



HAL
open science

Epidemiology and antifungal susceptibility testing of non-albicans *Candida* species colonizing mucosae of HIV-infected patients in Yaoundé (Cameroon)

Thierry Kammalac Ngouana, Rufin Kuipou Toghueo, I.F. Kenfack, Laurence Lachaud, A.K. Nana, L. Tadjou, C. Kouanfack, F.F. Boyom, Sébastien Bertout

► To cite this version:

Thierry Kammalac Ngouana, Rufin Kuipou Toghueo, I.F. Kenfack, Laurence Lachaud, A.K. Nana, et al.. Epidemiology and antifungal susceptibility testing of non-albicans *Candida* species colonizing mucosae of HIV-infected patients in Yaoundé (Cameroon). *Journal of Medical Mycology = Journal de Mycologie Médicale*, 2019, 29 (3), pp.233-238. 10.1016/j.mycmed.2019.06.003 . hal-02648973

HAL Id: hal-02648973

<https://hal.umontpellier.fr/hal-02648973v1>

Submitted on 20 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Epidemiology and antifungal susceptibility testing of non-*albicans* *Candida* species colonizing mucosae of HIV-infected patients in Yaoundé (Cameroon)

^{1,2,3*}Thierry Kammalac Ngouana, ³Rufin Marie Kuipou Toghueo, ³Ide Flavie Kenfack, ⁴Laurence Lachaud, ¹Adrien Kombou Nana, ¹Lorentz Tadjou, ⁵Charles Kouanfack, ³Fabrice Fekam Boyom, ²Sébastien Bertout.

¹Unité de Recherche Biomédicale, Laboratoire Sion, Yaoundé, Cameroun

²IRD UMI 233 TransVIHMI - UM INSERM U1175 « TransVIHMI » Laboratoire de Parasitologie et Mycologie Médicale, UFR Pharmacie, Université Montpellier, France

³Antimicrobial and Biocontrol Agents Unit (AMBAU), Laboratory for Phytobiochemistry and Medicinal Plants Studies, Department of Biochemistry, Faculty of Science, University of Yaoundé I, Cameroon

⁴Département de parasitologie-mycologie, faculté de médecine de Montpellier-Nîmes, Université Montpellier, centre hospitalier universitaire de Montpellier, site Antonin-Balmès/La Colombière, 39, avenue Charles-Flahault, 34095 Montpellier cedex 5, France

⁵Day hospital, Yaoundé Central Hospital, Cameroon

*Corresponding author

Mailing address : Laboratoire de Parasitologie et Mycologie Médicale

UFR de Pharmacie, 15 Avenue Charles Flahault, 34093 Montpellier Cedex 05, France

Phone: +33 4 67 66 81 31.

Email: ngouanathi@yahoo.com

Abstract

Non-*albicans Candida* (NAC) species have emerged as potent pathogenic yeasts among HIV-infected patients. Authors evaluated the epidemiology and antifungal susceptibility testing of non-*albicans Candida* species colonizing Yaoundé (capital of the Republic of Cameroon, Central Africa) HIV-infected patients.

The mucosal specimens were collected and submitted to the mycological diagnosis. Yeast isolates were identified by the Matrix Assisted Laser Desorption Ionisation - Time of Flight Mass Spectrometry (MALDI-TOF MS). The antifungal susceptibility testing was achieved by the CLSI-M27 protocols, and the interpretation of clinical break points (CBPs) and epidemiological cutoff values were in accordance with the CLSI-M60 and M59 recommendations.

402 patients were recruited and 1218 samples collected. The colonisation frequency was 24.1 % and 304 yeasts isolated. Yeast isolates were 113 (37.2%) *C. albicans*, 2 (0.7 %) *C. africana* and 172 (56.6 %) NAC isolates. The NAC isolates were grouped into 13 species including *C. krusei* (18.1 %), *C. glabrata* (10.9 %), *C. tropicalis* (8.5 %) and *C. parapsilosis* (5.9 %) as the major ones. All the isolates appeared to be wild-type for amphotericin B and itraconazole. One (1/33) isolate of *C. glabrata* was resistant to fluconazole. *C. parapsilosis* isolates appeared all susceptible to fluconazole. *C. tropicalis* isolates presented 50 % (13/26) resistance to fluconazole.

The achieved results bring out new insights about epidemiology of NAC species in Cameroon. The results also highlight the resistance of NAC species to current antifungal drugs.

Key words: Non-*albicans Candida* species, MALDI-TOF, Antifungal, HIV-patients, Cameroon.

1. Introduction

Yeast infections caused by *Candida* species are the most frequent opportunistic infections among HIV infected patients and extend from superficial to systemic [1-4]. Although *Candida albicans* remains the most frequently isolated agent from candidiasis, non-*albicans Candida* (NAC) species now account for a substantial part of clinical isolates collected worldwide [1,5,6]. The most current species include *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei* [7,8]. The less-frequent are *Candida guilliermondii*, *Candida lusitaniae*, *Candida kefyr*, *Candida famata*, *Candida inconspicua*, *Candida rugosa* and *Candida norvegensis* [7-9]. The frequency is highly region-dependent. *C. glabrata* is more frequent in North America than in Latin America, *C. tropicalis* is frequently isolated in Asia-Pacific and less in the rest of the world, whilst *C. parapsilosis* remains 3-fold more commonly recovered in North America than in Europe. *C. guilliermondii* and *C. rugosa* are more frequent in Latin America, and *C. inconspicua* and *C. norvegensis* in Europe than in the rest of the world [1,8,9].

