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Research Article

Pattern of susceptibility to azoles by E test method in candidemia patients

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ABSTRACT

Background: Blood stream infections due to *Candida sp* have considerably increased in recent years, along with the increase of drug-resistant isolates in immunocompromised patients. This increase in resistance makes it important to determine the antifungal susceptibility profile of each *Candida* species isolated from blood prior to treatment. Hence, this study was done to detect the resistant strains of *Candida* causing candidemia.
Methods: About Seventy *Candida* species isolated from blood cultures were used for this study. These included 27 *Candida albicans*, 23 *Candida tropicalis*, 8 *Candida parapsilosis*, 3 *Candida krusei*, 2 *Candida glabrata* and 7 other candida species. Minimum inhibitory concentrations (MIC) of the most commonly used azoles like fluconazole, ketoconazole, itraconazole and voriconazole were determined by E test method.
Results: The resistance percentage of *Candida albicans* for fluconazole and itraconazole was 11.1% and 7.4%; fluconazole resistance in *Candida tropicals* was 8.7%. *Candida parapsilosis* had good activity against all azoles with only 12.5% resistance for itraconazole.
Conclusions: Fluconazole had good activity against most of the Candida sp except for *Candida glabrata* and *Candida krusei* with MIC 90 > 256 µg/ml. Itraconazole was less effective for *Candida albicans*, *Candida glabrata* and *Candida parapsilosis* (MIC 90 >32 µg/ml). Voriconazole was found to be the most effective drug against all species of *Candida* with low MIC values (MIC 90 < 0.25 µg/ml). Hence it can be used to treat blood stream infections caused by *Candida* species.

Keywords: *Candida* sp, E test, Fluconazole, Ketoconazole, Itraconazole, Voriconazole

INTRODUCTION

Candida sp is the fourth most common cause of bloodstream infections all over the world, which is invasive and life-threatening.[1] Invasive infections caused by *Candida* leads to increased mortality and morbidity in immunocompromised patients, in spite of recent advances in preventive, diagnostic, and therapeutic procedures. The antifungal resistance pattern in such high-risk patients is a major concern. Hence a better knowledge about the antifungal resistance pattern of such isolates becomes essential for prompt and efficient treatment to improve the outcome of such infections.[2]

If the *Candida* sp causing the invasive infection is known it is generally possible to predict its antifungal susceptibility pattern. But all the infecting isolates may not follow this general pattern.[3,4] Hence susceptibility testing should be done to aid in the management of candidiasis, particularly in situations where the patient fails to respond to the initial antifungal treatment. Expert opinion also suggests that it is essential to perform routine antifungal susceptibility testing where azole resistance is strongly suspected, like against fluconazole for *C. glabrata* isolates from blood and sterile sites.

The Clinical and Laboratory Standards Institute has published Standard reference guidelines for susceptibility testing of yeasts M27-A3.[4,5] This document provides data based interpretive breakpoints for testing the susceptibility of *Candida* species to fluconazole, itraconazole, voriconazole, flucytosine, and the echinocandins.[3,4]

Hence, this study was undertaken for detecting resistant strains causing candidemia using E-Strip by estimating their Minimum Inhibitory Concentration (MIC).This will help to provide prompt & effective antifungal therapy to the patients.

METHODS

The study was done at Sri Ramachandra Medical College and Research Institute, Chennai which is a tertiary care centre. It is a retrospective hospital based descriptive study.

Around 70 yeast isolates which grew from blood cultures were considered for this study. They were speciated using conventional phenotypic methods like gram stain, germ tube production, growth on TTZ(Tetrazolium reduction medium) and sugar fermentation and assimilation studies. *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were quality controls used for all antifungal agents, each time antifungal susceptibility test was performed.

E test method

MIC of the isolates were determined by using agar based E test method using Mueller-Hinton agar supplemented with 2% glucose and 0.5% µg/ml methylene blue (MH-GMB). This media is already proven to work well for determining the MIC of azoles by E test.[6] MH- GMB agar plates were inoculated by dipping a sterile swab into the inoculum suspension(adjusted to 0.5 McFarland standard i.e. 10^6 cells/ml) and streaking it along the agar surface in four directions to spread it as a lawn culture . The agar plates were then dried for sometime before applying the E test strips. The plates were then incubated at 35^0C for 24 – 48 hours or until visible growth was seen. The MIC value was read at the point of intersection between the zone edge and the E-test strip, if the end point was clear. When growth occurs along the entire strip i.e. no inhibition ellipse is seen, the MIC was reported as more than the highest value on the MIC scale. When the inhibition ellipse was below the strip i.e. the zone edge did not intersect the strip, the MIC was reported to be less than the lowest value on the MIC scale.[7]The organisms were categorized into susceptible (S), susceptible dose dependant (SDD) and resistant (R) based on the MIC reading. For azoles, significant inhibition, 80% decrease in growth density is required to visually select the end point.[8] Interpretation was done as per CLSI M27-A3 document guidelines.

