Antifungal susceptibility profile of yeast isolates from sterile sites at a tertiary hospital in South Africa

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Invasive infections caused by yeasts are associated with high mortality and morbidity, and resistance to antifungal agents is increasing. *Candida* spp. has emerged as the leading cause of systemic nosocomial fungal infections. The aim of this study was to identify yeast isolates from sterile site specimens to species level, and to determine their susceptibility to fluconazole and voriconazole, at the National Health Laboratory, Service Dr George Mukhari Tertiary Laboratory from March to August 2007. *Candida* isolates were identified to species level using a germ tube test and/or Api® ID 32C kits. Antifungal susceptibility testing to fluconazole and voriconazole was performed using the disc diffusion method in accordance with the Clinical and Laboratory Standards Institute guidelines. All of the *Candida* isolates were from the neonatal intensive care unit (NICU), with the exception of two. The distribution of yeast isolates was as follows: *C. krusei* (41.9%), *C. albicans* (32.3%), *C. inconspicua* (5.5%), *C. parapsilosis* (2%), *C. tropicalis* (1.5%), *C. sake* (1.5%), *C. lambica* (1.5%), and *C. valida* (0.5%). *Cryptococcus neoformans* (11%), *C. albidus* (0.5%), *Rhodotorula glutinis* (1%), and *C. humicola* (0.5%) were also isolated. Of the isolated *C. albicans*, 61% were susceptible to fluconazole. A possible *C. krusei* outbreak could have occurred in the NICU during the study period. Voriconazole was the most susceptible antifungal agent to various yeast pathogens. The results of this study on azoles susceptibility testing of yeasts show that voriconazole may prove to be a valuable alternative antifungal agent in this tertiary hospital for the treatment of infections caused by yeasts, including *Candida* spp.

Introduction

Systemic fungal infections are on the increase. *Candida* spp. is the most prevalent causative agent.[1,2] Treatment failures mostly due to underlying diseases in patients with invasive candidiasis, as well as drug resistance, have been reported from different centres. As a result, the mortality rate is high.[3-5] Furthermore, the emergence of non-albicans *Candida* spp. and the prevalence of antifungal resistance, especially against azole antifungal agents, pose a challenge for the management of patients.[6]

This prospective study was carried out at Dr George Mukhari Hospital, a tertiary academic referral hospital situated in Ga-Rankuwa, north-west of Pretoria. This hospital serves a large indigent population from three adjoining provinces viz. the northern part of Gauteng, Limpopo and part of North West. South Africa has a very high human immunodeficiency virus (HIV) burden and this predisposes infected persons to opportunistic infections, such as cryptococcal meningitis. Fluconazole and ampho-tericin B are the most frequently used antifungal agents in this hospital.

There is poor documentation of data that indicate the species distribution of yeast infections and antifungal susceptibility profiles of yeast isolates in the area. Good and continuous surveillance programmes are needed in our hospital as this will help to produce data that document gradual shift trends in the antifungal susceptibility profile. To determine this, we collected consecutive yeast isolates from sterile body sites [blood culture and cerebrospinal fluid (CSF)] specimens, in order to identify them to species level and determine their susceptibility profiles to fluconazole and voriconazole.

Methods

This study was carried over a six-month period, from March to August 2007, during which blood culture and CSF yeast isolates were collected from National Health Laboratory Service Dr George Mukhari Tertiary Laboratory.

Yeast isolates

The isolates were stored as water suspensions until use and standard methods were used for identification. Isolates were subcultured on Sabouraud Dextrose® agar (Thermo Scientific, Basingstoke, UK). Germ tube testing was performed for the identification of *C. albicans*. Germ tube-negative isolates were further identified to species level by the Api® ID 32C (bioMérieux, Johannesburg, South Africa) identification kits. *Cryptococcus gattii* isolates were differentiated from *C. neoformans* using the urease test and L-canavanine-glycine bromothymol blue medium.

Disc diffusion susceptibility testing

The in-vitro activity of the antifungal agents fluconazole (25 μg) and voriconazole (1 μg) was determined by the disc diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI)[7,8] document, M44-A. Plates were read using an electronic image analysis BIOMIC® Plate Reader System (Giles Scientific, New York, USA) which further correlates the zone diameter with the minimum inhibitory concentration (MIC). Reference strains *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, *C. tropicalis* ATCC 750 and *C. neoformans* ATCC 66031 were used for quality control.

