**Original Research Article**

**Genotyping and antifungal susceptibility profile of *Candida albicans* isolated from HIV-infected women in Niamey, Niger**

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ABSTRACT

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| **Aims:** *C. albicans* infection is one of the most frequent mycosis among HIV-positive women, posing a serious health risk and significant economic burden for its management. The aim of this study was to genotype and assess the fluconazole susceptibility of *C. albicans* in women infected with HIV/AIDS in Niamey, Niger Republic.  **Study design:** Cross-sectional study  **Place and Duration of Study:** The study was conducted from March 2023 to April 2024 at the Department of Microbiology, Nassarawa State University, Keffi, and three tertiary hospitals of HIV management in Niamey, Niger.  **Methodology:** Three hundred and eighteen (318) high vaginal swab (HVS) and oral swab samples were collected from women who presented with signs and symptoms of vulvovaginal and/or oral candidiasis, aged between 12 and 58 years, and provided informed consent to be part of the study. The isolates were identified using the germ tube test (GTT) and chromogenic *Candida*-selective medium (ChromaticTM Candida). Confirmatory identification and fluconazole susceptibility testing were done using VITEK 2. The fungal isolates were genotyped using *Candida* species-specific genes for *C. albicans* group A, B, and C based on their 25S rDNA with multiplex PCR amplification.  **Results:** Twenty *C. albicans* strains were isolated from oral and vaginal swabs of 318 HIV-positive women. Tests for genotypes among *C. albicans* strains revealed that genotype A was the most prevalent (55%), followed by genotypes B (25%) and C (20%). Fluconazole resistance was detected in only two isolates of genotype A among the *C. albicans* strains.  ***Keywords****: C. albicans*, genotypes, fluconazole, Vulvovaginal candidiasis (VVC), Niamey |

INTRODUCTION

Candidiasis is a significant infectious disease in individuals with HIV, particularly those with acquired immunodeficiency syndrome (AIDS), and is considered an independent predictor of immunodeficiency 1. Candidiasis is an opportunistic infection that occurs when the immune system is weakened and the host is in a vulnerable state 1. *C. albicans* is the most common agent isolated from vulvovaginal candidiasis (VVC) in HIV-infected and uninfected women, appearing in 80-90% of cases in some reports 2,34. In the United States, candidemia costs approximately $1.7 billion annually 5. Candida infections significantly impact morbidity and mortality rates in HIV-positive patients, particularly in low and middle-income countries. In Niger, there is limited data on the epidemiological surveillance of candidiasis in persons living with HIV (PLWH). Only one study has been conducted since 2002, which revealed a high prevalence of *C. albicans* in these patients 6. In Niger, fluconazole is primarily used alone for preventing and treating yeast infections. Due to its use in AIDS prophylaxis and treatment, resistance to fluconazole is a growing concern that needs to be addressed.7. There have been no recent studies on *C. albicans* genotypes in Niger. While molecular genotyping is not currently standard practice, it should be introduced for diagnosing *C. albicans* strains. Molecular typing is crucial for gathering epidemiological data and developing effective strategies to control *C. albicans* infections. Molecular typing of *C. albicans* is essential for understanding epidemiological data and developing strategies to control *C. albicans* infections. The study aimed to investigate the genotypic diversity of *C. albicans* and its correlation with fluconazole susceptibility in women co-infected with HIV/AIDS in Niamey, Niger Republic. This information can help clinicians make more accurate diagnoses and tailor antifungal treatments. Additionally, it can be used to monitor the genetic relatedness of *Candida albicans* during surveillance.

materialS and methods

**Study design and period**

This descriptive cross-sectional study was conducted from March 2023 to October 2024.

**Sources of isolates**

318 swabs were collected from the study population of women aged between 12 and 58 years, with signs and symptoms of vulvovaginal and/or oral infection, who consented to be enrolled in the study. Twenty (20*) C. albicans* strains isolates were obtained from oral and vaginal swabs of patients presenting with candidiasis in two HIV management centres. These centres are: Poudriere Regional Hospital, Issaka Gazoby Maternity, and Amirou Boubacar Diallo National Hospital in Niamey, Niger.

