Trends in Azole Susceptibility of *Candida albicans* Isolated from Clinical Samples at a Tertiary Care Hospital in Georgetown, Guyana

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ABSTRACT

Objective: This study sought to examine the frequency of isolation and azole susceptibility patterns of clinical *Candida albicans* isolates from a tertiary hospital in Georgetown, Guyana during a three-month period.

Methods: Isolation and germ-tube identification of *Candida sp* were done by the hospital Microbiology Department. Further identification was made by assessing the colour and morphology of *Candida* isolates subcultured from SDA onto HardyCHROM™ Candida. Antifungal susceptibility testing and results interpretation were performed in accordance with the CLSI M44-A2 guidelines.

Results: sixty-two non-duplicate isolates of *Candida* were analysed from multiple patient sources. The majority of these isolates were *Candida albicans* (56.5%), while the remainder (43.5%%) were non-*C albicans* species of which *C glabrata* (32.3%) and *C krusei* (8.1%) were the predominant species. 28.6% of the *C albicans* isolates were resistant to fluconazole and voriconazole, respectively, while 40% of the isolates were resistant to itraconazole.

Conclusion: Azole resistance is a common phenomenon among *C albicans* isolates within the setting of the Georgetown Public Hospital Corp (GPHC).

Keywords: Antifungal susceptibility testing, azole resistance, *candida albicans*, candidiasis, Guyana, South America

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INTRODUCTION

In recent decades, fungal hospital infections have increased worldwide, with *Candida* species being amongst the most frequently isolated opportunistic fungal pathogens in the clinical laboratory. Studies have shown that *Candida spp* can account for as much as 90% of fungal infections encountered in the hospital setting (1). These micro-organisms exist as commensal fungi that primarily colonize human mucosal surfaces within the body (2–4). *Candida*, as a genus, is comprised of a heterogeneous group of organisms of approximately 200 species with only a few known to cause human infections, which are collectively referred to as candidiasis (3).

Infections caused by species of *Candida* are increasingly being reported worldwide. This has been attributed to increases in the at-risk population which include transplant recipients, cancer patients and other patients who receive immunosuppressive therapy (5). Women also represent a significant demographic in which *Candida* infections occur (6). Additionally, 90–95% of persons infected with the human immunodeficiency virus (HIV) develop clinical infections caused by *Candida* species (7).

The latter finding is especially important in the context of Guyana where HIV continues to be a significant burden with 7800 persons affected by the virus countrywide, according to 2015 estimates (8). *Candida albicans* remains a predominant isolate from these and other immunocompromised patients.

Traditionally, *C albicans* has been the most commonly isolated *Candida* species causing candidiasis and it is associated with significant morbidity and mortality (9). Azoles, the largest family of antifungal drugs that work by lanosterol 14-α-demethylase inhibition, *ie*, inhibition of the enzyme involved in the biosynthesis of ergosterol, thereby resulting in the disruption of the cell

membrane (10, 11) are the most frequently used class of drug for *Candida albicans* infections. However, the extensive use of azoles to treat these infections has led to an increasing number of reports of azole antifungal resistance among other South American countries (12) and worldwide.

This study was the first to be done in Guyana to examine the frequency of isolation and azole susceptibility patterns of clinical *Candida albicans* isolates. The aim was to ascertain whether azole resistance existed among *C albicans* isolated in the clinical setting and provide data with which evidence-based guidance could be given to clinicians that encounter *C albicans* infections in the tertiary care.

SUBJECTS AND METHODS

Isolation and Identification of Candida spp

Sixty-two *Candida* non-duplicate isolates were collected from multiple clinical sources during the period of June to August 2016 from the Georgetown Public Hospital Corporation (GPHC). The GPHC is a 600-bed tertiary healthcare facility located in the city of Georgetown, Guyana that provides specialized services for a varied population, including immunosuppressed patients.

Isolation of *Candida* was performed by the staff of the Micro-biology Department of the GPHC using standard microbiological procedures. Isolates were inoculated onto Sabouraud Dextrose Agar [SDA] (Hardy Diagnostics, Santa Maria, CA) before identification was performed using the germ tube test. Identification was also made by assessing the distinctive colour and morphology of *Candida* isolates subcultured from SDA onto HardyCHROM™ Candida chromogenic agar (Hardy Diagnostics, Santa Maria, CA). All isolates were tested within five days

of isolation before being stored at -70 ˚C in Tryptic Soy Broth with 15% glycerol (Hardy Diagnostics, Santa Maria, CA).