The revised interpretive susceptibility of the fluconazole zone diameter and minimum inhibitory concentration breakpoints

C. albicans, C. parapsilosis and C. tropicalis

- ≥ 17 mm (≤ 2 μg/ml), susceptible.
- 14-16 mm (4 μg/ml), intermediate.
- ≤ 13 mm (≥ 8μg/ml), resistant).

C. krusei

- ≥ 19 mm (≤ 8 μg/ml), susceptible.
- 5-18mm (16-32 μg/ml), intermediate.
- ≤ 14 mm (≥ 64 μg/ml, resistant.[7,8]

The revised interpretive susceptibility of the voriconazole zone diameter and minimum inhibitory concentration breakpoints

C. albicans, C. parapsilosis and C. tropicalis

- ≥ 17 mm (≤ 0.125 μg/ml), susceptible.
- 15-16 mm (0.25-0.5 μg/ml), intermediate.
- ≤ 14 mm (≥ 1 μg/ml), resistant.

C. krusei

- ≥ 15 mm (≤ 0.5 μg/ml), susceptible.
- 13-14 mm (1 μg/ml), intermediate.
- ≤ 12 mm (≥ 2 μg/ml), resistant.[9,10]

The voriconazole zone diameter and MIC breakpoints for *C. glabrata* were not established because of lack of a clear relationship between the MIC data and the clinical outcomes.[10]

The demographic (age, sex and clinical) information for the patients from whom the isolates were cultured was documented from the laboratory information system, DISA.

Ethical approval

Approval for the study was obtained from the Medunsa Research and Ethics Committee, University of Limpopo (Medunsa Campus). There were no patient identity links to the specimens used. Patients were not directly involved.

Results

During the six-month study period, a total of 589 consecutive yeast isolates were collected. Duplicate isolates were excluded and 198 isolates eventually analysed. The majority were from blood culture specimens 178/198 (90%), while 10% were from CSF (Table I). With the exception of two, all blood culture isolates were from neonates admitted to the NICU.

Overall, *Candida* spp. accounted for 172/198 (86.9%), and *Cryptococcus* spp. for 22/198 (11.1%) (Table I).

C. neoformans 20/198 (10.1%) was the most prevalent species of the isolated *Cryptococcus* spp. Two *C. gattii* were isolated from young female patients. One of the patients was confirmed to be HIV-positive. The other patient's HIV status was unknown. The mean age of the patients was 33.1 years ± 28.3 standard deviation. The youngest patient was 13 years old and the oldest 62. There were more females (13/22, 59.1%) than males. CD4 T-cell counts were available for 13 patients only. All available CD4 T-cell counts were less than 200 cells/mm^3, with a range of 20-163 cells/mm^3 and a mean of 38 cells/mm^3.

C. neoformans was isolated mainly from adult patients admitted to the medical wards. The clinical data documented on most of these patients were suggestive of meningitis. Two patients had *C. neoformans* isolated from CSF, as well as blood culture. Other species of isolated *Cryptococcus* were *C. albidus* and *C. humicola*.

Candida isolates

Of the 172/198 (86.9%) *Candida* spp. isolates, the germ tube test was positive for 64 (32.3%), hence presumed to be *C. albicans*. One hundred and twelve isolates were further identified to species level using Api® ID 32 C kits. *C. krusei* was found to be the most common isolate, accounting for 83/198 (41.9%) (Table I). *Rhodotorula glutinis* 2/198 (1%) was another uncommon isolated yeast.

