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Characterization and Susceptibility of Non-Albicans Candida Isolated from Clinical Samples at the National Public Health Laboratory in Congo

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Abstract:- The rising prevalence of non-albicans Candida infections, attributed to their increasing antimicrobial resistance, prompted an investigation into the prevalence of these infections within Candidiasis and the identification of the most effective antifungal treatment. A prospective cross-sectional study gathered 298 vaginal swab samples at the National Public Health Laboratory in Congo. Initial direct diagnostics excluded samples negative for candidiasis. Candida species were isolated using Sabouraud Chloramphenicol agar and identified via various phenotypic methods, including microscopic characterization, the germ tube test, and sugar fermentation tests. Antifungal susceptibility testing was conducted using antibiotic discs on Muller Hinton agar through the diffusion method. The prevalence of candidiasis among patients was 33%. Species other than Candida albicans and non-albicans Candida had a prevalence of 33% and 67%. Non-albicans Candida species accounted for 67%, with C. tropicalis being the most prevalent (31%), followed by C. glabrata (27%), C. parapsilosis (18%), and C. krusei (10%). Other species were also identified but with lower frequencies. There was notable resistance to azole antifungals in certain species like C. tropicalis, C.glabatra, C. glabrata and C. krusei. The emergence of non-albicans Candida species resistant to azole antifungals necessitates an antifungal susceptibility test before determining a therapeutic regimen.

Keywords:- *Candidiasis, Non-Albicans Candida, Antifungal Drugs, Susceptibility Profile.*

I. INTRODUCTION

Candidiasis stands as one of the most common fungal infections in women, especially in those who are sexually active (Kombade et al, 2021). It's an opportunistic pathogen typically considered a part of the commensal flora. However, when it becomes pathogenic, it can lead to severe manifestations. Vaginal candidiasis is a sexually transmitted infection of the vaginal mucosa caused by various species within the Candida genus, with Candida albicans being the most prevalent. Recently, the majority of candidiasis cases have been attributed to a significant number of non-albicans Candida species, showing an increase in both immunocompromised and healthy women. Among these, frequently implicated species include Candida glabrata, Candida tropicalis, Candida krusei and Candida parapsilosis (Botelho et al, 2022). Although azoles are commonly used as a preferred treatment, cases of resistance, especially in non-Candida albicans species, have been reported. Given the varied susceptibility of non-albicans Candida species to antifungal agents, accurately identifying the specific Candida species is critical (Husni et al, 2023). Diagnosis relies on examining vaginal secretions and smears, but clinical symptoms might overlap with other conditions, highlighting the need for microbiological diagnosis to confirm yeast presence. A deeper understanding of risk factors, mycological profiles, and antifungal susceptibility of non-albicans Candida species could aid clinicians in devising more effective therapies. This study endeavors to fill this critical knowledge gap in Brazzaville, Congo.

II. MATERIAL AND METHODS

A. Study Design

A cross-sectional study was adopted and antifungigrams were performed at the National Public Health Laboratory, Brazzaville, Congo.

B. Study Population

Study population consisted of women with vaginal infections who attended the National Public Health Laboratory. All participants gave prior consent to take part in the study.

C. Sample Collection

A total of 298 vaginal samples were collected using sterile swabs, selecting patients exhibiting typical symptoms of vaginal infections. These symptoms included a foul odor, scanty or purulent vaginal discharge, a burning sensation, pain during urination, as well as itching and irritation of the vagina. The samples were appropriately labelled and immediately transported to the laboratory for analysis. Collection of these samples took place from January to July 2020, with the aim of isolating non-albicans Candida strains for further study.

D. Sample Processing

Samples were submitted to the microbiology department, where they were rapidly processed to identify

pathogenic yeasts using standardized laboratory techniques. Vaginal swab samples were immediately examined fresh and directly using methylene blue-stained smears. They were observed under the microscope for the presence of yeast, thus establishing an initial diagnosis of vaginal infection. In cases where the samples were positive, the yeasts were cultured on Sabouraud Chloramphenicol agar and incubated at 37°C for 24 to 48 hours to promote growth and isolation (ElFeky et al, 2015).