MIC50 and MIC90 (the MIC at which 50% and 90% of the isolates are inhibited) were also calculated.[9]

RESULTS

A total of 70 *Candida* spp isolated from blood were used for this study. *C. albicans* (39%) was the most common species isolated followed by *C. tropicalis* (33%), *C. parapsilosis* (11%), *C. krusei*(4%), *C .glabrata*(3%) and other *Candida* sp (10%) (Table 1).

Table 1: Distribution of candida species isolated from blood.

Species	No. of samples	Distribution
Candida albicans	27	39%
Candida tropicalis	23	33%
Candida parapsilosis	8	11%
Candida krusei	3	4%
Candida glabrata	2	3%
Other Candida species	7	10%
Total samples	70	

Out of the 27 *Candida albicans* isolated, 26(96.3%) isolates were susceptible to voriconazole, 24(88.9%) to fluconazole, and 21 (77.8%) to itraconazole. Among the 23 strains of *Candida tropicalis*, 23(100%) were susceptible to voriconazole, 21(91.3%) to fluconazole and about 18 (78.3%) to itraconazole. Among the eight isolates of *Candida parapsilosis*, all the eight (100%) were susceptible for voriconazole and fluconazole and 4 (50%) to itraconazole. Out of the 3 *Candida krusei*, all of them (100%) were susceptible to voriconazole and 1 (33.3%) was susceptible and 2(66.7%) were in susceptible dose dependent category to itraconazole. None of the *Candida krusei* were susceptible to fluconazole, one isolate (33.3%) was in the susceptible dose dependent category and rest of the 2 (66.7%) were resistant to fluconazole. Among the 2 *Candida glabrata* strains both were susceptible to voriconazole and resistant to itraconazole and fluconazole respectively (Table 2).

The MIC ranges and MIC 50, MIC 90 with their resistant percentage for different Candida isolates to the azoles are listed in Table 3.

Fluconazole*:* The MIC 90 for *Candida krusei* and *Candida glabrata* was found to be high (>256µg/ml) with highest resistance percentage of 66.7% and 100% respectively. The MIC 90 for *Candida albicans, Candida parapsilosis, Candida tropicalis* were 4, 6, 1 µg/ml

respectively. *Candida parapsilosis* was not found to be resistant to fluconazole whereas *Candida albicans* and *Candida tropicalis* showed lesser resistance percentage of 11.1 and 8.69 respectively.

Table 2: Antifungal susceptibility pattern of candida isolates to azoles by E-test.

Species (No. of isolates)	Antifungal Agents	No. (%) of isolates		
		Susceptible	Susceptible dose dependent	Resistant
Candida albicans (27)	F	24(88.9)	-	3(11.1)
	V	26(96.3)	-	1(3.7)
	I	21(77.8)	4(14.8)	2(7.4)
Candida tropicalis (23)	F	21(91.3)	-	2(8.7)
	V	23 (100)	-	-
	I	18(78.3)	5(21.7)	-
Candida parapsilosis (8)	F	8(100)	-	-
	V	8(100)	-	-
	I	4(50)	3(37.5)	1(12.5)
Candida krusei (3)	F	-	1 (33.3)	2(66.7)
	V	3(100)	-	-
	I	1(33.3)	2((66.7)	-
Candida galbrata (2)	F	-	-	2 (100)
	V	2(100)	-	-
	I	-	-	2(100)

F- Fluconazole; V- Voriconazole; I- Itraconazole

Voriconazole*:* The MIC 90 for *Candida albicans*, *Candida tropicalis* was found to be 0.25 µg/ml and for *Candida paropsilosis*, *Candida krusei* and *Candida glabrata* were 0.047µg/ml, 0.19µg/ml and 0.125µg/ml respectively. None of the *Candida* sp isolated were resistant to voriconazole, whereas *Candida albicans* had a resistance percentage of 3.7%.

Ketoconazole: The MIC 90 for, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei* was 0.19 µg/ml whereas for *Candida albicans* and *Candida glabrata* were (0.50 and 2) µg/ml. None of them showed resistance to ketoconazole.

Itraconazole: The MIC90 for Candida glabrata was >32µg/ml with all the strains showing resistance to itraconazole (100%). The MIC90 values for *Candida albicans*, *Candida krusei* was found to be 0.50µg/ml and for *Candida tropicalis*, *Candida parapsilosis* it was found to be 0.38 and 0.75 µg/ml. None of the *Candida tropicalis* or *Candida krusei* were found to be resistant to itraconazole, whereas *Candida albicans*, *Candida parapsilosis* showed a minimum resistance of 7.4%, 12.5% respectively.

Table 3: Distributions of MIC by E test method.