Antifungal susceptibility testing

Disk diffusion antifungal susceptibility tests were performed for three azole antifungal drugs and results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) M44-A2 guidelines as well as manufacturers' instructions (Table 1). The antifungal agents that were used were fluconazole [25 µg] (Becton Dickinson, BBL), voriconazole (Becton Dickinson, BBL) [1 µg] and itraconazole (50 µg) (Liofilchem®). *Candida albicans* ATCC 90028 was used for quality control.

Briefly, antifungal susceptibility testing was conducted for all isolates identified as *Candida albicans* using Mueller Hinton Agar with 2% glucose and 0.5 µg/mL methylene blue (HiMedia Laboratories, India). Plates were inoculated using suspensions of each isolate prepared in 5 mL of sterile 0.85% saline and adjusted to a final concentration equivalent to a 0.5 McFarland standard. After drying for 15 minutes, the disks were applied to each plate. The plates were incubated at 35 ˚C ± 2 and were read at 24 hours. The inhibitory zone diameter for the disks were measured at the transition point where growth abruptly decreased as determined by a marked reduction in colony size, number and density.

Ethical considerations

Approval to carry out this research was granted by the Georgetown Public Hospital Corporation (GPHC) and the Institutional Review Board (IRB) of The Ministry of Public Health, Guyana.

Statistical analysis

For descriptive analyses mean and standard deviation (SD) for continuous variables and percentages for categorical data were calculated using SPSS version 19.

RESULTS

During the three-month study period, 62 *Candida spp* were isolated from hospitalized patients at the GPHC. Most of the isolates were obtained from vaginal swabs (n = 39) followed by urine (n = 18) and blood (n = 5). *Candida albicans* accounted for the highest percentage of *Candida* species isolated (n = 35; 56.5%) with the remainder (n = 27; 43.5%) being non-*C albicans* species.

Table 1: Criteria for the interpretation of susceptibility results

Antifungal agent	**Disk content (µg)**	**Zone diameter (ZD) (nearest whole mm)**			**Control ZD Candida ATCC90028**
		*R	*SDD	*S	
Fluconazole	25	≤ 14	≥ 19	15-18	28-39
Voriconazole	1	≤ 13	≥ 17	14-16	31-42
Itraconazole	50	≤ 9	≥ 16	10-15	16-20

*Abbreviations: R, Resistant; S, Susceptible; S-DD, Susceptible-dose dependent

The use of chromogenic agar allowed for characterisation of the non-*C albicans* species (Table 2) with *Candida glabrata* (n = 20) being the most commonly isolated species of this group followed by *Candida krusei* (n = 5) and *Candida tropicalis* (n = 2), respectively. *C glabrata* (n = 3) and *C krusei* (n = 2) were the only *Candida* species isolated from cases of candidemia.

Table 2: Identification of *Candida* isolates

Species	No of Isolates (n = 62) (%)	Result on Chromogenic Agar[a]	Positive by Morphological ID[c]	Positive by GTT
C tropicalis	2 (3.2)	Blue to dark metallic blue	2	2[b]
C krusei	5 (8.1)	Pink to medium pink	5	0
C glabrata	20 (32.3)	Pink with mauve centres	20	0
C albicans	35 (56.5)	Green to dark metallic green	35	35

[a] Interpretation of results on chromogenic agar was based on manufacturer's instruction
[b] These were false-positive results revealed after identification with chromogenic agar.
[c] ID: Identification

In-vitro antifungal susceptibility testing (AFST) was conducted on the 35 *Candida albicans* isolates identified using the antifungal agents: fluconazole, voriconazole and itraconazole. Inhibitory zone diameters were interpreted using the manufacturers' instructions and guidelines established by the CLSI M44-A2 (Table 3).