**Specimen Collection**

For oropharyngeal swabs,the samples were taken by swabbing the oral and pharyngeal mucosa using with two sterile cotton swab

For vaginal swabs, the patient(s) must be outside her menstrual period, having had no sexual intercourse the day before and not having done vaginal cleansing in the morning, the sample was taken using two sterile cotton swabs at the level of the vaginal cul-de-sac after placement of a speculum not moistened. The specimens were collected from each participant during the clinician's visit, and were labelled accordingly with their Identification Number on the specimen container. All samples were transported to the Issaka Gazoby Maternity Clinical Biology Laboratory as soon as possible without delay, and processed on the same day of collection.

***C. albicans* isolation and antifungal susceptibility testing**

Preliminary identification of *C. albicans* grown on Sabouraud’s Dextrose Agar (SDA)was done using the Germ tube test; chromogenic Candida-selective medium (Chromatic™ Candida). Confirmatory identification and fluconazole susceptibility testing were performed using VITEK 2.

**PCR confirmation of *C. albicans***

***C. albicans* DNA Extraction**

To extract genomic DNA from each colony of *C. albicans* isolate, they were grown on SDA from the primary culture and then added to 1.5ml tubes, containing 100μl of lysis buffer (100mM Tris–HCl of pH = 7.5, 30mM EDTA pH = 7.5, 0.5% (w:v) SDS). The tubes were boiled in water for 10 minutes. After adding 100μl of potassium acetate (2.5M) buffer of pH = 4.8, the tube was vortexed and placed in an ice box for 60 minutes. Cellular debris and precipitated proteins were removed by centrifugation at >12,000 rpm for 5 minutes. The supernatant was transferred to another 1.5ml tube and centrifuged twice. After transferring the supernatant to a new 1.5ml tube, an equal volume of isopropyl alcohol was added. The tube was placed in the freezer for 30 minutes, briefly mixed by inversion, and then centrifuged at >10,000 rpm for 15 minutes. The supernatant was discarded, and the DNA pellet was washed in 300μl of 70% (v:v) ethanol. After centrifugation at 10,000g for 5 minutes, the supernatant was discarded, and the DNA pellet was air-dried and dissolved in 50μl of distilled water 8. All reagents were purchased from Zymo Research USA.

**DNA quantification by spectrophotometer**

The genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The equipment was calibrated with 2 µl of sterile distilled water and blanked using normal saline. Subsequently, 2µl of the extracted DNA was loaded onto the lower pedestal, and the upper pedestal was lowered to make contact with the DNA. The DNA concentration was then measured 9.

**Candida species 18S rRNA ITS-region amplification**

The PCR was conducted in a 50µl final reaction volume. 1µl of DNA template was added to a 49µl master reaction mixture containing 5µl of 10X PCR buffer, 36.6µl of sterile distilled H2O, 1µl of 10mM MgCl2, 2µl of 2mM dNTPs, 2µl of 10pM ITS1 primer, 2µl of 10pM ITS4 primer, and 0.4µl of Taq polymerase (Zymo Research USA). All reactions were overlaid with sterile mineral oil before thermal cycling. The sequences of the ITS1 primers were 5′ -TCCGTAGGTGAACCTGCGG-3‘and ITS4 primers were 5′ TCCTCCGCTTATTGATATGC-3′. Thermal cycling parameters were initial denaturation at 96°C for 2 minutes, followed by 35 cycles consisting of denaturation at 96°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes, then final extension at 72°C for 10 minutes.

**Genotyping by multiplex PCR amplification**

The fungal isolates were genotyped using *Candida* species-specific genes for *C. albicans* group A, B and C based on their 25S rDNA region *CA-INTA-F* (5′- ATA AAG GGA AGT CGG CAA ATA GAT CCG TAA -3′) *CA-INTA-R* (5′- CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3′) *CA-INTB-F* (5′- ATA AAG GGA AGT CGG CAA ATA GAT CCG TAA-3′) *CA-INTB-R* (5′- CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3′) *CA-INTC-F* (5′- ATA AAG GGA AGT CGG CAA ATA GAT CCG TAA-3′)CA*-INTC-R* (5′- CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3′). The multiplex polymerase chain reaction (mPCR) was prepared in a final volume of 25μl using the ABI 9700 Applied Biosystems thermal cycler containing 15μl of PCR Master Mix, 1.5μl of each primer, and 2.5μl of genomic DNA. The amplification conditions were as follows: initial denaturation at 95°C for 5 minutes; 32 denaturation cycles at 95°C for 45 seconds, annealing at 63°C for 45 seconds, and extension at 72°C for 45 seconds; and a final extension at 72°C for 7 minutes.