E. Phenotypic Identification of Candida

Phenotypic identification of yeast isolates was carried out using morphological, physiological, and biochemical criteria. The morphological characterization of yeast relied on macroscopic criteria related to colony appearance, color, texture, as well as microscopic criteria concerning yeast cells and their shape. Physiological characterization by germ tude test to exclude Candida albicans (Ali et al, 2019). Lastly, biochemical characterization was performed using The Liofilchem® Integral System YEASTS Plus gallery (Abdelaziz et al, 2013).

F. Antifungal Susceptibility Testing

Antifungal susceptibility testing of non-albicans Candida was performed on Mueller-Hinton agar using the Kirby-Bauer disk diffusion method, following the criteria outlined by the National Committee for Clinical Laboratory Standards Interpretative. The agar surface was inoculated using a swab dipped in a cell suspension adjusted to a turbidity of 0.5 McFarland standard. The following antifungal disks were utilized: Nystatin (50µg), Amphotericin B (10µg), Voriconazole (1µg), Clotrimazole (50µg), Econazole (50µg), Fluconazole (25µg), Itraconazole (10µg), Miconazole (50µg), Ketoconazole (50µg), and Flucytosine (1µg) (Abdelaziz et al, 2013).

G. Data Analysis

The data were recorded on an Excel sheet. The Graphs were plotted using OriginPro 2023 (version 10.0.5.157) and GraphPad Prism (version 9.5.1).

III. RESULTS

A. Sample Characteristic

Vaginal samples totaling 298 were collected, representing ages from 15-70 years old. The highest frequency was observed among women aged 30-40 years (44%), while the lowest frequency was found in women under 20 years (9%) (Figure 1).

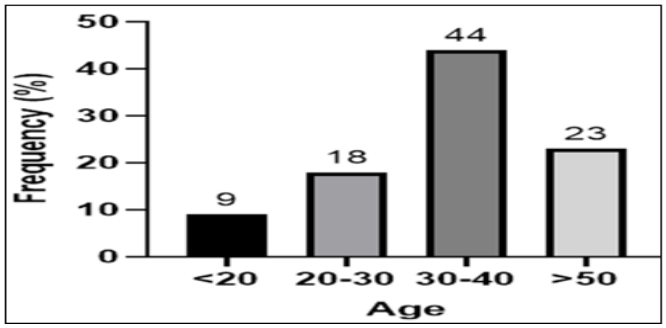

Fig 1 Age Distribution of the Samples

B. Sample Processing

Direct examination of vaginal samples detected the presence of *Candida* yeasts in specific samples, identified by their ovoid forms (Figure 2). Among the 298 collected samples, 99 (33%) were positive for this etiology (Table 1).

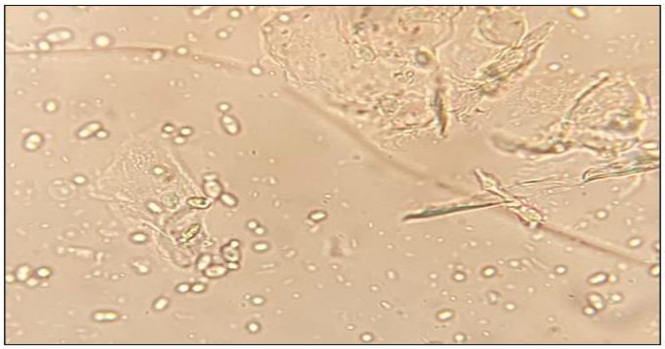

Fig 2 Yeast Micromorphology Observed on a Sample

- Predominantly among the diagnosed positive samples, the demographic analysis highlighted the highest occurrence among women aged 30-40 years (32%) (Figure 4).