Although biochemical profile-based and time-consuming microscopy assisted methods have dominated mycology diagnostic laboratories for many years, proper species identification is sometimes hindered by several factors, such as complex taxonomy, genetic relatedness of species, and misleading microscopic evidences. In the past few years, the MALDI-TOF mass spectrometry has revolutionized the medical microbiology [8], enabling rapid and accurate fungal species identification (within few minutes) with simple and reliable procedures [10]. Since its first application to yeast identification [11], there has been a growing body of

evidence indicating that microbial fingerprint by MALDI-TOF can represent a robust and fast tool for routine identification of clinically important fungi [12–20].

The treatment of candidiasis due to NAC species is currently done with antifungal agents belonging to three families including polyenes, azoles, and echinocandins [21]. Nowadays, most of the NAC species exhibit particular patterns of primary resistance or reduced susceptibility toward these antifungals. In fact, a high level of resistance against azoles was observed for *C. krusei*, *C. inconspicua*, *C. rugosa*, and *C. norvegensis* [21]. In addition, a decreased susceptibility of *C. glabrata*, *C. parapsilosis* and *C. guilliermondii* to echinocandins was observed [22]. In a recent study by Castanheira et al. involving 3,557 invasive yeasts and moulds collected in 29 countries worldwide during 2014-2015, Epidemiological cutoff values (ECVs) published in the CLSI M59 document were applied for species with no clinical breakpoints. Their results stated that echinocandins susceptibility rates were 95.9 % to 100 % for the 5 most common *Candida* species except anidulafungin and *C. parapsilosis* (88.7 % susceptible/100 % wild-type). Fluconazole resistance was 8 % for *C. glabrata*. Azole resistance among *C. tropicalis* and *C. parapsilosis* and echinocandin resistance in *C. krusei* was higher in Denmark compared to other regions [23].

For a global view of *Candida* distribution and their antifungal susceptibility profile, almost all the countries should contribute by publishing their local statements. However, such information about isolates from sub-Saharan region, especially in Cameroon is scarce. Authors investigated the NAC species distribution isolated from Yaoundé HIV infected patients by MALDI-TOF MS as well as the antifungal susceptibility of these isolates to some antifungal drugs.

2. Materials and methods

2.1. Ethical considerations and enrollment of participants

The survey was carried out at the Yaoundé Central Hospital (capital of the Republic of Cameroon, Central Africa), and involved HIV-infected patients presenting or not clinical signs of any mucosal candidiasis. This study was approved by the Cameroonian National Ethical Committee (N°128/CNE/CNM/2011). The survey took place from January 2012 to October 2013. Patients enrolled for this study were HIV-infected individuals of both genders, between 18 and 66 years old, who did not receive any antifungal treatment during the last 3 months preceding their enrollment. The purpose of the study and potential benefits were explained to patients, and those willing to participate were required to sign a written informed consent form prior to their registration as participant.

2.2. Determination of 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, antiretroviral therapy). Samples collected included: Vaginal discharge, oropharyngeal swab, 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. Colonization was identified as described by Bouchara et al. [24] in urine (culture $\geq 10^4$ cfu/ml), in vaginal collections (culture ≥ 10 colonies), in oropharyngeal mucosae (culture ≥ 5 -10 colonies/cm²) and in stool (culture $\geq 10^4$ cfu/g of stool).

Primary identification was assessed by combination of culture on chromogenic medium (ChromID CAN2 from Biomerieux, Marcy l'Etoile, France), germ tube test, evidence of

chlamydospores production and biochemical analysis (ID32C kit from Biomerieux, Marcy l'Etoile, France). Reference *C. albicans* ATCC90028 strain was used throughout the mycological diagnosis as quality control strain.

2.4. Identification by Mass Spectrometry

The second identification of yeasts isolates by the MALDI-TOF was achieved as described by the manufacturer of the Vitek MS (Biomerieux, Marcy l'Etoile, France). Briefly, yeast cells were grown on Sabouraud dextrose agar medium plates for 24h, at 37°C. A loopful (1µL) of yeast cells was directly transferred from the culture medium onto each position of the 48-well flex target plate, and 0.5µL of 25 % formic acid was immediately mixed with the yeast. After evaporation, 0.5µL matrix solution (75mg/mL 2,5-dihydroxybenzoic acid in ethanol/water/acetonitrile [1:1:1] with 0.03 % trifluoroacetic acid) was added and gently mixed. All sample mixtures were air dried at room temperature. Each isolate was spotted in duplicate. Analyses were performed on a Vitek MS (Biomerieux, Marcy l'Etoile, France) equipped with a nitrogen laser (337nm). The mass range from 2,000 to 20,000 Da was recorded by using the linear mode. An *Escherichia coli* ATCC 8739 strain was used for external calibration of the spectra. *C. albicans* ATCC 90028, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used as quality control strains. Spectra were exported to the SARAMIS™ software package where the final identifications were achieved. Cluster analysis of the MALDI-TOF ICMS mass spectral data was performed using the SARAMIS™ database by comparing database peak lists of individual samples with Super Spectra and/or reference spectra.

