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Epidemiology of *Candida* Species Colonizing Mucosae of HIV-Infected Patients in Two Healthcare Centers of Cameroon During 2018-2020

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Authors' contributions

This work was carried out in collaboration among all authors. Author JPD were involved in the conception and design of the study. Author CLK were involved in experiments, data analysis and drafted and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to investigate *Candida* species colonizing HIV-infected patients in Bafoussam and Yaounde in Cameroon.
Study Design: Cross-sectional study.

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Place and Duration of Study: Yaoundé Central Hospital (YCH) and Bafoussam Regional Hospital (BRH) between October 2018 and December 2020.
Methodology: We included 804 HIV-infected (681 women; 123 men; age range 21-81 years). Vaginal discharge, oral swab, stools, and urine were collected, and mycological diagnosis including direct macroscopic and microscopic analyses, culture on Sabouraud chloramphenicol medium, culture on chromogenic medium, germ tube test, evidence of chlamydospores production, biochemical analysis was performed. Yeast isolates were identified using matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS).
Results: Eight hundred and four patients were recruited, and 2754 samples were collected. The colonization frequency was 17.35%, and 513 yeasts were isolated. Overall, *Candida albicans* 251 (48.92%) was the most frequently isolated. Non-*albicans Candida* (NAC) isolates have been classified into 16 species, including *Candida krusei* (14.23*%), Candida glabrata* (9.94%), *Candida parapsilosis* (8.18%), and *Candida tropicalis* (7.99%) as the major ones. There was a relationship (*P*-value= 0.00) between antiretroviral therapy and *Candida* species colonization.
Conclusion: The results provide information on the epidemiology of *Candida* species in HIV-infected patients in Cameroon.

Keywords: Candida species; mucosae; HIV patient in Cameron; MALDI-TOF MS.

1. INTRODUCTION

Candida species are the most common opportunistic pathogens in human immunodeficiency virus (HIV) infection [1]. Although *Candida albicans* remains the most common pathogen overall, non-*albicans Candida* species (NAC) has emerged as a potent pathogenic yeast in HIV-infected patients [2]. The most common types include *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei* [3,4]. The rarer ones are *Candida guilliermondii*, *Candida lusitaniae*, *Candida kefyr*, *Candida famata*, *Candida inconspicua*, *Candida rugosa* and *Candida norvegensis* [3-5]. The relative frequency of *Candida* species other than *Candida albicans* also varies with region [6]. *Candida albicans* was most common in Europe (52.5%) and least common in North America (42.7%), whereas *Candida glabrata* was most common in North America (24.3%) and least common in Latin America (7.1%). *Candida parapsilosis* and *Candida tropicalis* were more common than *Candida glabrata* in Latin America (24.3% and 17.0% vs 7.1%, respectively). *Candida* tropicalis was most common in the Asia-Pacific region (14.1%). *Candida krusei* was more common in North America (2.9%) and Europe (3.0%), and other miscellaneous species of *Candida* were common in the Asia-Pacific region (7.3%) and North America (7.3%) [7]. Some epidemiological studies have been carried out in Cameroon and have shown that the prevalence of *Candida* species in people living with HIV remains high [2,8]. However, an important fact observed during the study of *Candida* species in Cameroon is that the identification of *Candida* species is generally limited based on their phenotypic and biochemical characteristics. These classic techniques for identifying pathogenic yeasts are sometimes inadequate and have limitations such as their lack of sensitivity, specificity, and rapidity. In mycology, rapid detection and accurate, reliable identification are needed to confirm a diagnosis and implement appropriate antifungal treatment. Conventional identification techniques must therefore be accompanied by extremely precise molecular biology techniques to confirm a diagnosis. The present study was conducted to determine the *Candida* species distribution isolated from Cameroon HIV-infected patients by MALDI-TOF MS.

2. METHODOLOGY

2.1 Ethical Considerations and Enrollment of Participants

The survey was carried out at Yaoundé Central Hospital (YCH) and Bafoussam Regional Hospital (BRH). The study involved HIV-infected patients presenting or not presenting clinical signs of any mucosal candidiasis and was approved by the Cameroonian National Ethical Committee. The survey took place from October 2018 to December 2020. Patients enrolled for this study were HIV-infected individuals of both genders, between 21 and 81 years old, who did not receive any antifungal treatment during the last 3 months preceding their enrollment.