Table 3: Susceptibility profile for *Candida albicans* against the antifungal drugs tested

Antifungal Agents	Antifungal Susceptibility Results (n = 35)			
	S[a] n (%)	SDD[a] n (%)	R[a] n (%)	Mean ZD[a]
Fluconazole	25 (71.4)	0	10 (28.6)	21.9
Itraconazole	21 (60)	0	14 (40)	14.9
Voriconazole	24 (68.6)	1 (2.9)	10 (28.6)	14.9

[a] Abbreviations: S, susceptible; SDD, susceptible dose dependent; R, resistant; ZD, zone diameter

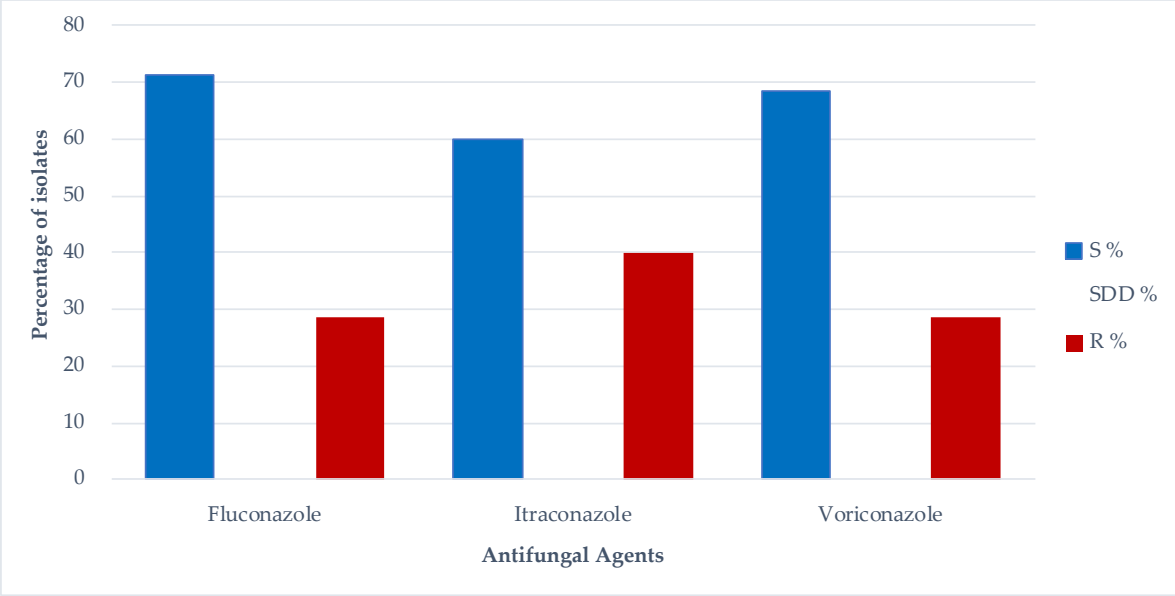

Figure 1. Showing the Azole susceptibility of *Candida albicans.*

Antifungal susceptibility testing indicated that the majority of *C albicans* isolates were susceptible to the azole antifungal agents assessed. Interestingly, all of the isolates that exhibited resistance to fluconazole also exhibited resistance to itraconazole and voriconazole. A single isolate was deemed to be susceptible dose-dependent to voriconazole.

DISCUSSION

Candidiasis, caused by various species of *Candida*, remains a persistent challenge worldwide and is an increasingly common finding in hospitalised patients. Although the epidemiology of these infections varies geographically, the most commonly isolated aetiological agent is *Candida albicans* (13, 14). In Latin America and the Caribbean, much work has been done to understand the species distribution and antifungal susceptibility profile of *Candida* causing serious cases of

candidiasis, especially those cases related to candidemia. However, in Guyana species characterisation is often limited to *Candida albicans* and information on the susceptibility profiles of these isolates is never provided.

In our study, *C albicans* was the most commonly isolated cause of candidiasis, a finding that was consistent with those of other South American and Caribbean countries (15–18). For the non-*C albicans* species, *C glabrata* was the most commonly isolated species followed by *C krusei* and *C tropicalis*. These results differ somewhat from those seen across Latin America, where *C parapsilosis*, *C tropicalis* and *C glabrata*, respectively are the most commonly isolated non-*C albicans* species (12). However, the finding of *C glabrata* as the most commonly isolated non-*C albicans* species is similar to results from a multi-center study done by Pfaller *et al* in the United States (19).