2.5. Antifungal susceptibility testing

The antifungal susceptibility testing was assessed as described by the CLSI M27-S4 protocol [25]. Amphotericin B, fluconazole, and itraconazole provided by Sigma-Aldrich (USA) were serially diluted in a 96 wells microtiter plate with RPMI 1640 (Sigma Aldrich) broth medium. Final range concentrations were as follow: Amphotericin B (0.0313-16 μ g/mL), fluconazole (0.125-64 μ g/mL), and itraconazole (0.0313-16 μ g/mL). A standardized fungal inoculum (spectrophotometrically calibrated) was then added into each well to yield 0.5 to 2.5x10³CFU/mL in 200 μ L as final volume. Plates were incubated at 37°C for 24 hours and results read afterwards. *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used as quality control strains.

3. Results

3.1. Patients characteristics

From January 2012 to October 2013, 402 patients were included in the study. They were 306 (76.1 %) females and 96 (23.9 %) males. The mean age was 40.2 \pm 9.5 years with extremes at 23 and 66 years. Only one patient was infected by two types of HIV strains (HIV 1-M and HIV 1-O), the others were all infected by the HIV 1-M strain. Among the 402 included patients, 250 (62.1 %) were colonized by yeasts on at least one of the collected sites. Non-*Candida* isolates were obtained from 16 patients and *Candida* species isolated from 232 (57.6 %) patients. The mean body mass index (BMI) of patients colonised by *Candida* was 25.0 \pm 5.2. The mean CD4+ count among patients colonised by *Candida* species was 371 \pm 222 CD4/mm³ with extremes at 34 and 1127 CD4/mm³. There was no relationship between CD4 counts and *Candida* colonisation (p-value >0.05), results are presented in table 2.

3.2. Mycological diagnosis and yeasts identification

Candida colonisation was observed in 88.9 % of HIV patients naïve to antiretroviral therapy and was also observed in 59. % of patients on antiretroviral therapy. Statistical analysis showed that there is no relationship between *Candida* colonisation and antiretroviral therapy (p-value >0.05).

From the 402 recruited patients, 1218 samples were collected and analysed, including 322 oropharyngeal swabs, 262 vaginal swabs, 308 stools and 326 urine samples. The table 3 contains results of colonisation at each collected site. When considering collected samples, the frequency of colonisation is 24.1 %. The intestinal tract was the most colonized site, followed by buccal mucosae.

The use of identification tools allowed identification of 304 yeast isolates, from which 113 (37.2 %) *C. albicans*, 2 (0.7 %) *C. africana*, 172 (56.6 %) NAC isolates, 15 (4.9 %) non *Candida* yeasts species, and 2 (0.7 %) non identified yeasts as presented in table 4. The most important NAC species were *C. krusei* (18.1 %), *C. glabrata* (10.9 %), *C. tropicalis* (8.5 %) and *C. parapsilosis* (5.9 %). *C. krusei* and *C. parapsilosis* were mostly identified in stools. *C. glabrata* was important in vaginal collections and *C. tropicalis* had a high frequency in urine samples. There were patients with two or more species in a particular sample. *C. albicans* was associated with *C. krusei* (40 %), *C. glabrata* (13.3 %), *C. parapsilosis* (6.7 %) and *C. rugosa* (6.7 %).

3.3. Antifungal susceptibility testing

The MIC results were interpreted according to the updated clinical breakpoints (CBPs) recommended by the Clinical and Laboratory Standards Institute [26] or epidemiological cut-off values (ECVs) [27-30] as presented in table 1.

All the NAC isolates appeared to be wild-type for amphotericin B and itraconazole, where ECVs have been established. CBPs for fluconazole have been published only for *C. glabrata*, *C. parapsilosis* and *C. tropicalis* amongst NAC species [31]. Isolates of *C. krusei* are assumed to be intrinsically resistant to fluconazole [25]. One (1/33; 3.0 %) isolate of *C. glabrata* was resistant to fluconazole. *C. parapsilosis* isolates appeared all susceptible to fluconazole. *C. tropicalis* isolates presented 50 % (13/26) resistances to fluconazole (table 5).

4. Discussion

The very nature of infectious diseases has undergone profound changes in the past few decades. Hitherto unknown microorganisms with no pathogenic role have emerged as important causes of morbidity and mortality worldwide. In recent years, *Candida* spp. have emerged as principal pathogens of a variety of human infections [28-31]. In the present study, data for the population characteristics are close to those published for HIV infected patients in sub-Saharan region in general and particularly in Cameroon [32-38].