2.2 Determination of the Clinical Status of Patients and Collection of Study Samples

Prior to sample collection, the patient's information was registered, including age and HIV status (CD4+ count, type of HIV, stage of the HIV infection, and antiretroviral therapy). Samples collected included vaginal discharge, oral swabs, stools, and urine.

2.3 Mycological Diagnosis

Samples were submitted to direct macroscopic and microscopic analyses using routine laboratory protocols prior to culture on Sabouraud chloramphenicol medium for 24 to 48 hours at 37 °C. Primary identification was assessed by a combination of culture on chromogenic medium (Chromagar Candida from Biomerieux, Marcy l'Etoile, France), germ tube test, evidence of chlamydospores production, and biochemical analysis (ID32C kit from Biomerieux, Marcy l'Etoile, France). The reference *Candida krusei* ATCC 6258 strain was used throughout the mycological diagnosis as a quality control strain.

2.3.1 Macroscopy

Macroscopic examination of the urine consisted of noting its appearance and color. The appearance, consistency, color, presence or absence of mucus and odor of the feces are the elements observed during macroscopic examination of the stools. The appearance, color, and odor of vaginal leucorrhoea were noted. The presence or absence of erythema in the buccal mucosa is the macroscopic element of the oral swab.

2.3.2 Microscopy

A drop of urine was mounted on a Malassez cell and observed under a microscope with a 10x and then 40x objective. Leukocytes, red blood cells, and yeasts were looked for and counted. A drop of feces, buccal swab, or vaginal swab was mounted between the slide and coverslip in physiological water and then observed under the microscope.

2.3.3 Culture

The biological product was deposited on the surface of the Sabouraud chloramphenicol medium and then spread over the entire surface of the agar. The plates were incubated at 37°C for 24 to 48 hours.

2.3.4 Germ tube test

A loop of pure culture was introduced into a tube containing 0.5 ml of sheep serum. The whole was homogenized and incubated at 37°C for 3 hours. The suspension was then observed between the slide and coverslip under a microscope with a 10x and 40x objective.

2.3.5 Chlamydosporulation test

Using a sterile platinum loop, a pure yeast culture was taken and spread on the agar by creating slits in the agar surface. A sterile coverslip was then placed over the slits. The petri dish was then closed and the edges were covered with parafilm paper. The Petri dish was incubated at 28°C for 72 hours. After 72 hours of incubation, readings were taken using a 10x and then a 40x objective.

2.3.6 Chromagar *Candida* medium

The sample was streaked onto the surface of the medium to create isolation. For samples grown from a swab, the swab was gently rolled over a small area of the surface near the edge and then spread from this area. After inoculation, the plates were incubated in an inverted position under aerobic conditions at 37°C for 48 h.

2.3.7 Biochemical identifications using the ID32C gallery

The yeast to be tested is suspended in a semi-solid medium. Readings are taken after 24 to 48 hours of incubation at 30°C. Identification is obtained using identification software. A fungal inoculum is prepared at a concentration corresponding to a density of 2 on the McFarland standard. Transfer 250µl of this inoculum into 7ml of ID32C medium and homogenize. 135µl of the above suspension is added to each well and incubated at 30°C for 24 to 48 hours.

2.4 Identification by Mass Spectrometry

The second identification was performed by MALDI-TOF MS achieving as described by Cassagne et al. (2016) [9]. Briefly, analyses were performed on a MicroflexLT (Bruker Daltonics GmbH, Germany) equipped with a nitrogen laser (337nm). The mass range from 2000 to 20000 Da was recorded by using the linear mode. *C. krusei* ATCC 6258 was used for quality control.

The spectra were compared to the MALDI Biotyper v3.0 software (Bruker Daltonics GmbH) containing the Bruker Daltonics database supplemented with our in-house yeast reference spectra for identification [9].

3. RESULTS

3.1 Patients Characteristics

From October 2018 to December 2020, 804 HIV-infected patients from the two hospitals agreed to participate in the study by signing an informed consent form. At BRH, of the 322 participants recruited, 273 (84.78%) were women and 49 (15.21%) were men. At the YCH, of the 482 participants, 408 (84.64%) were women and 74 (13.35%) were men.

The mean age of the general study population in the two hospitals was 42.39 ± 7.5 years, with extremes ranging from 21 to 81 years. 99.87% (n = 803/804) of patients in the study population were infected with HIV 1-M. 0.12% (n = 1/804) of HCY were coinfected with types 1-M and 1-0.

