ACTIVITY OF TRIAZOLES AND ECHINOCANDINS AGAINST CANDIDA BLOODSTREAM ISOLATES AT PHRAMONGKUTKLAO HOSPITAL, THAILAND

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Abstract

Background: Candidemia is a major cause of morbidity and mortality which can be treated using antifungal agents, triazoles and echinocandins.

Objectives: We aimed to determine *Candida* species and their sensitivities to triazoles (fluconazole, itraconazole, voriconazole, and posaconazole) and echinocandins (caspofungin, micafungin, and anidulafungin) among patients with candidiasis to guide future treatment of patients with candidemia or invasive candidiasis.

Methods: All firstly isolated *Candida* spp. from patients admitted at Phramongkutklao Hospital, Bangkok, Thailand from January 2012 to December 2013 were included in this study. The antifungal susceptibility testing of *Candida* spp. isolates was assessed based on micro-dilution method.

Results: During the 24-month study period, a total of 66 *Candida* isolates from 66 patients were identified. Of the 66 isolates, 35 (53%) were *C. albicans*, 18 (27.3%) were *C. tropicalis*, 10 (15.2%) were *C. glabrata* and 3 (4.5%) were *C. parapsilosis*. Fluconazole resistant *Candida* isolates were found in *C. glabrata* (100%), *C. albicans* (14.3%), *C. tropicalis* (22.2%) and *C. parapsilosis* (66.7%). Most *Candida* spp. isolates were mainly susceptible to echinocandins (>90%). Notably, 10%-20% of *C. glabrata* isolates showed resistance to echinocandins.

Conclusion: Fluconazole, an empirical therapy, has been cautiously used due to resistant non-albicans *Candida* species especially, *C. glabrata*, *C. tropicalis* and *C. parapsilosis*. However, the emerging echinocandins resistant *C. glabrata* isolates need to be closely monitored.

Keywords: Triazoles, Echinocandins**,** *Candida* spp., bloodstream infection

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Introduction

At present, the incidence of fungal infections has been increasing due to an increase of immunocompromised patients regarding cancer, organ transplantation and human immunodeficiency virus (HIV). *Candida* is one of the most common pathogenic fungi causing invasive and noninvasive infections.[1] A meta-analysis of epidemiological studies from Europe from January 2000 to February 2019 showed that invasive candidiasis, particularly candidemia, was associated with high morbidity and mortality rates. [2] A 30-day mortality rate of candidemia among hospitalized patients was approximately 40%. Candidiasis may cause infection in the liver, spleen and brain. *Candida* infections of the liver or spleen have been major complications among patients with neutropenic cancer.[1]

C. albicans is the most common *Candida* species isolated from clinical specimens. However, prevalence of nonalbicans *Candida* species has been increasing during the past decade. Among nonalbicans *Candida* species, prevalence of *C. glabrata* and *C. krusei* infections remained unchanged while those of *C. parapsilosis* and *C. tropicalis* infections have been increasing.[3] Prevalence of *C. glabrata* and *C. krusei* which harbor intrinsic resistance to triazole antifungul drugs, such as fluconazole [1] have been increasing from 4.9% in 2001 to 12.3% in 2010.[4] Thus, changing etiological agents may affect empiric treatment of invasive candidiasis.

From 1999 to 2002, a study at Siriraj Hospital, Thailand, revealed *Candida* infections totaled 44.6% while nonalbicans *Candida* infections totaled 55.4%.[5] The study showed that *C. albicans*, *C. tropicalis* and *C. parapsilosis* isolates were universally susceptible to fluconazole. However, itraconazole resistant isolates were detected from 16.7 to 19.8% whereas *C. glabrata* isolates were predominately resistant to fluconazole. From 2013 to 2015, a multi-center prospective observational study was conducted in seven countries in Asia/Pacific region; the results showed approximately one fourth of *C. tropicalis* isolates was not susceptible to fluconazole and voriconazole. [6] Moreover, approximately 5% of *C. glabrata* were nonsusceptible to caspofungin, micafungin and anidulafungin.

As described above, the study of antifungal susceptibility in Thailand was limited, so this study aimed to determine *Candida* species and activity of triazoles and echinocandins against *Candida* spp. bloodstream isolates to represent the situation of antifungal options for invasive candidiasis treatment.

