

# Genotyping and Antifungal Drug Susceptibility of the Pathogenic Yeast *Trichosporon asahii* Isolated from Thai Patients

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Received: 10 March 2009 / Accepted: 29 June 2009 / Published online: 16 July 2009  
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**Abstract** Trichosporonosis due to *Trichosporon asahii* is a life-threatening infection with a very poor prognosis. We analyzed the genotype of intergenic transcribed spacer (IGS) region 1 of the rRNA gene and determined the drug susceptibility of 101 *T. asahii* isolates obtained from Thai patients to collect basic information on trichosporonosis in Thailand. Of the five genotypes in the IGS region identified in this study, types 1 and 3 were predominant in Thailand. The distribution in Thailand differs from that in other countries, suggesting that there is a geographic sub-structure among *T. asahii* clinical isolates. Voriconazole appeared to be the most active drug.

**Keywords** *Trichosporon asahii* · Genotype · Intergenic spacer region · Antifungal drug susceptibility

## Introduction

The genus *Trichosporon* currently contains 37 recognized species, of which 8 are associated with infection or allergy: *T. asahii*, *T. asteroides*, *T. cutaneum*, *T. inkin*, *T. mucoides*, *T. ovoides*, *T. domesticum*, and *T. montevidense* [1, 2]. *T. asahii* is the most common in disseminated infections in immunocompromised patients, particularly in patients with hematological diseases [3]. The prognosis of infection due to *T. asahii* is very poor [4]. The microorganism has relatively low susceptibility to the so-called golden standard amphotericin B. Recently, azole-resistant *T. asahii* strains have been isolated from patients [5, 6].

In Thailand, the first case report of disseminated trichosporonosis was published in 2001 and described a 27-month-old Thai patient with chronic granulomatous disease [7]. The patient developed a systemic fungal infection that resulted in death despite rigorous medical care. The postmortem findings showed extensive granulomatous inflammation in several internal organs. Tissue culture led to a diagnosis of disseminated trichosporonosis, an uncommon infection in immunocompromised patients. Recent studies of fungemia in adult, non-HIV-infected patients admitted to Khon Kaen University Hospital in northeast Thailand

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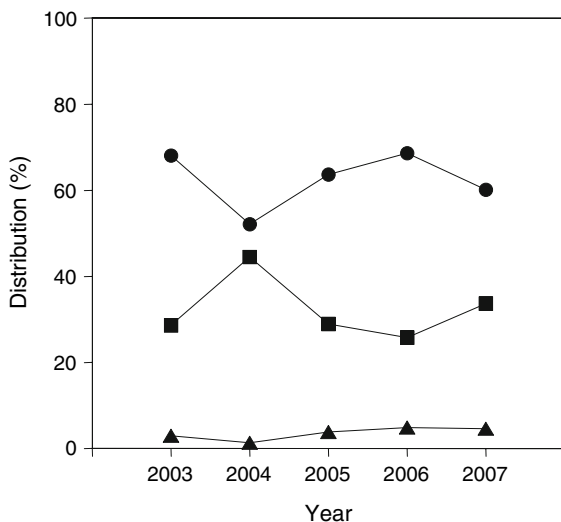
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between 1999 and 2003 indicate that *Candida* was the most common isolate, at a frequency of 93.9%, while fungemia caused by *Trichosporon* accounted for 6.1% of cases [8]. However, the clinical features of the patients with *Trichosporon* and *Candida* could not be distinguished. The overall in-hospital mortality rate of trichosporonosis was 56.1%.

A retrospective investigation into the isolation frequency of yeast pathogens from Thai patients conducted by the Mycology Laboratory of the Thailand National Institute of Health between 2003 and 2007 showed that *Candida* species predominated, followed by *Cryptococcus neoformans*. *Trichosporon* species accounted for ~2–7% of the infections (Fig. 1). Although the number of trichosporonosis patients has been increasing and trichosporonosis is life-threatening, there is little basic information on *T. asahii* isolated from Thai patients.