Antifungal agents & Candida spp. (no.)	MIC (µg ml^{-1})			Percentage resistant
	50%	90%	Range	
Fluconazole				
Candida albicans **(27)**	0.50	4	0.125 - >256	11.1 %
Candida tropicalis **(23)**	0.75	6	0.19 - >256	8.69 %
Candida parapsilosis **(8)**	0.75	1	0.50 - 1.5	0
Candida krusei **(3)**	48	>256	24 - >256	66.7 %
Candida glabrata **(2)**	8	>256	8 - >256	100%
Voriconazole				
Candida albicans **(27)**	0.047	0.25	0.008 - >32	3.70 %
Candida tropicalis **(23)**	0.064	0.25	0.012 - 0.25	0
Candida parapsilosis **(8)**	0.032	0.047	0.023 - 0.064	0
Candida krusei **(3)**	0.125	0.19	0.125 - 0.19	0
Candida glabrata **(2)**	0.064	0.125	0.064 - 0.125	0
Ketoconazole				
Candida albicans **(27)**	0.094	0.50	0.016 - 0.75	0
Candida tropicalis **(23)**	0.047	0.19	0.016 - 0.125	0
Candida parapsilosis **(8)**	0.032	0.19	0.016 - 0.75	0
Candida krusei **(3)**	0.125	0.19	0.125 - 0.19	0
Candida glabrata **(2)**	0.047	2	0.047 - 2	0
Itraconazole				
Candida albicans **(27)**	0.094	0.50	0.012 - >32	7.40 %
Candida tropicalis **(23)**	0.094	0.38	0.012 - 1	0
Candida parapsilosis **(8)**	0.19	0.75	0.19 - >32	12.5 %

Candida krusei **(3)**	0.25	0.50	0.19 - 0.50	0
Candida glabrata **(2)**	2	>32	2 - >32	100%

DISCUSSION

Blood stream infections due to *Candida* sp have increased worldwide, which are major contributors of high mortality. Majority of them are due to *Candida albicans*, although *non albicans Candida* causing blood stream infections have also increased.[10] The rise may be attributed to the increased use of azoles.[11] This study was undertaken in view of recent increase in *non-albicans Candida* species causing blood stream infections and the changing pattern of their antifungal susceptibility profile.

In this study, the antifungal susceptibility testing was performed using agar based E test method (MH GMB), as there are few reports about the effectiveness of this method[6] and good agreement between the E-test and the broth micro-dilution method.[6,12]

In our study the majority of the isolates were found to be *Candida albicans* (39%), the next most common isolate was *Candida tropicalis* (33%) followed by *Candida parapsilosis* (11%). In previous studies *Candida parapsilosis* was next to *Candida*,[10,13] whereas in few Indian studies *Candida tropicalis* was the most common species isolated followed by *Candida albicans.*[14,15] The distribution of the species is different in various regions and studies. Our data also suggested that *non-albicans Candida* (61%) was the predominant species when compared to *Candida albicans* (39%), which establishes the importance of *non-albicans Candida* in causing blood stream infections. This increase may be attributed to intensive, long term use of antifungals which may in turn contribute to their high level resistance.[16-18]

Susceptibility of *Candida albicans* to fluconazole in this study was 88.9% (MIC 90 $\leq$ 4µg/ml) comparable with susceptibility pattern of other studies(79, 87.5).[20,21] *Candida albicans* , *Candida tropicalis* and *Candida parapsilosis* had decreased resistance to azoles when compared to other *Candida* sp. Different rates of resistance were detected in different *non albicans Candida* sp.[22,23] *Candida krusei* and *Candida glabrata* had increased resistance to fluconazole (66.7 and 100%) with MIC 90 >256 µg/ml. *Candida glabrata* had increased resistance to both fluconazole and Itraconazole (100%). From the perspective of antifungal resistance, *Candida glabrata* and *Candida krusei* are clearly the *Candida* species with the greatest potential to acquire resistance to fluconazole and Itraconazole.[22,24]

All the *Candida* species exhibited a very good activity against voriconazole except for one isolate of Candida albicans (resistance of 3.7%). Voriconazole was found to be an effective azole among all the Candida isolates tested, with the MIC90 being $\leq$ 0.25µg/ml. Susceptibility of voriconazole was similar to that seen in other surveillance studies done by Hoda et al.; Marshall lyon et al. and Madhavan et al.[10,25,26] Though ketoconazole also had a very good activity against *Candida* species they can be used to treat only mucocutaneous infections.

Interestingly in our study, isolates of Candida exhibiting resistance to fluconazole and itraconazole were sensitive to voriconazole which proves it as an effective azole. These findings implies that voriconazole, due to its wider species coverage, can be used in the treatment of candidemia caused by fluconazole resistant strains. This was also confirmed in the previous studies done by Madhavan et al. (2010) and Hoda et al (2009).[26,10]

Due to the increase in resistant *non albicans Candida sp* causing blood stream infections, it is mandatory to know the antifungal susceptibility pattern of commonly available antifungal agents for effective management of patients. Though newer antifungal agents are available and effective, they are very expensive and may not be affordable for all patients.

The findings of the in-vitro resistance pattern of the different Candida species implies that antifungal susceptibility should be carried out routinely for all invasive infections caused by Candida species to detect the emergence of antifungal resistance.

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