Methods

This study was reviewed and approved by the Ethics Committee of the Medical Department of the Royal Thai Army (approval no. S029b/57).

Fungal strains

All strains of *Candida* spp. isolated from blood specimens of patients admitted at Phramongkutklao Hospital, Bangkok, Thailand from January 2012 to December 2013 were obtained. All firstly isolated clinical *Candida* spp. isolates in each patient were included. *Candida* isolates were cultured on blood agar at 35^0C and species of *Candida* colonies were identified using colony characteristics, germ tube test, and differentiation on CHROMagar accompanied with biochemical tests using a conventional method. In addition, all isolates were processed for matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) identification. The pure clinical *Candida* isolates were kept at -70^0C until the antifungal susceptibility test. The regrowth of kept isolates for further testing were cultured on blood agar at 35^0C from 24 to 48 hours to obtain a pure colony.

Determining antifungal susceptibility

Antifungal susceptibility testing of *Candida* spp. isolates was assessed using Sensititre® YeastOne (Thermo Scientific, IL, USA) based on micro-dilution method. The Minimal Inhibition Concentration (MIC) values of tested antifungals obtained from commercial kit test consisted of triazole antifungul drugs (fluconazole, itraconazole, posaconazole, voriconazole) and echinocandins, a class of antifungal drugs (caspofungin, micafungin and anidulafungin). The MIC that inhibited the growth of *Candida* spp. isolates was determined by changes in the Alamar blue color. For the quality control antifungal test, *C. parapsilosis*

ATCC 22019 and *C. krusei* ATCC 6258 were used as the reference strains according to the Clinical and Laboratory Standards Institute (CLSI) Version M27.

For the MIC breakpoint regarding susceptibility interpretation, we used the epidemiologic cut-off values (ECVs) and clinical breakpoints based on the CLSI M59 2nd edition and CLSI M60 version, respectively. In the case of lacking breakpoints based on CLSI, we used the clinical breakpoints and ECVs based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2019 guidelines.

Results

During a 24-month study period, 66 *Candida* specimens were clinically isolated. Of these, 35 (53%) were *C. albicans*. Other nonalbicans *Candida* isolates comprised the following: *C. tropicalis* (18, 27.3%), *C. glabrata* (10, 15.2%) and *C. parapsilosis* (3, 4.5%). Generally, *C. glabrata* isolates were mostly resistant to triazoles such as fluconazole (susceptible rate 0%) and posaconazole (susceptible rate 40%), except itraconazole (susceptible rate 90%) and voriconazole (susceptible rate 90%). *C. tropicalis* was resistant to fluconazole and itraconazole with resistant rates of 22.2 and 33.3%, respectively. However, *Candida* spp. isolates were mainly susceptible to echinocandins with susceptible rates from 80 to 100% (**Table 1**).

Not all *C. albicans* isolates were susceptible to fluconazole. The results of MIC range, MIC50 and MIC90 for fluconazole against *C. albicans* were as follow: 0.12 to >256 µg/mL, 1 µg/mL and 128 µg/mL, respectively. Moreover, MIC range, MIC50 and MIC90 for fluconazole against *C. tropicalis* were: 0.12 to 8 µg/mL, 1 µg/mL and 4 µg/mL, respectively. **Table 1** shows the ranges of antifungal MIC values against each *Candida* spp. isolate**.**

Discussion

Studies of *Candida* bloodstream isolates among patients admitted in tertiary care hospitals have been reported in Thailand. From 1999 to 2002, the prevalence of *C. albicans* comprised 44.6% while nonalbicans *Candida* spp. isolates accounted for 55.4% [5] Additionally, from 2004 to 2009, *C. albicans* and nonalbicans *Candida* accounted for 40.3 and 59.7%, respectively.[7] Prevalence of non-albicans *Candida* species over *C. albicans* was also similar to recent reports among countries in the Asia-Pacific region[6, 8]

