

Determination of minimum inhibitory concentrations of various antifungal agents in clinical isolates of *Candida* species

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Abstract

Objective: To evaluate the occurrence and in vitro minimum inhibitory concentrations of different *Candida* species against nine antifungal agents in blood and urine.

Methods: The prospective descriptive study was conducted at The Children's Hospital, Lahore, Pakistan, from June 2015 to May 2016. Identification of the *Candida* species was done by API (Analytical Profile Index) *Candida* (bioMérieux) and in vitro minimum inhibitory concentration break points were reported by using Sensititre Yeast One. Data was analysed using SPSS 20.

Results: Of the 87 samples, 68(78.2%) were isolated from blood and 19(21.8%) from urine specimens. Also, 66 (75.9%) samples were non-albicans *Candida*, 31(35.6%) *Candida parapsilosis* and 21(24.1%) were *Candida albicans*. Besides, 83(95.4%) strains exhibited excellent susceptibility pattern with the three echinocandins at minimum inhibitory concentration endpoint of $\leq 2\mu\text{g/ml}$ and all (100%) the strains inhibited by $\leq 1\mu\text{g/ml}$ of voriconazole. None (0%) of the *C. albicans* isolates was resistant to an antifungal agent.

Conclusion: Excellent susceptibility pattern against antifungal agents was found with an increasing resistance trend towards non-albicans *Candida* species.

Keywords: *Candida albicans*, *Candida* infections, Antifungal susceptibility, Minimum inhibitory concentrations. (JPMA 69: 1131; 2019)

Introduction

Over 90,000 people in the United States (US) are affected by invasive bloodstream infection due to *Candida* (C.) species.¹ C. species are responsible for 8-10% of nosocomial bloodstream infections. C. albicans most commonly cause bloodstream infections, but increased incidence of bloodstream infections by other C. species has also been reported in studies.^{2,3} Non-albicans C. species (NAC) is mainly associated with candidaemia rather than C. albicans.⁴ Epidemiological studies of bloodstream infections due to C. species have provided the information about antifungal drug resistance, species involved and risk factors for mortality.⁵ The hospital stay of patients become longer due to candidaemia, therefore cost for medical care of patients is also increased. There is about 30-40% mortality caused by candidaemia.⁶

Anidulafungin and caspofungin have been approved for treatment of candidaemia and invasive candidiasis by US Food and Drug Administration (FDA). Micafungin is also an effective treatment for candidaemia.⁷

The minimum inhibitory concentration (MIC) is defined as the minimum amount of drug that inhibits the growth of

the organism.⁸ The standardised methods are present which provide the susceptibility of echinocandins against C. species.⁹ Recently, the Clinical and Laboratory Standards Institute (CLSI) described that the clinical efficacy of each antifungal agent relates to the MIC as it gives the MIC breakpoint for susceptibility of $\leq 2\mu\text{g/ml}$ for C. species depending on which infecting strain is involved. More than 99% of all C. species tested against each agent encompasses this breakpoint.¹⁰

Automated and semi-automated methods for antifungal susceptibility testing have been developed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) which are simple, affordable and easy to perform.^{11,12} There is a fully automated commercial method called Vitek 2 antifungal susceptibility system that indicates yeast growth spectrophotometrically.¹³

The current study was planned to find out the bloodstream and urinary tract C. infection and to determine the effective therapeutic dose for their treatment. Also, the aim was to assess the occurrence of different C. species and their MICs against various antifungal agents.

Subjects and Methods

The prospective descriptive study was conducted at The Children's Hospital, Lahore, Pakistan, on from June 2015 to May 2016, and comprised paediatric patients. After

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approval from the institutional ethics committee, a proforma was used to collect patient information, the name of *C. species* and corresponding MIC endpoints, and results were interpreted as susceptible, intermediate and resistant using CLSI guidelines.¹⁴

Blood and urine samples were collected from different wards of the hospital and were cultured using the routine microbiological methods on blood, cystine lactose electrolyte deficient (CLED) and sabouraud dextrose agar (Oxoid, UK). Incubation temperature was set at 37°C for 48-72 hours in an aerobic incubator (Mettler, Germany) and the fungal species was identified by colony morphology, biochemical characteristics and APIC andida (bioMerieux, France). The cases with positive *C. growth* were included in the study, while specimens with bacterial growth were excluded.