The study of mucosal *Candida* infection among HIV infected patients is mostly limited to oropharyngeal location as it is a major indicator of the AIDS state [32-38]. However, all the human mucosae can be affected by *Candida* species. We found that the intestinal mucosa is more affected than others (41.5 %) and this result was different from findings by Khan et al. [39] who obtained 5 % of gastro-intestinal colonization in their study. These later also obtained 65 % of oral colonization [39] whilst we found 28.6 % of oro-pharyngeal colonization in our study. Merenstein et al. [33] obtained 18.8 % of vaginal colonization among HIV infected patients, which is not far from our own findings (14.5 %). Mukasa et al [40] obtained 45.4 % of vaginal candidiasis in Uganda. Another study in Ethiopia stated 41 % of vaginal candidiasis in their study population [41]. In a recent study in South Africa, authors

obtained following features; candiduria 46.5 %, vaginal colonization 30.6 %, oral colonization 11 % [42]. In Cameroon, Lohoue et al. [43] identified candiduria in 36.2 % of HIV infected patients. A team in Buea found 18.2 % of *Candida* urinary tract colonization among 207 HIV positive patients [44].

From the 1218 analyzed samples, we identified 304 yeast isolates. NAC species represented 56.7 % of *Candida* isolates with one of the most important being *C. krusei* (18.1 %). This is of particular interest since it is rare to observe *C. krusei* at a percentage in humans. A study in South America established the presence of *C. krusei* among 30 % of the population [45]. Of the total 132 neonates included in a study by Juyal et al. [46], NAC species were responsible for 80.3 % candidemia cases with *C. parapsilosis* (25.0 %) and *C. tropicalis* (22 %) as the most predominant species. Mohandas and Ballal [47] when studying a total of 111 isolates of *Candida* species found NAC species with 60.4 %; they also observed *C. krusei* (50.0 %) being commonly isolated in urinary tract of hospitalized patients, followed by *C. albicans* (25.0 %). When evaluating the prevalence of *Candida* species among patients with vulvo-vaginal candidiasis, Kumari et al. [48] obtained 32.4% *C. albicans*, 45.1 % *C. parapsilosis* and 22.5 % of *C. glabrata*. Another study obtained 41.4 % of NAC species from vaginal isolates [41]. Although *C. albicans* is the most prevalent species involved in fungal infections, the incidence of infections due to NAC species is increasing, as we observed in the present work. This changing in epidemiology could be associated with severe immunosuppression or illness, exposure to broad-spectrum antibiotics and intensive use of azoles that are less effective against NAC species [49,50].

The Clinical and Laboratory Standards Institute (CLSI) developed new *Candida* species-specific clinical breakpoints (CBPs) for fluconazole, voriconazole, and echinocandins [25,51].

A recent report indicated that resistance to azoles and echinocandins of *Candida* species may be increased using the new CLSI CBPs [52,53]. Therefore, the new CBPs of the CLSI may be applied to antifungal susceptibility studies as sensitive tools for detecting emerging resistance in *Candida* isolates [25]. We applied new species-specific CLSI CBPs associated to epidemiological cut-off values to determine the antifungal susceptibility of *Candida* species isolates in Cameroon.

All the isolates appeared to be wild-type for amphotericin B. This is in accordance with previous studies [53,54]. Till date, very few species have developed reduced susceptibility to amphotericin B [55,23]. Khotari et al. [56] from North India reported the susceptibility profile of *Candida* isolates as 92% were sensitive to amphotericin B, 36 % to fluconazole, and 24 % to itraconazole. *C. tropicalis* isolates presented 50 % (13/26) resistance to fluconazole. Xiao et al. [57] obtained 11.2 % of resistance by *C. tropicalis* isolates to fluconazole in China. We obtained 3.0 % resistance to fluconazole by *C. glabrata*, while Castanheira et al. [23] observed 8.0 %.

In the present study, we established the *Candida* species distribution amongst Yaoundé HIV infected patients. The achieved results bring out new insights about epidemiology of *Candida* in Cameroon as well as their antifungal susceptibility profiles. The susceptibility profiles of those isolates to other antifungal drugs need to be carried out to give a global idea about the behavior of these isolates to antifungal drugs.

5. Acknowledgment

We thank Dominique Castel for its technical help.