**Detection of all the amplified genes by Agarose Gel Electrophoresis**

1% agarose gel was used to resolve DNA fragments. This was prepared by combining 1g agarose in 10 X (10ml 10XTB-EDTA) buffer and 90ml sterile distilled water in 250ml beaker flask and heating in a microwave for 2 minutes until the agarose is dissolved. Exactly 0.7µl of ethidium bromide was added to the dissolved agarose solution with swirling to mix. The gel was then poured onto a mini horizontal gel electrophoresis tank, and casting combs were inserted. The gel was allowed to set for 30 minutes. The casting combs were carefully removed after the agarose gel had solidified completely. 1X (TBE buffer) was added to the reservoir until it covered the agarose gel. Precisely 8µl of gel tracking dye (bromophenol blue) was added to 10µl of each sample with gentle mixing. The sample was loaded onto the gel wells at a concentration of 10µl, the mini horizontal electrophoresis gel setup was covered, and electrodes were connected. Electrophoresis was carried out at 100- 200mA for one hour. After electrophoresis, the gel was removed from the buffer, visualized under UV light, and documented.

**Data Collection**

A standardized questionnaire was administered to each participant to gather data on patient demographics and clinical manifestations

results

A total of 20 *C. albicans* strains were isolated from (1/17) oral swabs and (19/301) vaginal swabs of 318 HIV-positive women in my study population. The PCR products for genotypes A, B, and C were obtained using 25S rDNA primer at 450bp, 840bp, and 440bp respectively. Genotype A, with 11 isolates (55%) were the most predominant followed by genotype B (5 isolates, 25%) and genotype C (4 isolates, 20%).Fluconazole resistance was found in only two (2) genotype A *C. albicans* isolates.

NC PC M 4 5 6 9 12 15 16 17 18 20 21 22 24 25 26 120 229 238 239 251



*C.albicans*18S-ITS region (600bp)

**Plate 1:** Agarose gel electrophoresis of the amplified 18S-ITS *C. albicans* gene region. Lane NC=Negative control, lane PC=Positive control. Lane M represents the 1kb DNA molecular ladder. Lane 4, 5, 6, 9, 12B, 15, 16, 17, 18, 20, 21, 22, 24, 25, 26, 120, 229, 238, 239, and Lane 251 represent the expression of the 18S-rRNA-ITS region (600bp) gene for *C. albicans.*

**ncnc**

M 4 CC5 6 9 12B 15 16 17 18 20 21 22 24 25 26 120 229 238 239 251

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**Plate 2:** Agarose gel electrophoresis of the amplicons of *C. albicans* genotypes. Lane M represents the 1kb DNA molecular ladder. Lanes 4, 17, 21, 22 and 251 represent the expression of the Genotype B (840bp), Lanes CC5, 6 18 and 20 represent the expression of the Genotype C (440bp) and Lanes 9, 12B, 15, 16, 24, 25, 26, 120, 229, 238 and 239 represent the expression of the Genotype A (450bp)*.*

200bp

100bp

900bp

500bp

Group B (840bp)

Group A (450bp)

300bp

Group C (440bp)