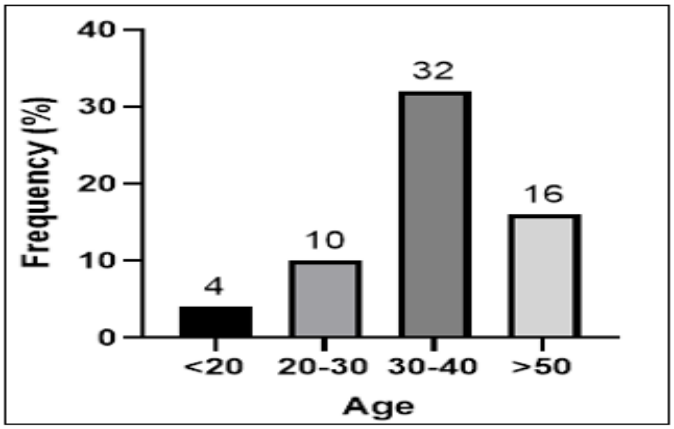

Fig 3 Demographic Characteristics of Patients with Positive Samples

C. Phenotypic Identification of Candida

Isolation of the yeasts responsible for candidiasis showed white to cream-colored colonies (Figure 4).

Fig 4 Morphology of Yeast Colonies on Sabouraud Agar After 48h of Aerobic Incubation At 37 °C

Candida albicans was distinguished from other non-albicans Candida species by the formation of germ tubes from its cells after 1h (Figure 5).

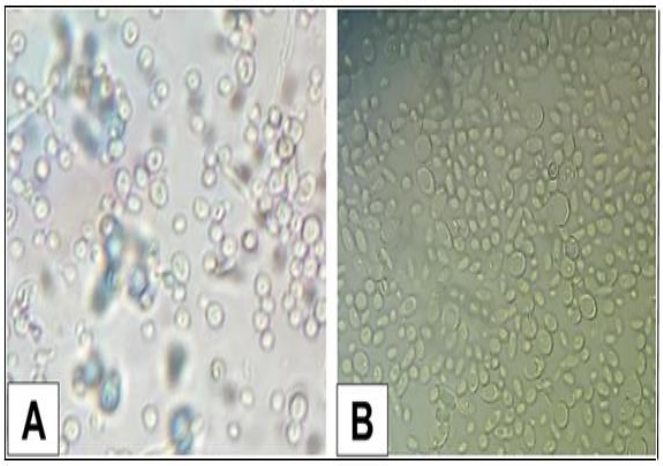

Fig 5 Germ Tube Formation Test of Candida
(A)Candida Albicans, (B) Non-Albicans Candida

Out of a total of 99 positive samples for candidiasis tested, 37 (37%) were identified as Candida albicans, while 63 (63%) were classified as non-albicans Candida (Table 1).

Table 1 Frequency of Candida Causing Candidiasis

Candidiasis	C.albicans	Non-alb. Candida
Distribution (%)	37 (37%)	63 (63%)

Carbohydrate assimilation test identified the 63 (63%) non-albicans Candida species (Figure 6). Each of them adopted a particular profile compatible with a specific data.

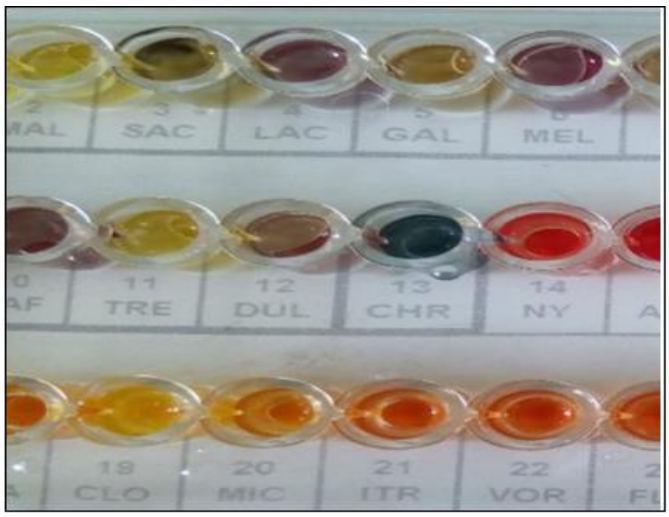

Fig 6 Carbohydrate Fermentation Test by Candida Dubliniensis on Integral System YEASTS Plus Gallery

Results from the carbohydrate assimilation test using the YEAST gallery revealed 7 species. Among the identified non-albicans Candida species, 19 (31%) were attributed to Candida tropicalis, followed by 17 (27%) Candida glabrata, 11 (18%) Candida parapsilosis, and 6 (10%) Candida krusei. Other species included 4 (6%) Candida famata, 4 (6%) Candida dubliniensis, and 1 (2%) Candida stellatoidea (Figure 7).