6. Conflict of interest

No conflict of interest to be mentioned

7. References

1. Papon N, Courdavault V, Clastre M and Bennett RJ. Emerging and Emerged Pathogenic *Candida* Species: Beyond the *Candida albicans* Paradigm. *PLoS Pathog.* 2013; 9(9): e1003550, PMID: PMC3784480.
2. Esebelahie NO, Enweani IB, Omoregie R. *Candida* colonisation in asymptomatic HIV patients attending a tertiary hospital in Benin City, Nigeria. *Libyan J Med.* 2013; 8:20322. doi: 10.3402/ljm.v8i0.20322.
3. Yongabi KA, Mbacham WF, Nubia KK, and Singh RM. Yeast strains isolated from HIV-seropositive patients in Cameroon and their sensibility to extract of eight medicinal plants. *Af J Microbial Res.* 2009; 3(4): 133-136.
4. Oliveira P, Mascarenhas R, Lacroix C, et al. *Candida* species isolated from the vaginal mucosa of HIV infected women in Salvador, Bahia, Brazil. *Braz J Infect Dis.* 2011; 15 (3):2239-2244.
5. Newton O, Esebelahie, Ifeoma B, Enweani, Omoregie R. *Candida* colonisation in asymptomatic HIV patients attending a tertiary hospital in Benin City, Nigeria. *Libyan J Med.* 2013; 8: 20322 doi.org/10.3402/ljm.v8i0.20322.
6. Krcmery V, Barnes AJ. Non-*albicans Candida* spp. causing fungaemia: pathogenicity and antifungal resistance. *J Hosp Infect.* 2002; 50: 243–260, doi:10.1053/jhin.2001.1151.

7. Silva S, Negri M, Mariana Henriques M, *et al.* *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiol Rev.* 2012; 36: 288–305.
8. Pfaller MA, Diekema DJ, Gibbs DL, *et al.* Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of *Candida* Species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. *J Clin Microbiol.* 2010, 48: 1366–1377, doi:10.1128/JCM.02117-09.
9. Pfaller MA, Moet GJ, Messer SA, Jones RN, Castanheira M. Geographic variations in species distribution and echinocandin and azole antifungal resistance rates among *Candida* bloodstream infection isolates: Report from the SENTRY Antimicrobial Surveillance Program (2008 to 2009). *J Clin Microbiol.* 2011 ; 49:396 -399
10. Croxatto A, Prod'hom G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *FEMS Microbiol. Rev.* 2012, 36: 380–407.
11. Amiri-Eliasi B, Fenselau C. Characterization of protein biomarkers desorbed by MALDI from whole fungal cells. *Anal Chem.* 2001, 73: 5228–5231.
12. Bader O. MALDI-TOF-MS-based species identification and typing approaches in medical mycology. *Proteomics.* 2013, 13: 788–799.
13. Marklein G, Josten M, Klanke U *et al.* Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. *J Clin Microbiol.* 2009, 47: 2912–2917.
14. Becker PT, de Bel A, Martiny D *et al.* Identification of filamentous fungi isolates by MALDI-TOF mass spectrometry: Clinical evaluation of an extended reference spectra library. *Med Mycol.* 2014, 52: 826–834.

15. Chen JH, Yam WC, Ngan AH et al. Advantages of using matrix-assisted laser desorption ionization-time of flight mass spectrometry as a rapid diagnostic tool for identification of yeasts and mycobacteria in the clinical microbiological laboratory. *J Clin Microbiol.* 2013, 51: 3981–3987.
16. De Almeida JN, Figueiredo DS, Toubas D et al. Usefulness of matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry for identifying clinical *Trichosporon* isolates. *Clin Microbiol Infect.* 2014, 20: 784–790.
17. De Carolis E, Vella A, Vaccaro L et al. Application of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *J Infect Dev Ctries.* 2014, 8: 1081–1088.
18. Posteraro B, Vella A, Cogliati M et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based method for discrimination between molecular types of *Cryptococcus neoformans* and *Cryptococcus gattii*. *J Clin Microbiol.* 2012, 50: 2472–2476.
19. Triest D, Stubbe D, de Cremer K et al. Use of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of molds of the *Fusarium* genus. *J Clin Microbiol.* 2015, 53: 465–476.
20. Cassagne C, Ranque S, Normand AC et al. Mould routine identification in the clinical laboratory by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *PLoS ONE.* 2011, 6:e28425.
21. Pfaller MA, Espinel-Ingroff A, Canton E, et al. Wild-type MIC distributions and epidemiological cutoff values for amphotericin B, flucytosine, and itraconazole and *Candida* spp. as determined by CLSI broth microdilution. *J Clin Microbiol.* 2012; 50:2040–2046.

22. Walker LA, Gow NA, Munro CA. Elevated chitin content reduces the susceptibility of *Candida* species to caspofungin. *Antimicrob Agents Chemother*. 2013; 57: 146-154, doi:10.1128/AAC.01486-12.
23. Castanheira M. Fungemia surveillance in denmark demonstrates emergence of *Candida non-albicans*, higher antifungal usage and resistance rates when compared to other nations. *J Clin Microbiol*. 2018; doi:10.1128/JCM.01907-17.
24. Bouchara JP, Pihet M, de Gentile L et al. *Les levures et les levuroses*, Cahiers de formation Biologie médicale, N°44, imprimerie vert, Paris France, 2010.
25. Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts; fourth informational supplement. Wayne: Clinical and Laboratory Standards Institute. 2012 (Document M27-S4)
26. CLSI (Clinical and Laboratory Standard Institute). *Performance standards for Antifungal Susceptibility Testing of Yeasts*. 1st edition M60. Wayne, P A, 2017.
27. CLSI (Clinical and Laboratory Standard Institute). Epidemiological cutoff values for antifungal susceptibility testing. 2nd ed. CLSI supplement M59. Wayne, P A, 2018
28. Pfaller MA, Andes D, Arendrup MC et al. Clinical breakpoints for voriconazole and *Candida* spp. revisited: review of microbiologic, molecular, pharmacodynamic, and clinical data as they pertain to the development of species-specific interpretive criteria. *Diagn Microbiol Infect Dis*. 2011; 70:330–343.
29. Pfaller MA, Boyken L, Hollis RJ et al. Wild-type MIC distributions and epidemiological cutoff values for posaconazole and voriconazole and *Candida* spp. as determined by 24-hour CLSI broth microdilution. *J Clin Microbiol*. 2011; 49:630–637.
30. Pfaller MA, Castanheira M, Diekema DJ et al. Triazole and echinocandin MIC distributions with epidemiological cutoff values for differentiation of wild-type strains