Infections with these yeasts greatly influence the choice of antifungal therapy as well as the patient's clinical outcome. Therefore, knowledge of species distribution in clinical isolates as well as their antifungal susceptibility profiles help in selecting early empirical treatment choices.

Azole antifungal agents, such as fluconazole and voriconazole, are one of three drug classes recommended for the treatment of patients with invasive candidiasis (20), with fluconazole being considered the drug of choice for initial therapy for most adult patients with candidemia (20). For this study, only the *C albicans* isolates were examined for antifungal activity against azole agents using the methodology outlined in the CLSI M44-A2 guidelines. This method was chosen because it has been shown to correlate well with testing methods that employ broth microdilution or E-test (21, 22) and is more cost-effective. Testing indicated that of the 35 *C albicans* isolated, 28.6%, 40% and 28.6% expressed resistance to fluconazole, itraconazole and voriconazole, respectively. Only one isolate expressed susceptible-dose dependence to voriconazole.

Based on these results, it was evident that our study had a higher rate of resistance to fluconazole as compared similar studies conducted by Khan *et al* in India, which showed that 12.5% of the isolates tested were resistant to fluconazole (23) and Rodero *et al* in Argentina, which showed that the rate of fluconazole resistance for *C albicans* isolates was 15.7% (24). This finding may be representative of the increased likelihood associated with harbouring fluconazole-resistant *Candida* species following fluconazole exposure, especially in Guyana, where *in vitro* resistance tests and the adequacy of dosing for this azole are not traditionally investigated.

Voriconazole, a derivative of fluconazole, is intended to be a more-effective alternative to fluconazole particularly for combatting *C albicans*-related infections (25). Coincidently, the results from our study suggested that both drugs had the same degree of effectiveness. Although the finding of similar resistance rates between fluconazole and voriconazole is not uncommon (26, 27), our results seem to underline the more serious phenomenon of cross resistance. This finding may be related to the common mode of action of these drugs, which act through inhibition of the cytochrome P450-dependent 14α-sterol demethylase, as well as the increased use of these drugs (28, 29).

For itraconazole, 40% of the isolates tested in our study exhibited resistance. This resistant rate was much higher than that of fluconazole or voriconazole, and similar to findings by other studies (30, 31). As other authors have pointed out, this higher potential for resistance may be associated with the presence of fluconazole-resistant *Candida* species and a prior exposure to fluconazole therapy (32).

A worrying finding from our study was the isolation of *C glabrata and C krusei*, particularly from cases of bloodstream infections. Importantly, these non-*C albicans* species represent potentially fluconazole-resistant isolates with some authors suggesting that this may be related to

prior, low-dose fluconazole prophylaxis either alone or in combination with amphotericin B therapy (32).

Our study was limited by the number and types of antifungal agents that were used. We focussed primarily on azole agents and did not investigate other agents that are commonly regarded as initial empirical treatment options, such as echinocandins. Also, our study was only conducted at the GPHC and makes no accommodation for *Candida* isolates from other hospitals across the country. Additionally, our focus on *Candida albicans* limited our ability to properly evaluate the clinical significance of the non-*C albicans* species.

Further studies with more clinical data, larger numbers of isolates, and more antifungal agents should be conducted on both *Candida albicans* and non-*C albicans* species to understand the true impact of infections with these organisms and identify key measures to restrict the empirical use of currently administered antifungal agents.

CONCLUSION

Our results indicate that azole resistance among *Candida albicans* should be a major concern in the hospital setting in Guyana. Therefore, improving surveillance mechanisms for detecting aetiological agents of candidiasis and conducting further investigations to understand the risk factors for acquisition of resistant strains will be essential to restricting the spread of these resistant pathogens. Furthermore, while the choice of an initial antifungal agent for empirical therapy may be a complicated one, the results from disk diffusion testing and similar methodologies offer a means of alleviating the risks associated with the administration of inappropriate therapies.

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AUTHOR'S NOTE

PC was the principal investigator, conceptualised the study, and defined the study parameters. VDR, AKR, and LAS participated in the planning and execution of the study, performed data entry and data analysis, laboratory work, and were involved in writing of the study. All authors have read and approved the final manuscript. The authors declare no conflicts of interest.

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