**DISCUSSION**

Candidiasis is one of the most frequent infections among HIV-positive women, representing a severe pathology with a considerable economic burden related to its management, and it can be fatal to both immunocompromised and immunocompetent subjects 2,3*. C. albicans* is the most common agent isolated from Candidiasis in HIV-infected women, appearing in 80-90% of cases in some reports 2,3. *C. albicans* molecular typing is very important to help us better understand or deepen knowledge on the epidemiology or pathogenesis of *C. albicans* by identifying the factors that promote the risk of developing candidiasis. In Niger, health services do not show any interest in medical mycology, hence the absence of molecular typing of *C. albicans*. There are no resources or programs to conduct epidemiological research activities to control the emergence of candidiasis. The genotypic identification of *C. albicans* is highly desired in clinical and research biology laboratories as it improves diagnosis. Molecular genotyping is crucial for enhancing our comprehension of fungal disease outbreaks, antifungal resistance, risk factors, transmission patterns, pathogenesis, and the genomic and genetic characteristics of the fungi responsible 10. Till date, no study dealing with the prevalence of *C. albicans* genotypes in Niger has been published. We investigated the susceptibility of *C. albicans* isolated from HIV Women in Niamey, Niger to fluconazole, and genotyped them into: A, B and C. This is the first study documenting the genotypic distribution of *C. albicans* in Niger. A, B and C genotyping of 20 *C. albicans* strains resulted in predominantly 11 A genotypes (55%), followed by 5 B genotype (25%) and 4 C genotype (25%) isolates. A similar observation was reported by 7,8,11,12. Contrary to this study, Da Silva-Rocha et *al*. 13 showed that genotypes A and C were more common. A high resistance of *C. albicans* to fluconazole (2; 10%) was seen, which was in agreement with the studies 12. Analysis of the relationship between genotypes and antifungal sensitivity showed that only two (2), A genotypes developed resistance to fluconazole. The mechanisms by which fluconazole develops resistance include: mutations in the ERG11 gene 14–17, overexpression of ERG11 14,15, efflux of drug from the cell 14,15, an increase in the expression of azole targets 14,17, development of bypass pathways 14, and increased expression of CDR1, CDR2 and MDR1 gene-coded specific multidrug efflux transporters preventing an intracellular azole-accumulation 15–18. Erg11p (lanosterol 14𝛼-demethylase) mutations G464S, I471T, R467K, S405F, T315A, and Y132H cause azole resistance 18. The mutations: D116E, D153E, D446N, E266D, E266Q, F105L, F72L, G129A, G303D, K128T, K147R, K287R, K342R, K99T, L305P, and V437I, on the other hand, exist in susceptible strains and are not known to cause resistance 18. Antifungal resistance may also be mediated by biofilms 18. Lastly, the sequestration of fluconazole within intracellular vacuoles may be a novel resistance mechanism 18. Additionally, this resistance of *C. albicans* to fluconazole in Niger could be explained by its use for prophylaxis and treatment for candidiasis in HIV-positive patients. Fluconazole is the most available and accessible antifungal to the public, which is why it is more widely used in self-medication. Antifungals in general and fluconazole in particular are prescribed without the results of the antifungal test due to the lack of a mycology laboratory in Niger. All these situations contribute to the proliferation of fluconazole-resistant strains of *C albicans.*

Our study had some limitations. The sample size was not large enough to allow for the generalization of our findings. It wasn't easy to obtain consent from a closed group of HIV-positive women. Analyses were only descriptive, and the p-value was not calculated. Despite these limitations, to the best of our knowledge, this study represents the first investigation of *C. albicans* genotyping andfluconazole resistance in Niger. Our results provide baseline information that will further aid in formulating a proper antimicrobial policy to combat infections caused by *Candida*.

Conclusion

Genotyping for *C. albicans* is crucial for precise infection diagnosis, targeted antifungal treatment, understanding pathogenesis and epidemiology, and tracking molecular epidemiology. Hence, authorities and policymakers should implement measures that offer the required structural and logistical assistance.

Consent

Informed consent was obtained from each participant. The purpose and nature of the study, as well as the method of sample collection, were explained to them properly. Afterwards, participants were requested to voluntarily complete the consent form in their handwriting and endorse it with their signatures as proof of their willingness to provide samples for the test. They were assured of confidentiality.

Ethical approval

Before the study began, the National Ethics Committee for Health Research in Niger (N° 15/2023/CNERS) approved the protocol.

References

1. Torabi, I., Sharififar, F., Izadi, A. & Ayatollahi Mousavi, S. A. Inhibitory effects of different fractions separated from standardized extract of Myrtus communis L. against nystatin-susceptible and nystatin-resistant Candida albicans isolated from HIV positive patients. *Heliyon* **8**, (2022).

2. Zanni, P. C. M. D. *et al.* Virulence factors and genetic variability of vaginal *Candida albicans* isolates from HIV-infected women in the post-highly active antiretroviral era. *Rev. Inst. Med. Trop. Sao Paulo* **59**, (2017).