- from non-wild-type strains of six uncommon species of *Candida*. *J Clin Microbiol*. 2011; 49:3800–3804.
31. Deorukhkar SC, Saini S, Mathew S. Non-*albicans* *Candida* Infection: An Emerging Threat. *Interdiscip Perspect Infect Dis*. 2014; 2014: 615958. doi: 10.1155/2014/615958.
 32. Lortholary O, Petrikkos G Akova M et al. ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: patients with HIV infection or AIDS. *Clin Microbiol Infect*. 2012; 18 (7): 68–77.
 33. Merenstein D, Hu H, Wang C, et al. Colonization by *Candida* Species of the Oral and Vaginal Mucosa in HIV-Infected and Non-infected Women. *Aids Res Hum Retrovir*. 2013; 29(1). Doi: 10.1089/aid.2012.0269.
 34. ONUSIDA. Diapositives clés sur les données épidémiologiques. 2012; www.unaids.org.
 35. ONUSIDA. Rapport mondial: Rapport ONUSIDA sur l'épidémie mondiale de sida. 2013; ISBN 978-92-92503-033-4.
 36. Kalpanadevi V, Geethalakshmi S and Sumathi G. A study on speciation and antifungal susceptibility pattern of *Candida* isolates from HIV patients with oropharyngeal candidiasis and correlation with CD4 count. *BMC Infect Dis*. 2012; 12(1): P19.
 37. Vazquez JA. Optimal management of oropharyngeal and oesophageal candidiasis in patients living with HIV infection HIV/AIDS. *Res Pal Care*. 2010; 2: 89–101
 38. Yang Y-L, Hung C-C, Wang A-H, et al. Oropharyngeal Colonization of HIV-Infected Outpatients in Taiwan by Yeast Pathogens. *J Clin Microbiol*. 2010; 48(7): 2609–2612.
 39. Khan AP, Malik A and Subhan Khan H. Profile of candidiasis in HIV infected patients. *Iran J Microbiol*. 2010; 4 (4): 204-209.

40. Mukasa KJ, Herbert I, Daniel A et al. Antifungal Susceptibility Patterns of Vulvovaginal *Candida* species among Women Attending Antenatal Clinic at Mbarara Regional Referral Hospital, South Western Uganda. *Br Microbiol Res J*. 2015; 5(4):322-331.
41. Bitew A, Abebaw Y. Vulvovaginal candidiasis: species distribution of *Candida* and their antifungal susceptibility pattern. *BMC Womens Health*. 2018;18(1):94. doi: 10.1186/s12905-018-0607-z.
42. Mnge P, Okeleye B I, Vasaikar S D, Apalata T. Species distribution and antifungal susceptibility patterns of *Candida* isolates from a public tertiary teaching hospital in the Eastern Cape Province, South Africa. *Braz J Med Biol Res*. 2017; 50(6): e5797 doi: 10.1590/1414-431X20175797.
43. Lohoué JP, Angwafo FF, Kechia FA, Noukeu ND. Candiduria in HIV Infected Patients in Yaoundé, Cameroon. *Af J Urol*. 2005; 11(1): 61-65.
44. Longdoh AN, Assob CN, Nsagha SD, et al. Oral and Urinary Colonisation of *Candida* Species in HIV/AIDS Patients in Cameroon. *B Sci Med*. 2013; 2(1):1-8. doi: 10.5923/j.medicine.20130201.01.
45. Angebault C, Djossou F, Abélanet S, et al. *Candida albicans* is not always the preferential yeast colonizing humans: A Study in Wayampi Amerindians. *J Infect Dis*. 2013; 208(10): 1705–1716. <https://doi.org/10.1093/infdis/jit389>.
46. Juyal D, Sharma M, Pal S, Rathaur VK and Sharma N. Emergence of Non-*albicans* *Candida* Species in Neonatal Candidemia. *N Am J Med Sci*. 2013; 5(9): 541–545. doi: 10.4103/1947-2714.118919.