3.2 Mycological Diagnosis and Yeast Identification

3.2.1 Distribution of mucosal samples by sites sampled for the detection of *Candida* in the study population

A total of 2754 samples (732 stool samples, 617 urine samples, 601 vaginal swabs, and 804 oral swabs) were collected from the entire study population (Table 1). Cases with positive cultures for germs other than *Candida* species were excluded from the study. In the two hospitals, 31.42% (n = 230/732) of positive cultures were found in stools, 16.47% (n = 99/601) in vaginal swabs, 10.69% (n = 66/617) in urine, and 10.32% (83/804) in oral swabs. When considering the collected samples, the frequency of colonization in the study population is 17.35%.

3.2.2 Distribution of *Candida* species isolated within the study population of the 02 hospitals

At BRH, the 5 most represented *Candida* species were in order *Candida albicans* 52.75% (n= 134/254), *Candida parapsilosis* 11.02% (n=28/254), *Candida krusei* 8.66% (n= 22/254), *Candida glabrata* 8.66% (n= 22/254) and *Candida dubliniensis* 7.87% (n=20/254). At the YCH, the 5 most common *Candida* species were *Candida albicans* 45.17% (n= 117/259), *Candida krusei* 19.69% (51/259), *Candida glabrata* 11.19% (29/259), *Candida tropicalis* 8.49% (22/259) and *Candida parapsilosis* 5.40% (14/259). In the 02 hospitals, *Candida albicans* was the germ isolated in the majority of cases, whatever the sample**.** Table 2 shows the sample distribution of *Candida* species isolated and identified within the study population of the 02 hospitals.

Table 3 illustrates the distribution of *Candida* species isolates according to gender, age, sample type, CD4 count, ARV status, and locality. Although *Candida* species were predominantly isolated from stools in the 02 hospital study population, there was no relationship (*P*-value= 0.39) between *Candida* colonization and the different specimens collected. Within the study population of the 02 hospitals, the majority of *Candida* isolates were in the 31-40 age group. There was no relationship (*P*-value= 0.09) between the *Candida spp.* colonisation and age. *Candida* species were predominantly isolated from patients who were not on antiretroviral therapy compared to those who were under antiretroviral therapy. There was a relationship (*P*-value= 0.00) between antiretroviral therapy and *Candida* spp. Colonization.

Table 1. Distribution of mucosal samples by sites sampled in the study population

		Oral swabs	Vaginal swabs	stool	Urine	Total (%)
BRH	Number of collected samples	322	231	279	205	1037
	Number of colonized samples	30	61	118	29	238 (22.95)
YCH	Number of collected samples	482	370	453	412	1717
	Number of colonized samples	53	38	112	37	240 (13.97)

**BRH : Bafoussam Regional Hospital; YCH : Yaounde Central Hospital*

Table 2. Distribution of *Candida* species isolated in the study population of the 02 hospitals

***Candida* species**	**BRH n(%)**	**YCH n(%)**
Candida albicans	134(52.75)	117 (45.17)
Candida parapsilosis	28(11.02)	14 (5.40)
Candida krusei	22(8.66)	51 (19.69)
Candida glabrata	22(8.66)	29 (11.19)
Candida dubliniensis	20(7.87)	0
Candida tropicalis	19(7.48)	22 (8.49)
Candida guilliermondi	7 (2.75)	1(0.38)
Candida catenulata	1(0.39)	0
Candida rugosa	1(0.39)	11(4.24)
Candida kefyr	0	4(1.54)
Candida intermedia	0	1(0.38)
Candida famata	0	1(0.38)
Candida sake	0	3(1.15)
Candida lusitaniae	0	2(0.77)
Candida norvegensis	0	2(0.77)
Candida valida	0	1(0.38)
Total	254 (49.51)	259 (50.48)

**BRH : Bafoussam Regional Hospital; YCH : Yaounde Central Hospital*

Table 3. Distribution of *Candida* species isolates according to gender, age, sample type, CD4 count, ARV status, and hospitals

Parameter	**Category**	***Candida* species (N= 513)**	**Frequency (%)**	**Chi-Square(X^2)**	***p*-value**
Gender	Male	98	19.10	0.057	0.80
	Female	415	80.89		
Age range (years)	21-30	84	16.37		
	31-40	274	53.41		
	41-50	64	12.47	6.428	0.09
	51-60	70	13.64		
	≥61	21	4.09		
Sample Type	Oral swab	70	13.64	4.066	0.39
	Vaginal swab	64	12.47		
	Stool	344	67.05		
	Urine	35	6.82		
CD4 count ($CD4/mm^3$)	<200	84	16.44	1.688	0.68
	200-349	204	39.73		
	350-499	120	23.29		
	≥500	105	20.54		
Antiretroviral therapy status	ARV (+)	14	2.74		
	ARV (-)	499	97.26	14.261	0.00
The locality of sample collection	BRH	254	31.51	7.524	0.11
	YCH	259	68.49		