This study reported on the species distribution of *Candida* bloodstream isolates among patients admitted in Phramongkutklao Hospital and their antifungal susceptibilities from 2012 to 2013. During the study period, 66 *Candida* isolates were identified. Our study showed that *C. albicans* was the most predominant species followed by *C. tropicalis*, *C. glabrata* and *C. parapsilosis*. In contrast to a study of *Candida* species isolated from patient blood samples at Siriraj Hospital, Bangkok, Thailand from January 2016 to December 2017, *C. tropicalis* was the most predominant, followed by *C. albicans*, *C. glabrata* and *C. parapsilosis*. Species distribution of *Candida* spp. directly affected the optimal candidemia treatment. Thus, the close monitoring and surveillance in each hospital setting was important to appropriately design institutional guidelines for empiric treatment of invasive *Candida* infection.

According to 2016 clinical guidelines to manage candidiasis by the Infectious Diseases Society of America, either nonneutropenic or neutropenic patients, receiving a diagnosis of invasive candidiasis, are strongly recommended to undergo echinocandins treatment as first line empirical therapy. However, fluconazole should be used as an alternative drug in the case of non-critically ill or nonsuspected infection with fluconazole-resistant *Candida* spp., especially *C. krusei* or *C. glabrata* infections.[10] Our study showed that *C. glabrata*, accounted for 15% of all *Candida* bloodstream isolates, and was universally resistant to fluconazole. Moreover, fluconazole-resistant *Candida* spp. were detected in *C. albicans* (14.3%) and *C. tropicalis* (22.2%) isolates. Additionally, a recent study using *Candida* spp. isolated from blood samples collected from 2016 to 2017 reported that fluconazole resistance was significantly increased in *C. tropicalis* (37.8%). [11] Thus, treatment using fluconazole was concerned for fluconazole-resistant *C. tropicalis*.

Table 1. Minimum Inhibitory Concentration (MIC) distribution and susceptible rate (%) among *Candida* spp. isolates (N=66)

Antifungals	MIC (µg/mL)																	Susceptible rate (%)	Susceptible breakpoint (µg/mL)
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256		
Fluconazole																			
Candida albicans (n=35)	-	-	-	-	3	12	1	10	4	-	1	-	1	-	1	-	2	85.7	≤ 2[a]
Candida tropicalis (n=18)	-	-	-	-	2	1	2	9	-	3	1	-	-	-	-	-	-	77.8	≤ 2 [a]
Candida glabrata (n=10)	-	-	-	-	-	-	-	-	-	-	-	4	4	2	-	-	-	0	≤ 0.002[b]
Candida parapsilosis (n=3)	-	-	-	-	1	-	-	-	-	2	-	-	-	-	-	-	-	33.3	≤ 2[a]
Itraconazole																			
Candida albicans (n=35)	-	1	10	10	7	6	1	-	-	-	-	-	-	-	-	-	-	60	≤ 0.064[b]
Candida tropicalis (n=18)	-	1	-	4	7	3	3	-	-	-	-	-	-	-	-	-	-	66.7	≤ 0.125[b]
Candida glabrata (n=10)	-	-	-	-	-	-	2	7	-	-	-	1	-	-	-	-	-	90	≤ 4[c]
Candida parapsilosis (n=3)	-	1	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	66.7	≤ 0.125[b]
Voriconazole																			
Candida albicans (n=35)	17	3	3	8	3	-	-	1	-	-	-	-	-	-	-	-	-	97.1	≤ 0.12[a]
Candida tropicalis (n=18)	3	-	3	8	4	-	-	-	-	-	-	-	-	-	-	-	-	100	≤ 0.12[a]
Candida glabrata (n=10)	-	-	-	-	-	3	6	-	-	-	1	-	-	-	-	-	-	90	≤ 1[d]
Candida parapsilosis (n=3)	1	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	66.7	≤ 0.12[a]
Posaconazole																			
Candida albicans (n=35)	1	12	5	1	8	3	-	4	1	-	-	-	-	-	-	-	-	54.3	≤ 0.064[b]
Candida tropicalis (n=18)	1	-	-	1	5	7	-	4	-	-	-	-	-	-	-	-	-	11.1	≤ 0.064[b]
Candida glabrata (n=10)	-	-	-	-	-	-	-	4	1	4	1	-	-	-	-	-	-	40	≤ 1[d]
Candida parapsilosis (n=3)	1	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	66.7	≤ 0.064[b]
Caspofungin																			
Candida albicans (n=35)	-	-	15	15	5	-	-	-	-	-	-	-	-	-	-	-	-	100	≤ 0.25[a]
Candida tropicalis (n=18)	-	-	13	1	4	-	-	-	-	-	-	-	-	-	-	-	-	100	≤ 0.25[a]
Candida glabrata (n=10)	-	-	-	3	5	1	-	1	-	-	-	-	-	-	-	-	-	80	≤ 0.12[a]
Candida parapsilosis (n=3)	-	-	1	-	1	-	1	-	-	-	-	-	-	-	-	-	-	100	≤ 2[a]