47. Mohandas V, Ballal M. Distribution of *Candida* Species in different clinical samples and their virulence: Biofilm formation, proteinase and phospholipase production: A study on hospitalized patients in Southern India. *J Global Infect Dis.* 2011; 3:4-8.
48. Kumari V, Banerjee T, Kumar P, Pandey S, Tilak R. Emergence of non-*albicans Candida* among candidal vulvo-vaginitis cases and study of their potential virulence factors, from a tertiary care center, North India. *Indian J Pathol Microbiol.* 2013; 56:144-147.
49. Pereira GH, Müller PR, Szeszs MW et al. Five-year evaluation of bloodstream yeast infections in a tertiary hospital: the predominance of non-*C. albicans Candida* species. *Med Mycol.* 2010; 48:839–842.
50. Sardi JCO, Scorzoni L, Bernardi T et al. *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *J Med Microbiol.* 2013; 62: 10-24, doi: 10.1099/jmm.0.045054-0.
51. Pfaller MA, Diekema DJ. Progress in antifungal susceptibility testing of *Candida* spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. *J Clin Microbiol.* 2012; 50: 2846–2856. doi: 10.1128/JCM.00937-12.
52. Fothergill AW, Sutton DA, McCarthy DI, Wiederhold NP. Impact of new antifungal breakpoints on antifungal resistance in *Candida* species. *J Clin Microbiol.* 2014; 52: 994–997. doi: 10.1128/JCM.03044-13.
53. Morris A J, Rogers K, McKinney W P et al. Antifungal susceptibility testing results of New Zealand yeast isolates, 2001–2015: Impact of recent CLSI breakpoints and epidemiological cut-off values for *Candida* and other yeast species. *J Glob Antimicrob Resist.* 2018; 14:72-77.
54. Lockhart SR, Iqbal N, Cleveland AA, et al. Species Identification and Antifungal Susceptibility Testing of *Candida* Bloodstream Isolates from Population-Based

Surveillance Studies in Two U.S. Cities from 2008 to 2011. *J Clin Microbiol.* 2012; 50(11):3435-3442. doi: 10.1128/JCM.01283-12.

55. Pahwa N, Kumar R, Nirkhivale S, Bandi A. Species distribution and drug susceptibility of *Candida* in clinical isolates from a tertiary care centre at Indore. *Indian J Med Microbiol.* 2014;32:44-48
56. Kothari A, Sagar V. Epidemiology of *Candida* bloodstream infections in a tertiary care institute in India. *Indian J Med Microbiol.* 2008; 27:171-172.
57. Xiao M, Fan X, Chen SC, et al. Antifungal susceptibilities of *Candida glabrata* species complex, *Candida krusei*, *Candida parapsilosis* species complex and *Candida tropicalis* causing invasive candidiasis in China: 3 year national surveillance. *J Antimicrob Chemother.* 2015; 70(3):802-810. doi: 10.1093/jac/dku460.

Table 1: Epidemiological cut-off values and clinical breakpoints for antifungal agents and non-*Candida albicans* *Candida* species determined by 24 hours CLSI broth microdilution method [25-30]

Organism	Antifungals	CBPs ($\mu\text{g/mL}$)				ECVs ($\mu\text{g/mL}$)	
		S	SDD	I	R	WT	non-WT
<i>C. glabrata</i>	Fluconazole		≤ 32		> 32	≤ 32	> 32
	Itraconazole					≤ 4	> 4
	Amphotericin B					≤ 2	> 2
<i>C. parapsilosis</i>	Fluconazole	≤ 2	4		≥ 8	≤ 2	> 2
	Itraconazole					≤ 0.5	> 0.5
	Amphotericin B					≤ 2	> 2
<i>C. tropicalis</i>	Fluconazole	≤ 2	4		≥ 8	≤ 2	> 2
	Itraconazole					≤ 0.5	> 0.5
	Amphotericin B					≤ 2	> 2
<i>C. krusei</i>	Fluconazole					≤ 64	> 64
	Itraconazole					≤ 1	> 1
	Amphotericin B					≤ 2	> 2
<i>C. lusitaniae</i>	Fluconazole					≤ 2	> 2
	Itraconazole					≤ 1	> 1
	Amphotericin B					≤ 2	> 2
<i>C. guilliermondii</i>	Fluconazole					≤ 8	> 8
	Itraconazole					≤ 1	> 1
	Amphotericin B					≤ 2	> 2
<i>C. kefyr</i>	Fluconazole					≤ 1	> 1
	Itraconazole					NP	NP

Amphotericin B

NP

NP

ECVs, epidemiological cut-off values; CBPs, clinical breakpoints; WT, wild type; non-WT, non-wild type; S, susceptible; SDD, susceptible, dose dependent; I, intermediate; R, resistant; NP, non-published.

Table 2: Distribution of *Candida* colonisation according to the CD4+ count as defined by the WHO classification [35].

	CD4+ count (/mm ³)			
	<200 (severe ID)	200-349 (advanced ID)	350-499 (moderated ID)	≥500 (no ID)
<i>Candida</i> + (%)	53.8	65.8	65.6	57.5
<i>Candida</i> - (%)	46.2	34.2	34.4	42.5

ID = immuno-depression; *Candida* +, positive to colonisation by *Candida*; *Candida*-, negative to *Candida* colonisation.

Table 3: Distribution of *Candida* colonisation in function of collected site.