**BRH: Bafoussam Regional Hospital; YCH: Yaounde Central Hospital; ARV: Antiretroviral therapy*

4. DISCUSSION

HIV infection facilitates various opportunistic infections [10], including fungal infections [11], in particular candidiasis. Candidiasis is the most common opportunistic fungal infection in HIV-infected patients [11]. It is responsible for the high morbidity and mortality rates in these patients [12]. Mucosal candidiasis occurs in healthy individuals under the influence of several favorable factors but is most common in patients with impaired cellular immunity, such as HIV-infected patients [13]. The study of mucosal candidiasis in HIV-infected patients is very often limited to the oropharyngeal mucosa, as this is an important indicator of the state of HIV infection [14-19]. However, the results obtained by this study reveal that all human mucosae can be affected by *Candida* species. This result is similar to that obtained by Ngouana et al. [2] in Cameroon.

Nine and fourteen yeasts of the *Candida* genus were isolated at the BRH and YCH respectively. *C. albicans* was the most species frequently isolated in the 02 hospitals. Whatever the type of sample analyzed, *Candida albicans* was the species isolated in the majority. These results are in agreement with the results of studies carried out on mucosal candidiasis by Esebelahie et al. [11] in Nigeria, Maheshwari et al. [12] in India, and Ngouana et al. [2] in Cameroon.

Although *Candida albicans* remains the most frequently isolated agent in candidiasis studies, non-*albicans Candida* now accounts for a significant proportion of clinical isolates collected worldwide [2,20]. More and more non-*albicans Candida* species are being isolated during studies of candidiasis in people living with HIV worldwide, and the prevalence of species varies from study to study, and from region to region. This increased emergence of non-*albicans Candida* could be attributed to the increase in the number of immunocompromised patients, particularly HIV-infected patients, the use of narrow-spectrum antifungal agents that act essentially only on *Candida albicans* [21], increased resistance of species other *than Candida albicans* to antifungal agents, improvements in diagnostic techniques, such as the use of MALDI-TOF MS and routine diagnosis with molecular techniques [22,23].

Candida species colonization was predominantly observed in HIV-positive patients not receiving antiretroviral therapy compared with patients receiving antiretroviral therapy. In fact, *Candida* species colonizing mucosae is common in HIV-positive patients who are naive to antiretroviral therapy [19]. Furthermore, there has been a significant decrease in the incidence of *Candida* spp. colonization in HIV-infected patients since the discovery of antiretroviral therapy [24]. The intestinal tract was the most colonized site. This result is in agreement with that obtained by a previous study conducted in Cameroon on patients living with HIV [2].

Candida spp. colonization in the study population was predominantly observed in female patients compared to male patients. This result is similar to that obtained in South Korea by Yoon et al. [25], Chile by Cruz et al. [26], and Cameroon by Ngouana [27]. This difference may be explained by differences in activities, personal hygiene, and exposure to contamination between the female and male populations [28,29].

Our results showed that *Candida* spp*.* was most frequently isolated from stool samples in HIV-positive patients. This result is similar to that obtained by Ngouana et al. [2] in Cameroon. *Candida* spp. were also predominantly isolated from buccal swabs. This result is similar to that obtained in Cameroon by Ngouana et al. [2] and Ngwa et al. [19]. *Candida* spp isolated from oral swabs is responsible for oral candidiasis which is one of the most common fungal opportunistic infections in immunocompromised individuals. Oral candidiasis occurs in up to 95% of the human immunodeficiency virus (HIV)-infected individuals during their illness and is a prognostic indicator for acquired immune deficiency syndrome (AIDS) [30].

5. CONCLUSION

A total of 513 *Candida* species were isolated. The frequency of colonization in the study population was 17.35%. *Candida albicans* was the most common species isolated. *Candida krusei* was the most frequently isolated NAC species.

CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study involved HIV-infected patients presenting or not presenting clinical signs of any mucosal candidiasis and was approved by the Cameroonian National Ethical Committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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