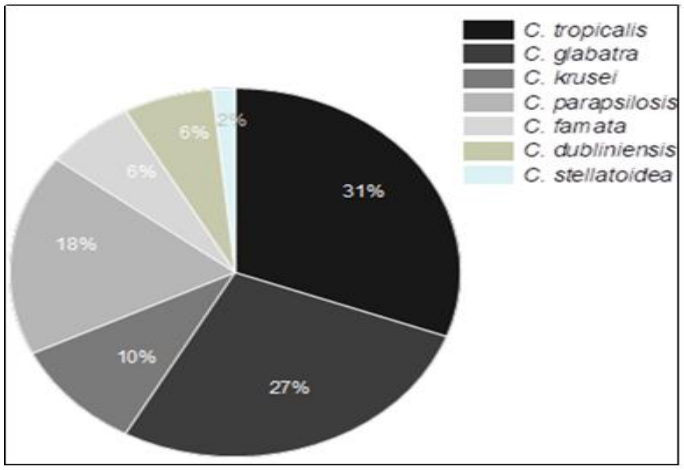

Fig 7 Distribution of Non-Albicans Candida Species Identified

D. Antifungal Susceptibility Testing

Disk diffusion method was used to assess the resistance of non-albicans Candida strains to common antifungal drugs. Antifungal susceptibility tests revealed both resistance and sensitivity, depicted by zones of inhibition around the wells (Figure 8).

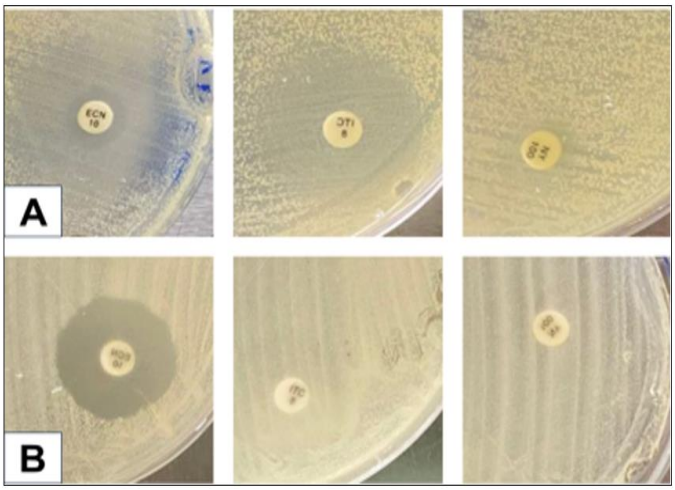

Fig 8 An Overview of the Antifungal Susceptibility Test Conducted with Three Antifungal Agents Against two Clinical Isolates of Non-Albicans Candida, Including Candida Krusei (A) and Candida Tropicalis (B). Econazole (ECN), Itraconazole (ITC), and Nystatin (NY)

Antifungal susceptibility tests conducted on non-albicans Candida species revealed that strains belonging to C. famata, C. dubliniensis, and C. stellatoidea were all susceptible to the tested antifungals. For C. parapsilosis strains, there was low resistance observed to azoles such as Fluconazole (88%), Itraconazole (88%), and Miconazole (88%). However, they exhibited susceptibility to Ketoconazole, Flucytosine, Nystatin, and Amphotericin B. In contrast, significant resistance was observed among C. tropicalis, C. glabrata, and C. krusei strains, particularly towards azoles like Fluconazole (59%, 65%, 0%), Itraconazole (68%, 65%, 16%), and Miconazole (53%, 59%, 64%) respectively (Table 2).