MIC endpoints were determined for nine antifungal agents: micafungin, caspofungin, flucytosine, posaconazole, voriconazole, itraconazole, fluconazole, anidulafungin and amphotericin B. The use of automated equipment for the antifungal MICs requires the use of costly equipment and highly skilled staff, but the current study used Sensititre Yeast One (TREK diagnostic systems, UK), which does not require the use of expensive

equipment. It is a colorimetric micro-dilution test. Each plate was incorporated with antifungal agents at appropriate dilutions and a colorimetric indicator was kept functional. Results were read manually by observing the lowest antifungal concentration showing inhibition of growth which was evidenced by no colour change. Using CLSI guideline, *C. isolates* were interpreted as resistant, intermediate and susceptible.¹⁴ The compilation and analysis of data was done using SPSS 20.

Results

Of the 87 samples isolated, 68(78.2%) were isolated from blood and 19(21.8%) from urine specimens. Overall, 66(75.9%) samples were NAC, 31(35.6%) *C. parapsilosis* and 21(24.1%) were *C. albicans* (Table-1).

Of the isolated samples, 51(58.6%) came from boys and 36(41.4%) from girls. The highest numbers of cases were

Table-1: Frequency of *Candida* species from blood and urine specimens (n=87).

<i>Candida</i> species	Blood n (%)	Urine n (%)	Total n (%)
<i>Candida albicans</i>	13 (14.9)	8 (9.19)	21 (24.1)
Non-albicans <i>Candida</i>	55 (63.2)	11 (12.6)	66 (75.9)
Total	68 (78.2)	19 (21.8)	87

Table-2: General characteristics of patients with candidal episodes.

Patient characteristics	No. (%) of episodes						
	Total	<i>C. albicans</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>	<i>C. krusei</i>	<i>C. glabrata</i>	<i>C. guilliermondii</i>
Age							
< 1 month	36 (41.4)	11 (30.5)	9 (25.0)	8 (22.2)	6 (16.7)	2 (5.5)	
1-12 months	16 (18.4)	4 (25.0)	5 (31.2)	5 (31.2)			2 (12.5)
1-15 years	35 (40.2)	6 (17.1)	17 (54.8)	4 (11.4)	6 (17.1)	2 (5.7)	
Gender							
Male	51 (58.6)	11 (21.6)	18 (35.3)	9 (17.6)	9 (17.6)	3 (5.8)	1 (1.9)
Female	36 (41.4)	10 (27.7)	13 (36.1)	8 (22.2)	3 (8.3)	1 (2.7)	1 (2.7)
Specimen							
Blood	68 (78.2)	16 (23.5)	23 (33.8)	14 (20.6)	10 (14.7)	3 (4.4)	2 (2.9)
Urine	19 (21.8)	5 (26.3)	8 (42.1)	3 (15.8)	2 (10.5)	1 (5.3)	
Location							
Neonatal nursery	36 (41.3)	12 (33.3)	16 (44.4)	4 (11.1)	1 (2.7)	2 (5.5)	1 (2.7)
MICU	13 (14.9)	1 (7.7)	5 (38.5)	7 (53.8)			
CICU	12 (13.8)	3 (25.0)	6 (50.0)	3 (25.0)			
Medical Ward	9 (10.3)	2 (22.2)	2 (22.2)		4 (44.4)		1 (11.1)
SICU	8 (9.2)	2 (25.0)		1 (12.5)	5 (62.5)		
Nephrology	4 (4.6)	1 (25.0)	1 (25.0)		1 (25.0)	1 (25.0)	
D/W	3 (3.4)			2 (66.6)		1 (33.3)	
H/O departments	2 (2.3)		1 (50.0)		1 (50.0)		
Total episodes	87	21 (24.1)	31 (35.6)	17 (19.5)	12 (13.8)	4 (4.6)	2 (2.3)

MICU: Medical Intensive Care Unit

CICU: Cardiac Intensive Care Unit

SICU: Surgical Intensive Care Unit

D/W: Developmental Ward

H/O: Haematology Oncology.