	Mouth	Vagina	Stool	Urine	Total
Number of collected samples	322	262	308	326	1218
Number of colonised samples	92	38	128	36	294
Frequency of colonisation (%)	28.56	14.5	41.5	11.0	24.1

Table 4: Distribution of isolates at different collection sites

Species	Collection site					Percentage (%)
	Mouth	Vagina	Stool	Urine	Total	
<i>C. africana</i>	0	2	0	0	2	0.7
<i>C. albicans</i>	44	22	40	7	113	37.2
<i>C. famata</i>	0	0	1	0	1	0.3
<i>C. glabrata</i>	3	10	14	6	33	10.9
<i>C. guilliermondii</i>	1	0	0	0	1	0.3
<i>C. intermedia</i>	0	0	0	2	2	0.7
<i>C. kefyr</i>	2	2	2	2	8	2.6
<i>C. krusei</i>	7	7	37	4	55	18.1
<i>C. lusitaniae</i>	0	0	3	0	3	1.0
<i>C. norvegensis</i>	0	0	3	0	3	1.0
<i>C. parapsilosis</i>	5	2	8	3	18	5.9
<i>C. rugosa</i>	3	0	9	2	14	4.6
<i>C. sake</i>	4	0	0	2	6	2.0
<i>C. tropicalis</i>	3	7	9	7	26	8.6
<i>C. valida</i>	0	0	2	0	2	0.7
<i>Cryptococcus sp.</i>	0	0	2	0	2	0.7
<i>Kodamaea</i>						0.3
<i>ohmeri</i>	1	0	0	0	1	
<i>Debaryomyces</i>	0	0	2	0	2	0.7

<i>etchelsii</i>						
<i>S. cerevisiae</i>	0	0	4	0	4	1.3
<i>Trichosporon</i>						0.7
<i>asahii</i>	0	0	2	0	2	
<i>Trichosporon</i>						1.3
<i>inkin</i>	0	0	3	1	4	
non identified	0	0	2	0	2	0.7
Total					304	100.0

Table 5: *In vitro* activities of antifungal agents against non-*albicans* *Candida* isolates collected from mucosae of HIV infected patients at the Yaoundé Central Hospital

Species (Number of isolates)	Antifungals	MIC range (µg/mL)	Percentage (Number) of isolates				
			CBPs ^a			ECVs ^a	
			S ^b	SDD ^b	R ^b	WT ^b	NWT ^b
<i>C. famata</i> (1)	Fluconazole	0.25					
	Itraconazole	0.03					
	Amphotericin B	0.25					
<i>C. glabrata</i> (33)	Fluconazole	0.25-64	97.0 (32)		3.0 (1)		
	Itraconazole	0.03-8				97(32)	3.07 (1)
	Amphotericin B	0.5-2				100 (33)	0 (0)
<i>C. guilliermondii</i> (1)	Fluconazole	1				100 (1)	0 (0)
	Itraconazole	0.125				100 (1)	0 (0)
	Amphotericin B	1				100 (2)	0 (0)
<i>C. intermedia</i> (2)	Fluconazole	2					
	Itraconazole	0.06					
	Amphotericin B	2					
<i>C. kefyr</i> (8)	Fluconazole	0.25-0.5				100 (8)	0 (0)
	Itraconazole	0.03-0.125					
	Amphotericin B	0.25-2					
<i>C. krusei</i> (55)	Fluconazole	4-32				100 (55)	0 (0)
	Itraconazole	0.03-0.06				100 (55)	0 (0)
	Amphotericin B	1-2				100 (55)	0 (0)
<i>C. lusitaniae</i> (3)	Fluconazole	1				100 (3)	0 (0)

	Itraconazole	0.06				100 (3)	0 (0)
	Amphotericin B	0.5				100 (3)	0 (0)
<i>C. norvegensis</i> (3)	Fluconazole	16					
	Itraconazole	0.03					
	Amphotericin B	1					
<i>C. parapsilosis</i> (18)	Fluconazole	0.25-1	100 (18)	0 (0)	0 (0)		
	Itraconazole	0.03-0.125				100 (18)	0 (0)
	Amphotericin B	0.125-1				100 (18)	0 (0)
<i>C. rugosa</i> (14)	Fluconazole	0.25-2					
	Itraconazole	0.03-16					
	Amphotericin B	1-2					
<i>C. sake</i> (6)	Fluconazole	0.25-1					
	Itraconazole	0.03					
	Amphotericin B	0.5-1					
<i>C. tropicalis</i> (26)	Fluconazole	0.25->64	46.17 (12)	3.87 (1)	50 (13)		
	Itraconazole	0.25->16				15.4 (4)	84.6 (22)
	Amphotericin B	1				100 (26)	0 (0)
<i>C. valida</i> (2)	Fluconazole	16					
	Itraconazole	0.25					
	Amphotericin B	2					

ECVs, epidemiological cut-off values; CBPs, clinical breakpoints; WT, wild type; non-WT, non-wild type; S, susceptible; SDD, susceptible, dose-dependent; R, resistant; ^aMICs are categorised according to published data; ^bpercentages (number of isolates).