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Table 2 Susceptibility of Non-Albicans Candida Strains to Antifungal Drugs

Antifungal agent	C.tropicas	C.glabatra	C. krusei	C.parapsiloss	C.famata	C.dubliniens	C.stellatoia
VRC (1µg)	17 (91%)	15 (88%)	5 (91%)	11 (100%)	4 (100%)	4 (100%)	1 (100%)
CTZ (50µg)	17 (91%)	14 (82%)	5 (91%)	11 (100%)	4 (100%)	4 (100%)	1 (100%)
ECN (50µg)	14 (74%)	12 (70%)	3 (50%)	11 (100%)	4 (100%)	4 (100%)	1 (100%)
FLC (25µg)	11 (59%)	11 (65%)	0 (0%)	9 (83%)	4 (100%)	4 (100%)	1 (100%)
ITC (10µg)	13 (68%)	11 (65%)	1 (16%)	9 (83%)	4 (100%)	4 (100%)	1 (100%)
MCZ (50µg)	10 (53%)	10 (59%)	4 (64%)	9 (83%)	4 (100%)	4 (100%)	1 (100%)
KCZ (50µg)	14 (74%)	11 (64%)	4 (65%)	11 (100%)	4 (100%)	4 (100%)	1 (100%)
5FC (1µg)	16 (84%)	16 (94%)	5 (91%)	11 (100%)	4 (100%)	4 (100%)	1 (100%)
NY (50µg)	18 (95%)	16 (95%)	4 (73%)	11 (100%)	4 (100%)	4 (100%)	1 (100%)
AMP (10µg)	17 (89%)	15 (88%)	5 (90%)	11 (100%)	4 (100%)	4 (100%)	1 (100%)

Voriconazole (VRC), Clotrimazole (CTZ), Econazole (ECN), Fluconazole (FLC), Itraconazole (ITC), Miconazole (MCZ), Ketoconazole (KCZ), Flucytosine (5FC), Nystatin (NY), Amphotericin B (AMP). Many non-albicans Candida species showed resistance to all the antifungals tested. Overall resistance levels were observed in C. tropicalis (20%), C. glabrata (20%), C. krusei (34%), and C. parapsilosis (7%) as part of the overall sensitivity profile of these species to the antifungals tested (Figure 9).

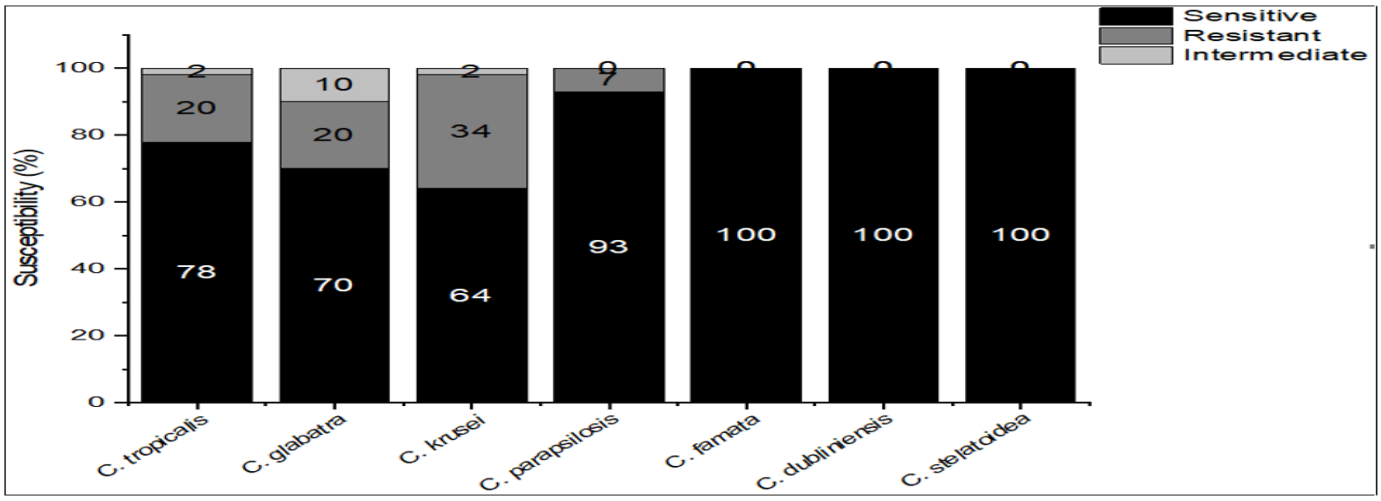

Fig 9 Profile of Non-Albicans Candida Species to Antifungal Drugs

Majority of non-albicans Candida species were found to be sensitive to seven (7) antifungal medications, including Voriconazole (95%), Clotrimazole (92%), Econazole (73%), Ketoconazole (74%), Flucytosine (95%), Nystatin (91%), and Amphotericin B (92%). However, azole compounds such as Fluconazole (53%), Itraconazole (41%), and Miconazole (26%) exhibited resistance in most cases among these species (Figure 10).

