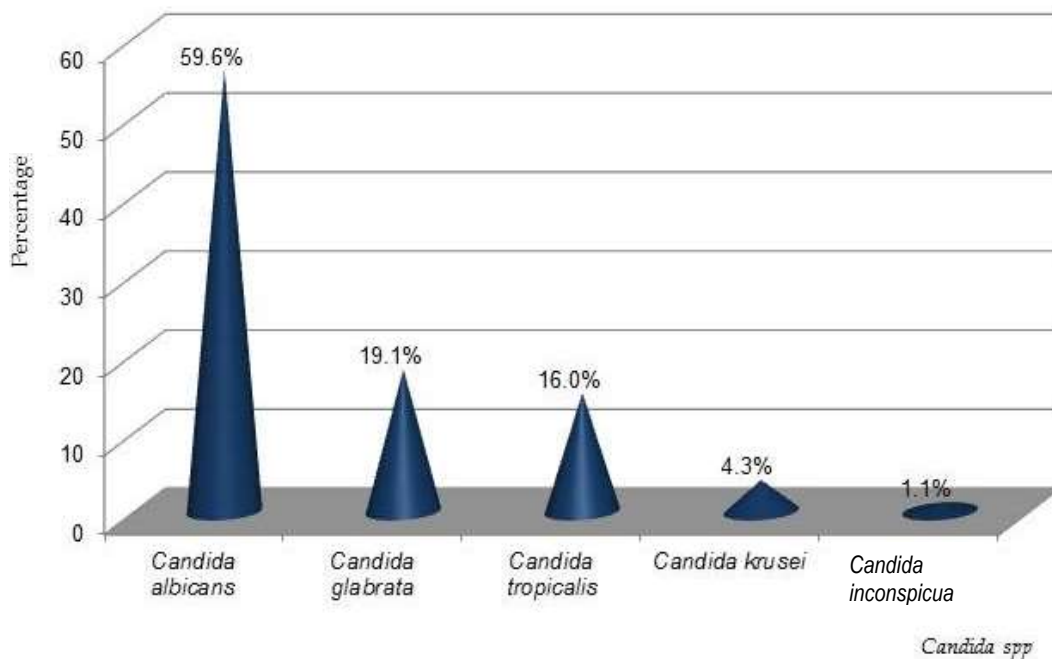
hospital admission for women that are admitted for gynecological visits. The cause of a recurrent form is linked to the exposure to some risk factors and to the chronicity of a first episode for vulvovaginal candidiasis (Fidel et al., 2004; Auler et al., 2010; Sobel, 2007). Different clinical symptoms take place in the development of recurrent vulvovaginal candidiasis. Thus, the symptoms documented in our work earlier stated by other authors (Bernstein and Seidu, 2015; Sobel, 2015).

In the survey, recurrent vulvovaginal candidiasis was found in 23.5% of the patients. Because recurrent vulvovaginal candidiasis varied from a country to another different prevalence rates from 8 to 23% were recorded in some other surveys (Corsello et al., 2003; Rylander et al., 2004; Grigoriou et al., 2006).

The frequent appearance of *C. albicans* in recurrent vulvovaginal candidiasis demonstrates its commensal nature from women genital mucous membrane (Sobel et al., 2004; Amouri et al., 2010; Hu et al., 2015). The present study also sheds light on the role of non albicans in the outbreak of recurrent vulvovaginal candidiasis with *C. glabrata* predominance.