Table-3: In vitro susceptibilities of 87 candidal isolates to nine antifungal agents.

Candida species	Antifungal drug	MIC ($\mu\text{g/ml}$)		No. (%) of resistant isolates (CLSI)
		Range	GM	
<i>C. parapsilosis</i> (31)	Amphotericin B	0.12-1	0.20	0
	Anidulafungin	0.015-0.12	0.04	0
	Caspofungin	0.015-0.12	0.08	0
	Micafungin	0.008-0.25	0.03	0
	Fluconazole	0.12-1	0.22	0
	Itraconazole	0.015-0.5	0.04	0
	Voriconazole	0.008-1	0.02	0
	Posaconazole	0.015-0.5	0.07	0
<i>C. albicans</i> (21)	Flucytosine	0.06-64	0.63	2 (6.5)
	Amphotericin B	0.12-0.25	0.14	0
	Anidulafungin	0.015-0.03	0.02	0
	Caspofungin	0.03-0.06	0.04	0
	Micafungin	0.008-0.015	0.008	0
	Fluconazole	0.12-0.5	0.21	0
	Itraconazole	0.015-0.03	0.02	0
	Voriconazole	0.008-0.03	0.01	0
<i>C. tropicalis</i> (17)	Posaconazole	0.008-0.015	0.01	0
	Flucytosine	0.12-0.5	0.20	0
	Amphotericin B	0.12-0.25	0.13	0
	Anidulafungin	0.015-0.06	0.03	0
	Caspofungin	0.008-0.03	0.02	0
	Micafungin	0.008-0.03	0.02	0
	Fluconazole	0.12-2	0.69	0
	Itraconazole	0.015-0.12	0.03	0
<i>C. krusei</i> (12)	Voriconazole	0.015-0.3	0.02	0
	Posaconazole	0.088-0.06	0.02	0
	Flucytosine	0.06-64	0.43	4 (23.5)
	Amphotericin B	0.5-1	0.70	0
	Anidulafungin	0.015-0.03	0.02	0
	Caspofungin	0.015-0.06	0.11	0
	Micafungin	0.008-8	0.07	4 (33.3)
	Fluconazole	64	64	12 (100)
<i>C. glabrata</i> (4)	Itraconazole	0.015	0.015	0
	Voriconazole	0.015-0.06	0.03	0
	Posaconazole	0.008-0.06	0.015	0
	Flucytosine	0.25-4	0.79	0
	Amphotericin B	0.12	0.12	0
	Anidulafungin	0.06	0.06	0
	Caspofungin	0.06	0.06	0
	Micafungin	0.008	0.008	0
<i>C. guilliermondii</i> (2)	Fluconazole	0.12	0.12	0
	Itraconazole	0.015	0.015	0
	Voriconazole	0.015	0.015	0
	Posaconazole	0.008	0.008	0
	Flucytosine	0.12	0.12	0
	Amphotericin B	0.5	0.5	0
	Anidulafungin	0.03	0.03	0
	Caspofungin	0.03	0.03	0
	Micafungin	0.015	0.015	0
	Fluconazole	0.12	0.12	0
	Itraconazole	0.015	0.015	0
	Voriconazole	0.008	0.008	0
	Posaconazole	0.008	0.008	0
	Flucytosine	0.06	0.06	0

MIC: Minimum inhibitory concentration. GM: Geometric mean. CLSI: Clinical and Laboratory Standards Institute.

36 (41.3%) seen in the Neonatal Nursery, 13 (14.9%) in Medical Intensive Care Unit (MICU), 12(13.8%) in Cardiac ICU (CICU), 9(10.3%) in the Medical Ward and 8(9.2%) in Surgical ICU. Neonates were predominantly infected with *C. albicans*, while children aged 1-5 years were infected with *C. parapsilosis* (Table-2).