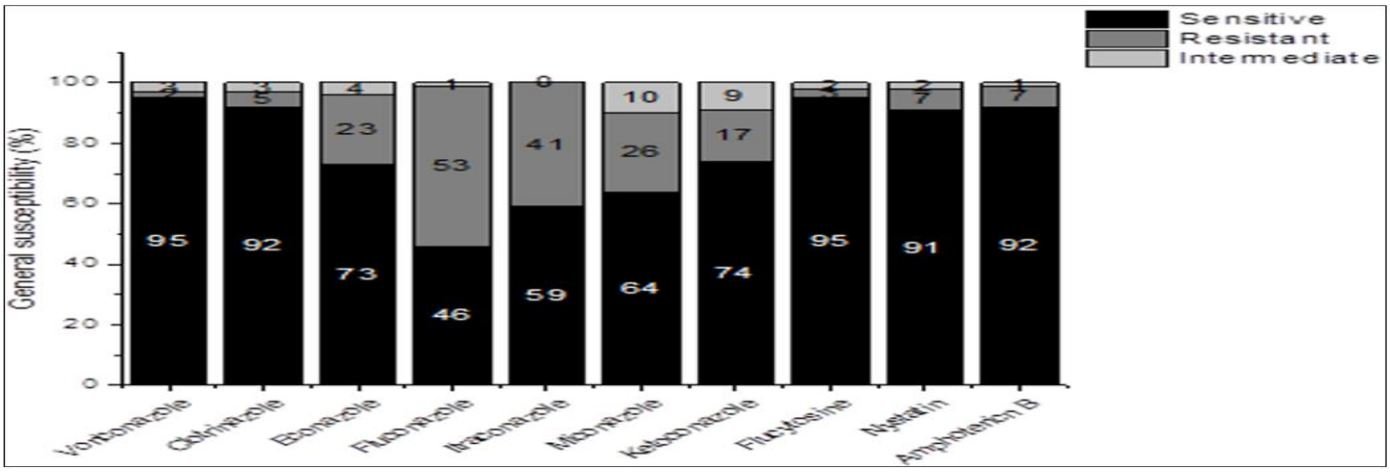

Fig 10 Profile of Antifungal Drugs on Non-Albicans Candida Species

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IV. CONCLUSION

The aim of this cross-sectional study was to determine the prevalence of vaginal candidiasis, identify non-albicans Candida species, and assess their antifungal susceptibilities. This study is significant because the diagnosis and treatment of vaginal candidiasis in Congo are primarily based on clinical presentation without any laboratory diagnosis.

Among the 298 subjects recruited in this study, the initial diagnosis of candidiasis revealed that 99 were confirmed to have vaginal candidiasis, resulting in a prevalence rate of 33% among the participants. A similar finding was reported by Waikhom et al, (2020), in Ghana, demonstrating a 30% prevalence of positive cases of vaginal candidiasis among participants. A higher frequency of vaginal candidiasis was also observed in the age group of 30-40 years, confirming the results of Susilawati et al, (2019). This suggests a potential predisposition to vaginal candidiasis in this age group, perhaps due to increased sexual activity.

Secondary diagnosis from the germ tube production test revealed a prevalence of candidiasis primarily caused by non-albicans Candida (63%), which contrasts with the findings of Tsega et al, (2019), regarding a prevalence of non- albicans Candida (44%). However, these results align with those Mokhtar et al, (2021), who also reported a predominance of non-albicans Candida (56%).

The emergence of non-albicans Candida in candidiasis prevalence could be attributed to certain resistance or adaptation to the vaginal environment, contributing to their predominance in cases of candidiasis. However, this filamentation test can present errors for non-experts, potentially leading to confusion between C. albicans and C. dubliniensis, as both can also form filaments Waikhom et al, (2020). Therefore, further in-depth analyses are necessary for rapid identification of non-albicans Candida .