In fact, recurrent vulvovaginal candidiasis is caused mostly by *Candida* spp., especially by *C. glabrata* (Sobel, 2015; Denning et al., 2018). The occurrence of these fungal agents in vulvovaginal candidiasis etiology was previously mentioned in Côte d'Ivoire with prevalence rates ranging from 10 to 32,7% (Bonouman-Ira et al., 2011; Djohan et al., 2012; Konaté et al., 2014) where *C. glabrata* was also considered predominant. The other species observed were *C. tropicalis*, *C. krusei*, *Candida parapsilosis* and seldom *Candida famata* (Richter et al., 2005)





**Figure 1.** Distribution of *Candida* species isolated from the infected patients.

**Table 2.** *In vitro* antifungal susceptibility patterns of *Candida* spp. isolated from patients.

<i>Candida</i> strains and antifungal agents	Sensitive		Resistant	
	n	%	n	%
<b><i>Candida albicans</i> (n=56)</b>				
Fluconazole	54	96.4	2	3.6
Itraconazole	53	94.6	3	5.4
Voriconazole	56	100	0	0
Amphotericin B	56	100	0	0
5-Fluorocytosine	50	89.3	6	10.7
<b><i>Candida glabrata</i> (n=18)</b>				
Fluconazole	14	77.8	4	22.2
Itraconazole	9	50.0	9	50.0
Voriconazole	15	83.3	3	16.7
Amphotericin B	18	100	0	0
5-Fluorocytosine	18	100	0	0
<b><i>Candida tropicalis</i> (n=15)</b>				
Fluconazole	15	100	0	0
Itraconazole	14	93.3	1	6.7
Voriconazole	15	100	0	0
Amphotericin B	15	100	0	0
5-Fluorocytosine	13	86.7	2	13.3

n: Total of *Candida* strains; %: percentage of *Candida* strains.

Ekpenyong et al., 2012; Brandolt et al., 2017). This situation could be linked to the self-medication with

antifungal agents in ambulatory treatment or to frequent risk factor exposures. The difference observed in

susceptibility rates vary according to the *Candida* spp. tested and to the antifungal agent used. Other reports about *C. albicans* susceptibility rates to fluconazole, to amphotericin B, voriconazole and clotrimazole gave similar results (Panizo et al., 2009; Khan et al., 2018). No case of resistance to fluconazole was seen in this study, similar results about susceptibility of *C. albicans* from recurrent vulvovaginal candidiasis were reported (Shahid and Sobel, 2009). *C. albicans* spp. isolated from recurrent vulvovaginal candidiasis remain still susceptible to antifungal agents. But recently, fluconazole resistant *C. albicans* was found from vulvovaginal candidiasis (Sobel and Sobel, 2018). Because of this, they deserve an accepted usage in order to avoid development of resistance.

Non *albicans* spp. identified in this study, a good susceptibility to *in vitro* tested antifungals, except for *C. glabrata* with resistance rates of 50% to itraconazole, 22.2% to fluconazole and 16.7% to voriconazole. Non *albicans* spp. was particularly reported in the recurrence pathogenicity with a predominance of *C. glabrata*. Cases of *C. glabrata in vitro* resistance to antifungals especially to fluconazole were also observed in some surveys (Sobel et al., 2004; Amouri et al., 2010; Sobel, 2015). Fluconazole is an azotic antifungal frequently used through oral route associated to local forms of other antifungals in case of recurrent vulvovaginal candidiasis outbreak (Amouri et al., 2010; Khan et al., 2018). Associating an attacking treatment with a simple health care measures over a period is an advisable management strategy. Suspension of treatment can lead to a relapse in 30 to 50% of patients after three months (Sobel, 2007, 2015). Thus, adequate treatment and compliance is essential for a reduction of recurrence and in an outbreak of resistance appearance of non *Albicans* spp. to antifungal agents.

## Conclusion

Recurrent vulvovaginal candidiasis is frequent and *C. albicans* is the most prevalent. The treatment requires a precise diagnosis based on a mycological analysis and on the determination of the species susceptibility to the antifungal agents. Thus, appropriate tests to detect the presence of *Candida* spp. and assessment of the risk factors linked to the outbreak of vulvovaginal candidiasis from women should help in infection prevention.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors are grateful to all patients who participated in

this study and the Department of Parasitology-Mycology of Institut Pasteur for its support in this work. Particular thanks to all the team from Gynecology Department of "Centre Hospitalier Universitaire (CHU) Cocody for assistance during data collection.

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