3. Dockrell, D., O’Shea, D., Cartledge, J. & Freedman, A. British HIV Association/British Infection Association guidelines on the management of opportunistic infection in people living with HIV: The clinical management of Candidiasis 2019. *HIV Med.* **20**, 2–24 (2019).

4. Mashaly, G. E. S. & Zeid, M. S. Candida albicans Genotyping and Relationship of Virulence Factors with Fluconazole Tolerance in Infected Pediatric Patients. *Infect. Drug Resist.* **15**, 2035–2043 (2022).

5. Song, N., Zhou, X., Li, D., Li, X. & Liu, W. A Proteomic Landscape of Candida albicans in the Stepwise Evolution to Fluconazole Resistance. *Antimicrob. Agents Chemother.* **66**, (2022).

6. Mamadou, S. *et al.* autres infections sexuellement transmissibles chez les professionnelles du sexe à Niamey , Niger . S ANTÉ PUBLIQUE. 19–22 (2006).

7. Tamai, I. A., Salehi, T. Z., Sharifzadeh, A., Shokri, H. & Khosravi, A. R. Repetitive sequences based on genotyping of *candida albicans* isolates obtained from iranian patients with human immunodeficiency virus. *Iran. J. Basic Med. Sci.* **17**, 831–835 (2014).

8. Jafarian, H., Gharaghani, M., Seyedian, S. S. & Mahmoudabadi, A. Z. Genotyping, antifungal susceptibility, enzymatic activity, and phenotypic variation in Candida albicans from esophageal candidiasis. *J. Clin. Lab. Anal.* **35**, e23826 (2021).

9. Victor, O., Ejiro, O. & Ejiro Agbagwa, O. The Prevalence of Fungal Infections in Six Communities in Akwa Ibom State Nigeria. *Afr. J. Health Sci.* **35**, 574–585 (2022).

10. Chen, M., Al-Hatmi, A. M. S., Xu, J. & De Hoog, G. S. Editorial: Molecular Epidemiology of Fungal Infections. *Front. Cell. Infect. Microbiol.* **12**, 10–12 (2022).

11. Tamai, I. A., Pakbin, B. & Fasaei, B. N. Genetic diversity and antifungal susceptibility of Candida albicans isolates from Iranian HIV-infected patients with oral candidiasis. *BMC Res. Notes* **14**, (2021).

12. Sawadogo, P. M. *et al.* Genotype Analysis of Clinical Candida albicans Isolates Using PCRs Targeting 25S rDNA and ALT Repeat Sequences of the RPS and Antifungal Susceptibility in Ouagadougou (Burkina Faso). *Infect. Drug Resist.* **12**, 3859 (2019).

13. da Silva-Rocha, W. P. *et al.* Candida species distribution, genotyping and virulence factors of Candida albicans isolated from the oral cavity of kidney transplant recipients of two geographic regions of Brazil. *Mycoses* **14**, 24–28 (2014).

14. Karthika, K., Premamalini, T., Vijayakishore, T. & Kindo, A. J. Standardization of polymerase chain reaction for detection of fluconazole resistance targeting Y132F mutation in ERG11 gene in Candida parapsilosis. *Curr. Med. Mycol.* **10**, (2024).

15. Ribeiro, M., Paula, C. R., Perfect, J. R. & Cox, G. M. Phenotypic and genotypic evaluation of fluconazole resistance in vaginal Candida strains isolated from HIV-infected women from Brazil. *Med. Mycol.* **43**, 647–650 (2005).

16. Accoceberry, I. *et al.* A CTG clade candida yeast genetically engineered for the genotype-phenotype characterization of azole antifungal resistance in human-pathogenic yeasts. *Antimicrob. Agents Chemother.* **62**, (2018).

17. Nim, S. *et al.* Atomic modelling and systematic mutagenesis identify residues in multiple drug binding sites that are essential for drug resistance in the major Candida transporter Cdr1. *Biochim. Biophys. Acta - Biomembr.* **1858**, 2858–2870 (2016).

18. Xu, Y., Chen, L. & Li, C. Susceptibility of clinical isolates of Candida species to fluconazole and detection of Candida albicans ERG11 mutations. *J. Antimicrob. Chemother.* **61**, 798–804 (2008).