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Original Research Article

In- vitro susceptibility of fluconazole resistant candidemial isolates to mycafungin and anidulafungin

R.Deepa[1]*, P.Sunil Rao[2] and Subbanayya Kottigade[3]

[1]Asst. Professor, Travancore Medical College, Kollam, India
[2]Professor & HOD, Yenepoya Medical College, Mangalore, India
[3]Professor, K.V.G Medical College, Sullia, India
Corresponding author

K e y w o r d s

Echinocandins; Candida; Antifungal susceptibility; Mycafungin; Anidulafungin; Fluconazole.

A B S T R A C T

Echinocandins like Mycafungin and Anidulafungin exhibitis broad-spectrum activity against *Candida* spp .The intrinsic or acquired resistance of *Candida* species to fluconazole is of increasing nowadays. We determined the MICs of Micafungin and Anidulafungin against 21 bloodstream isolates of Fluconazole-resistant *Candida* species obtained from medical centers between 2011 and 2013. MICs were determined using E test method according to the CLSI reference method M27-A2. RPMI 1640 was used as the test medium, and we used the MIC endpoint of prominent growth reduction at 24 h. Among the 21 Fluconazole-resistant *Candida* isolates,5 (23%) were *C. krusei*, 6 (29%) were *C. glabrata*, 4 (19%) were *C. albicans*, and C tropicalis, 2(10%) were C. parapsilosis. Micafungin and Anidulafungin had good in vitro activity against all Fluconazole-resistant *Candida* species tested, the MICs at which 50% (MIC50) and 90% (MIC90) of isolates were inhibited were 0.03 µg/ml and 0.06 µg/ml, respectively. All the Fluconazole-resistant *Candida* spp. were inhibited at a Micafungin MIC that was <1 µg/ml. So Echinocandins like Micafungin and Anidulafungin has excellent in vitro activity against 21 candidemial isolates of fluconazole-resistant *Candida* species.

Introduction

Over the two decades, Non-albicans *Candida* represented 10-40% of all candidemia and the ratio of Non-albicans *Candida* species among *Candida* species is increasing Gudlaugsson *et al*., (2003).“Little is known about the incidence of Echinocandin- resistance in Candida species. The Echinocandins (Anidulafungin [ANF], Caspofungin [CSF], and Micafungin [MCF])are lipopeptide antifungal agents that inhibit the synthesis of β 1,3-d-glucan in the fungal cellwall and exhibit concentration-dependent fungicidal activity against most species of Candida Cappelletty (2007), Chandrasekar and Sobel (2006).

Echinocandins inhibit β 1,3-d-glucan synthase (GS),which catalyzes the biosynthesis of β 1, 3-d-glucan, the major glucan component of *Candida* cell walls Douglas(2001).We examined the MICs of Micafungin and Anidulafungin against 21 bloodstream isolates of fluconazole-resistant *Candida* species. MICs were determined using E test method according to the CLSI reference method M27-A2.

Materials and Methods

Over the two decades, *Non-albicans Candida* represented 10-40% of all candidemia and the ratio of Non-albicans *Candida* species among *Candida* species is increasing Gudlaugsson et al (2003). Little is known about the incidence of Echinocandin-resistance in Candida species.The Echinocandins (Anidulafungin [ANF], Caspofungin [CSF], and Micafungin [MCF]) are lipopeptide antifungal agents that inhibit the synthesis of β 1,3-d-glucan in the fungal cellwall and exhibit concentration-dependent fungicidal activity against most species of Candida Cappelletty (2007), Chandrasekar and Sobel (2006). Echinocandins inhibit β 1,3-d-glucan synthase(GS),which catalyzes the biosynthesis of β 1, 3-d-glucan, the major glucan component of *Candida* cell walls Douglas(2001).We examined the MICs of Micafungin and Anidulafungin against 21 bloodstream isolates of fluconazole-resistant *Candida* species. MICs were determined using E test method according to the CLSI reference method M27-A2.

Results and Discussion

The susceptibilities of Anidulafungin, and Micafungin to 21 Fluconazole resistant *Candida* species from blood stream infections were determined. From the E test readings at 24 h, MIC 90 values for Anidulafungin and Micafungin were 0.004, and 0.008 µg/ml, for *C. albicans*; 0.004, and 0.008 µg/ml for *C. tropicalis*, 0.008 and 0.008 µg/ml, for *C. glabrata*. No resistance was detected for these species at 24hours.

C. parapsilosis MIC values were relatively high, especially for Anidulafungin. Among the Fluconazole-resistant *Candida* spp. tested, *C. glabrata* exhibited Micafungin MICs (MIC90, <0.016µ g/ml), followed by *C. albicans* (MIC90, 0.07 µg/ml) and *C. krusei* (MIC90, 0.06 µg/ml).

The two Echinocandins like Micafungin and Anidulafungin has excellent in vitro activity against 21 invasive clinical isolates of Fluconazole-resistant *Candida* species. Micafungin and Anidulafungin exhibited potent activity against azole-resistant *Candida* species. Pfaller *et al*.(2010a) Ostrosky-Zeichner *et al*., (2003) who reported similar activity of Micafungin against *Candida* spp despite using a more prolonged incubation time of 48 h. It is now well established that cross-resistance between Echinocandins and Fluconazole does not exist Pfaller and Diekema, (2007); Richards et al., (2008); Silver et al., 2008); Reboli et al., (2007); Pfaller et al., (2011). The fluconazole resistance and echinocandins sensitive that was observed in our study was considered a consequence of increased drug use ,so a close monitoring in the future has been strongly recommended.

The two Echinocandins like Micafungin and Anidulafungin has excellent in vitro activity against 21 Fluconazole-resistant *Candidemial* isolates. The sporadic

Table.1 Micafungin and Anidulafungin MIC profiles of Fluconazole resistant Candida isolates by E TEST read at 24 hrs

Species	No. of isolates tested	Antifungal agent	Range	MIC (µg/ml)											
				0.002	**0.007**	**0.016**	**0.032**	**0.064**	**0.125**	**0.25**	**0.50**	**1**	**2**	**4**	**8**
C.albicans	4	Micafungin	0.007–0.25		4										
		Anidulafungin	0.002–0.016		1	3									
C. tropicalis	4	Micafungin	0.008–0.5		3	1									
		Anidulafungin	0.008–2			3	1								
C. glabrata	6	Micafungin	0.007–0.06	1	5										
		Anidulafungin	0.004–0.016				6								
C. krusei	5	Micafungin	0.064–0.125					5							
		Anidulafungin	0.016–0.032		4	1									
C.parapsilosis	2	Micafungin	0.125–32								2				
		Anidulafungin	0.125–32									2			

occurrence of breakthrough infections associated with acquired resistance mechanisms is becoming increasingly recognized. Antifungal susceptibility testing of the Echinocandins against *Candida* species is becoming recognized as a useful aid in optimizing treatment of Candidemia and testing should be carried out routinely for all invasive infections caused by *Candida* species and other opportunistic fungal pathogens to monitor epidemiological trends and to detect the emergence of antifungal resistance.

The E-test procedure seems to be a feasible and trustworthy alternative. Standardized MIC of these agents can be performed accurately in the routine clinical laboratory providing real-time results for difficult clinical infections.

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