Table I: The distribution of yeast isolates from sterile site specimens (n = 198)

Species	Number of isolates (%)	
	Blood	CSF
Candida krusei	83 (42)	-
Candida albicans	64 (32.3)	-
Candida inconspicua	11 (5.6)	-
Candida lambica	3 (1.5)	-
Candida sake	3 (1.5)	-
Candida tropicalis	3 (1.5)	-
Candida parapsilosis	4 (2)	-
Candida valida	1 (0.5)	-
Cryptococcus neoformans[*]	2 (1)	18 (9)
Cryptococcus gattii	-	2 (1)
Cryptococcus humicola	1 (0.5)	-
Cryptococcus albidus	1 (0.5)	-
Rhodotorula glutinis	2 (1)	-
Total	**178 (90)**	**20 (10)**

CSF: cerebrospinal fluid
*Isolated from both the blood culture and the cerebrospinal fluid

Table II: Susceptibility of *Candida* isolates to fluconazole and voriconazole (n = 154)

Isolates	Fluconazole*			Voriconazole**		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Candida albicans (64)	39 (61)	20 (31.2)	5 (7.8)	60 (93.8)	3 (4.7)	1(1.5)
Candida krusei (83)	0 (0)	6 (7.2)	77 (92.8)	75 (90.4)	8 (9.6)	0 (0)
Candida tropicalis (3)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)
Candida parapsilosis (4)	4 (100)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)

I: intermediate, R: resistant, S: susceptible
* Fluconazole zone diameter and minimum inhibitory concentration breakpoints
C. albicans, *C. parapsilosis* and *C. tropicalis*
≥ 17 mm (≤ 2 µg/ml), susceptible
14-16 mm (4 µg/ml), intermediate
≤ 13 mm (≥ 8 µg/ml), resistant
C. krusei
≥ 19 mm (≤ 8 µg/ml)
15-18 mm (16-32 µg/ml)
≤ 14 mm (≥ 64 µg/ml), resistant.[7,8]
**: Voriconazole zone diameter and minimum inhibitory concentration breakpoints

C. albicans, *C. tropicalis* and *C. parapsilosis*
≥ 17 mm (≤ 0.125 µg/ml), susceptible
15-16 mm (0.25-0.5 µg/ml), intermediate
≤ 14 mm (≥ 1 µg/ml), resistant
C. krusei
≥ 15 mm (≤ .5 µg/ml), susceptible
13-14 mm (1 µg/ml), intermediate
≤ 12 mm (≥ 2 µg/ml), resistant.

Antifungal susceptibility profiles

Disc diffusion method

One hundred and ninety-eight yeast isolates were tested for susceptibility to fluconazole and voriconazole by the disc diffusion method. Table II shows the susceptibility profiles of the yeast isolates to both fluconazole and voriconazole, as determined by the CLSI guidelines.

Candida isolates were found to be more susceptible to fluconazole. *C. krusei* (100%) isolates were completely resistant to fluconazole and no resistance was detected with voriconazole in *C. krusei*. More than half of the *C. albicans* (61%) isolates were fully susceptible to fluconazole, whereas 7.8% were resistant. Only one isolate of *C. albicans* was resistant to voriconazole. All 4 (100%) *C. parapsilosis* isolates were susceptible to both fluconazole and voriconazole. Three isolates of *C. tropicalis* were fully susceptible to fluconazole, with no resistance to voriconazole (Table II).

The CLSI breakpoints are species specific. Thus, there are no clinical interpretative breakpoints for the routine susceptibility testing of uncommon *Candida* spp. and *Cryptococcus* spp. to date, but the zone diameters for fluconazole and voriconazole of uncommon species of *Candida* spp. and *Cryptococcus* spp. ranged from 6-35 mm.

Discussion and Conclusion

Nosocomial blood stream infections caused by yeasts, e.g. *Candida* spp. have been reported for more than three decades worldwide by many investigators, and have since increased substantially.[1,2,5,9,10] *C. albicans* is reported to be the most commonly isolated yeast from bloodstream infections.[2,11] Subsequently, there are increasing reports globally of infections caused by non-albicans *Candida* spp.[2,12-14] *C. albicans* was previously observed to be the predominant yeast isolated in the NICU of Dr George Mukhari Hospital (unpublished data, 2002). There has been a shift to non-albicans *Candida* spp. in this unit since 2003. This shift in epidemiology was demonstrated in this study, where *C. krusei* was the most common isolated species. Change in species from *C. albicans* to *C. krusei* could be attributed to the pressure exerted by the use of the antifungal agent, fluconazole, that may have led to the selection of isolates that were resistant or less susceptible to fluconazole.