Identification of non-albicans Candida species by fermentation showed that vaginal candidiasis could be caused by more than one species of non-albicans Candida . A total of 7 species of non-albicans Candida were identified, including C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, C. famata, C. dubliniensis, and C. stellatoidea. Similar research conducted by Mohammed et al, (2015), identified multiple species of non-albicans Candida responsible for vaginal candidiasis. Most cases of vaginal candidiasis caused by non-albicans Candida were attributed to C. glabrata (27%) and C. tropicalis (31%). These findings support the results of Yadav et al, (2016) on the species of non-albicans Candida most implicated in vaginal candidiasis.

Individual sensitivity tests suggest complete sensitivity of strains of C. famata, C. dubliniensis, and C. stellatoidea to the tested antifungals. This could indicate a widespread effectiveness of these antifungals against these specific Candida species, which is consistent with the recent findings from Maraki et al, (2019).

parapsilosis has exhibited overall sensitivity to the majority of antifungals, with exceptions noted for Fluconazole (17%), Itraconazole (17%), and Miconazole (17%). The emergence of certain strains of C. parapsilosis displaying resistance to these broad-spectrum azoles has also been highlighted in the research conducted by Branco et al, (2023), The partial resistance observed in C. parapsilosis to Fluconazole, Itraconazole, and Miconazole suggests the emergence of resistant strains over time, potentially through the acquisition of specific resistance genes, as demonstrated by Branco et al.

Regarding C. krusei, C. glabrata, and C. tropicalis, some strains have demonstrated sensitivity while others have shown resistance to the tested antifungals. For instance, certain strains of C. krusei were found to be sensitive to Voriconazole, Clotrimazole, Flucytosine, and Amphotericin B, but completely resistant to Fluconazole (100%), with also some resistance to Econazole (50%), and Itraconazole (84%). C. glabrata exhibited resistance clusters to Fluconazole (35%), Itraconazole (35%), Miconazole (41%), and Ketoconazole (36%). Similarly, C. tropicalis displayed resistance clusters to all three antifungals, particularly to Fluconazole (41%), Itraconazole (32%), and Miconazole (47%). These results align with those of Badiee et al, (2011), who observed an evolution of resistance in these species over the years. The differences in sensitivity among strains of C. krusei, C. glabrata, and C. tropicalis indicate significant variability. This variability could be attributed to genetic diversity within each species, suggesting complexity in the response of these Candida species to the various tested antifungals.

The overall assessment of species sensitivity to antifungals revealed that four species, namely C. parapsilosis (93%), C. famata (100%), C. dubliniensis (100%), and C. stellatoidea (100%), displayed strong sensitivity. In contrast, a more pronounced overall resistance was observed in Candida non-albicans, especially in C. krusei (34%), C. glabrata (20%), and C. tropicalis (20%), against azoles such as Fluconazole, Itraconazole, and Miconazole. This general resistance observed in C. krusei, C. glabrata, and C. tropicalis to the tested azoles could be attributed to the frequent and prolonged use of these antifungals, particularly Fluconazole. This finding aligns with previous research by Subramanya et al, (2017), highlighting a growing trend of Fluconazole resistance over time among non- Candida albicans.

The overall sensitivity rates of antifungals against non-albicans Candida species highlight that fluconazole exhibited the lowest sensitivity rate in this study, at 46%, followed by itraconazole at 59% and miconazole at 64%. These findings align with the conclusions drawn by Khan et al, (2018), who also noted resistance to these molecules. The complexity of varied sensitivities among different species of non-albicans Candida to antifungals underscores the importance of gaining a better understanding of these variations to optimize the management and treatment of fungal infections.

The study's findings indicated a rise in non-albicans species prevalence in vaginal candidiasis. These frequently implicated species displayed resistance to commonly prescribed azoles. The overall escalation in azole resistance within these species may pose a significant public health concern. The conclusions emphasize the necessity for ongoing monitoring of shifts in species distribution and their susceptibility to antifungal treatments, underscoring the importance of routine screening and appropriate treatment.

➢ *Conflicts of interests*

The authors declare that they have no conflicts of interests.

➢ *Acknowledgments*

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