In vitro susceptibility of *C.* strains were tested against 9 antifungal agents and all (100%) isolates were susceptible at the MIC endpoint of $\leq 2\mu\text{g/ml}$, and there was little variation in the degree of susceptibility of the isolates against the three echinocandins (Table-3).

Discussion

Hospitalised patients have more threat of being infected with fungi, but infections due to NAC species have increased during the last 30 years.¹⁵ Prompt identification, diagnosis of pathogenic yeast and MIC breakpoints are essential for the treatment of candidiasis.¹⁶

In the current study, *C.* infections were higher among male patients (58.6%) compared to female (41.4%). Similar results with higher proportion of fungaemia among males (60.6%) have been reported.¹⁷ Some studies reported higher infection rate among females.¹⁸ Our findings indicated that out of the 87 *C.* cases, the most abundant species isolated was *C. parapsilosis* (35.6%), which is similar to the results of studies conducted in Saudi Arabia (44%), Italy (35.5%) and Portugal (32%) and contrary to the studies reported from India where the proportion of this species varied from 9.5-20 %.¹⁹

We observed the dominance of NAC (75.9%) species over the *C. albicans* (24.1%). Our results are contradictory to a study which reported *C. albicans* as the most prevalent cause of fungal infections among all the other *Candida* species.²⁰ The occurrence of the higher rate of NAC species has also been reported.¹⁷ We isolated only a few cases of *C. glabrata* infections, but more cases of *C. glabrata* have also been reported in Europe (19.6-27%), Saudi Arabia (9.5-17%) and the United Arab Emirates (UAE) (5%).¹⁹ Majority of strains in the current study were isolated from neonates and ICU patients which is similar to the results observed in New York.²¹ A higher number of cases with fungaemia have also been reported in patients admitted to general paediatric and surgical wards of a hospital in Spain.¹⁷

The current study performed antifungal susceptibility testing against 9 crucial agents and found the majority of the strains susceptible to these drugs. Susceptibility to fluconazole remained high with the MIC range of 0.12-2 $\mu\text{g/ml}$ despite the higher use of the drug, except *C. krusei* which is intrinsically resistant to this antifungal

agent. Other studies have also reported similar results with good susceptibility rate of fluconazole.²¹ However, the results differ from some other studies.²² Amphotericin B was the most susceptible antifungal against all *C.* strains with an MIC breakpoint of 0.12-1 $\mu\text{g/ml}$ which is consistent with a study conducted in India.²³ NAC showed significant resistance to antifungal in contrast to *C. albicans* which has also been seen in Brazil.²⁴

Overall, 95.4% strains exhibited excellent susceptibility pattern with the three echinocandins at MIC endpoint of $\leq 2\mu\text{g/ml}$ whereas only 4.6% isolates of *C. krusei* showed resistance against micafungin in the current study which also indicated that resistance to flucytosine was moderately high. The results differ from those of a study in Spain where the rate of resistance to flucytosine was significantly low.¹⁷ In the present study, all the isolates tested revealed excellent susceptibility pattern against voriconazole, itraconazole and posaconazole.

All the *C.* strains in the current study inhibited by $\leq 1\mu\text{g/ml}$ of voriconazole which has also been reported in another study.² The efficiency of itraconazole was good against *C.* isolates with the lowest MIC value. These results are contradictory to a study where 12.3% isolates demonstrated decreased susceptibility with itraconazole.¹¹

In terms of limitations, the current study was done at a single centre and only comprised paediatric patients. The molecular characterisation could not be performed due to the unavailability of funds.

Conclusions

Higher occurrence of NAC than *C. albicans* was noted. Overall, there was an excellent susceptibility pattern established against the commonly used antifungal agents. The outcome of the patients with candidaemia can be improved by the treatment of fluconazole and itraconazole. Increasing resistance to NAC species makes it essential to correctly identify the strains and report the antifungal susceptibility pattern to control the fungal infections efficiently.

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