C. krusei has been associated with nosocomial outbreaks.[5] Bloodstream infections caused by *C. krusei* are associated with a high crude mortality because of the poor response to conventional therapy.[15] Clinically, this organism is of particular importance because of its well known intrinsic resistance to fluconazole, and its decreased susceptibility, even to amphotericin B.[15]

In this study, a possible outbreak could have occurred during the period of the study, since *C. krusei* was isolated in high numbers. Currently, *C. parapsilosis* and *C. glabrata* are the most prevalent isolates from sterile sites in the study centre (unpublished data, 2013). Empiric therapy in the NICU at the study centre was switched from fluconazole to amphotericin B. Unusual *Candida* spp. isolates, e.g. *C. sake*, *C. valida* and *C. inconspicua*, were also isolated in low numbers from the blood culture of premature neonates who presented with sepsis, which is consistent with other studies.[11,16,17]

The prevalence of fluconazole resistance in non-albicans *Candida* spp. isolates varies over time in different countries. In the current study, most of the non-albicans *Candida* spp. isolates were resistant to fluconazole, but uniformly susceptible to voriconazole. As expected, all *C. krusei* isolates were resistant to fluconazole. Despite intrinsic resistance to fluconazole, most of the isolated *C. krusei* remained susceptible to voriconazole, showing lack of cross-resistance between the two azoles. Decreased susceptibility to fluconazole was shown, even in *C. albicans* isolates. Five isolates showed complete resistance. In keeping with other studies,[11,18,19] the unusual *Candida* spp. were mostly resistant to fluconazole.

Before the acquired immune deficiency sydnrome (AIDS) epidemic, cryptococcosis was a rare disease. However, now it is one of the major opportunistic infections in HIV-infected patients. Seventy-five to 90% of AIDS patients develop cryptococcal meningitis.[20] Usually, these highly immunocompromised patients have very low CD4 cell counts of < 100 cells/m³. In this study, patients with cryptococcal meningitis who were tested were HIV-positive with CD4 counts below 200 cell/m³. HIV-positive patients with meningitis due to this pathogen require lifelong maintenance prophylaxis with fluconazole,[21,22] but the duration of exposure to fluconazole in such patients raises concerns with regard to the development of fluconazole resistance. In this study, fluconazole zone diameters ranged from 17-40 mm in 9 (41%) isolates.

Although C. neoformans causes infections worldwide, mainly in immunocompromised hosts, by contrast, C. gattii is geographically restricted to tropical and subtropical regions.[23] Infections caused by C. gattii often have a worse prognosis than those caused by C. neoformans.[24-26] In the north and north-east regions of Brazil, C. gattii is endemic, prevailing in 62.7% of cryptococcosis cases.[26] C. gattii had fluconazole zone diameters of ≤ 13 mm in 2 (1%) isolates.

Generally, infections caused by C. gattii are not well studied. This makes it critical for the diagnostic laboratories to establish in-vitro susceptibility testing to antifungal drugs. The zone diameters of C. neoformans isolates to fluconazole in this study suggest the importance of determining the variant of C. neoformans infecting patients. A rare species, C. albidus,[27-29] was isolated, and showed higher zone diameters of voriconazole than those of fluconazole. The 2 (1%) isolates of Rhodotorula glutinis showed a range of 6-13 mm of zone diameter to both fluconazole and voriconazole, which is consistent with other studies.[30-33]

The germ tube method used to confirm C. albicans in this study, although accepted for non-sterile site isolates, is not sufficiently accurate when confirming these isolates as misidentifications are a distinct possibility, e.g. C. dubliniensis isolates might be misidentified as C. albicans. High proportion of C. albicans isolates were observed to be in the intermediate category. Therefore, ideally, this should be confirmed by MIC determination.

This study suggests that routine susceptibility testing is of high importance for diverse yeast pathogens isolated from sterile site specimens, and more surveys need to be conducted at Dr George Mukhari Hospital from time to time. The knowledge of the yeast genus and its species, as well as the antifungal pattern, is a useful guide to the sound management of patients. The susceptibility testing results of yeasts to azoles showed that voriconazole proved to be a valuable alternative antifungal agent for the treatment of infections caused by yeasts, including non-albicans Candida spp. Thus, consideration should be given to changing the empiric treatment of yeast infections, also bearing in mind that azole cross-resistance could progressively develop during treatment.

Acknowledgements

Declarations

Conflict of interest

The authors declare no conflict of interest.

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