Table 1. Minimum Inhibitory Concentration (MIC) distribution and susceptible rate (%) among *Candida* spp. isolates (N=66) (ext.)

Antifungals	MIC (µg/mL)																	Susceptible rate (%)	Susceptible breakpoint (µg/mL)
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256		
Micafungin																			
Candida albicans (n=35)	12	10	11	1	-	-	1	-	-	-	-	-	-	-	-	-	-	97.1	≤ 0.25[a]
Candida tropicalis (n=18)	1	9	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	100	≤ 0.25[a]
Candida glabrata (n=10)	-	9	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	90	≤ 0.06[a]
Candida parapsilosis (n=3)	-	-	1	-	-	-	-	1	1	-	-	-	-	-	-	-	-	100	≤ 2[a]
Anidulafungin																			
Candida albicans (n=35)	-	8	11	4	9	0	0	2	1	-	-	-	-	-	-	-	-	91.4	≤ 0.25[a]
Candida tropicalis (n=18)	-	7	5	5	1	0	0	0	0	-	-	-	-	-	-	-	-	100	≤ 0.25[a]
Candida glabrata (n=10)	-	0	9	0	0	1	0	0	0	-	-	-	-	-	-	-	-	90	≤ 0.12[a]
Candida parapsilosis (n=3)	-	0	0	1	0	0	1	0	1	-	-	-	-	-	-	-	-	100	≤ 2[a]

For the MIC breakpoint for susceptibility interpretation, [a] clinical breakpoints were based on the Clinical and Laboratory Standards Institute (CLSI); [b] epidemiologic cut-off values were based on CLSI; [c] clinical breakpoints were based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST); [d] and epidemiologic cut-off values were based on the EUCAST.

Consequently, this study showed over 80% of *Candida* spp. isolates remained susceptible to caspofungin, micafungin, and anidulafungin. As a result, echinocandins seemed to be the preferable choice for *Candida* bloodstream infection. However, 20% of *C. glabrata* isolates were resistant to caspofungin compared with 5% reported from countries in the Asia/Pacific region.[6] Thus, further studies need to closely monitor the echinocandins resistant Candida species when echinocandins has been used as empirical therapy.

The oretically, MICs to echinocandins against *C. parapsilosis* usually tend to be higher due to intrinsic resistance that should lead to less successful treatment by echinocandins. *C. parapsilosis* isolates still comprised echinocandins susceptible strains. In Spain, among 200 episodes of *C. parapsilosis* bloodstream infection, initial use of an echinocandin-based regimen had no impact on clinical failure.[12] Thus, echinocandins remains a preferably empiric choice regarding *C. parapsilosis* infection as one of the etiologic candidemia.

In this study, results were obtained from in vitro assay of antifungal activity against identified *Candida* spp. Practically, for each healthcare setting, selection of antifungal therapy has to be based on *Candida* species identification, antifungal susceptibility pattern, patients' severity of illness and underlying diseases, and co-administered medications including certain drugs prescribed in some patients' conditions.

Conclusion

Prevalence of *Candida* bloodstream species and the role of echinocandins as empirical therapy were investigated. Due to drug resistance of nonalbicans *Candida* spp., especially, C. glabrata, *C. tropicalis* and *C. parapsilosis,* fluconazole, an alternative choice for invasive candidiasis, should be used cautiously. Echinocandins remains the preferable choice for candidemia; however, the increase of echinocandins resistant *C. glabrata* isolates needs to be closely monitored.

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