In this study, we analyzed the genotypes of the rRNA gene of the isolates and compared them with genotypes in other countries from an epidemiological perspective to collect basic information about Thai trichosporonosis. The drug susceptibility of *T. asahii* isolates obtained from Thai patients was also determined.



**Fig. 1** Isolation frequency of pathogenic yeasts from 2003 to 2007 in Thailand. Circles, *Candida* species; squares, *Cryptococcus neoformans*; triangles, *Trichosporon* species. Total numbers of the strains are 335, 301, 418, 535, and 306 in 2003, 2004, 2005, 2006, and 2007, respectively

## Materials and Methods

### Clinical Isolates

Between 1997 and 2007, 101 *T. asahii* strains were isolated from 101 Thai patients with trichosporonosis. The isolates were stocked in the Mycology Laboratory of the Thailand National Institute of Health and were tentatively identified as *T. asahii* using a commercial kit (API 20 C AUX; BioMérieux, France).

### Genotyping the IGS Region of the rRNA Gene

Genomic DNA was extracted using the method by Makimura et al. [9]. Intergenic spacer (IGS) region 1, which is located between the 26S and 5S rRNA genes, was amplified by PCR using the primer pairs 26SBF (5'-ATCCTTTGCAGACGACTTGA-3') and 5SR (5'-AGCTTGACTTCGCAGATCGG-3') [10]. The PCR products were sequenced using both primers (26SBF and 5SR).

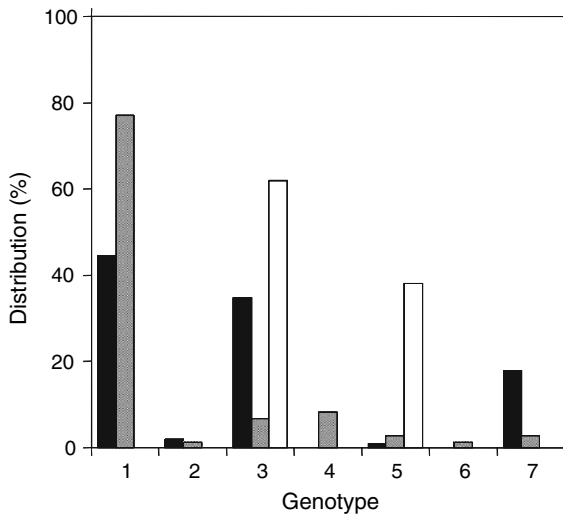
### Antifungal Drug Susceptibility Testing

The minimum inhibitory concentrations (MICs) of six antifungal agents were determined according to the M 27-A2 broth microdilution method published by the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) [11]. These were amphotericin B (AMPH-B; Wako Pure Chemical, Osaka, Japan), flucytosine (5-FC; Wako), fluconazole (FLCZ; Wako), miconazole (MCZ; Wako), itraconazole (ITCZ; Janssen Pharmaceutical, Tokyo, Japan), and voriconazole (VCZ; Pfizer Pharmaceuticals, Tokyo, Japan).

## Results

### IGS 1 Genotypes of the *T. asahii* Isolates

Of the nine IGS genotypes [12], five were identified in the 101 *T. asahii* isolates: 45 (44.5%) were genotype 1, 2 (2.0%) were genotype 2, 35 (34.7%) were genotype 3, 1 (1.0%) was genotype 5, and 18 (17.8%) were genotype 7. Genotypes 4 and 6 were not found in the isolates. The genotype distributions of Japanese and US isolates are shown in Fig. 2. The



**Fig. 2** Distribution of the IGS1 genotypes of *T. asahii*. Black, Thailand; gray, Japan [10, 12]; white, USA [10, 12]. Total number of isolates in Thailand, Japan, and USA are 101, 74, and 31, respectively

major genotypes of the Thai isolates were types 1 and 3, while those of the Japanese and US isolates were types 1 and 3, respectively.

#### Antifungal Drug Susceptibility

Table 1 shows the geometric means (GMs), MIC<sub>50</sub>, MIC<sub>90</sub>, and range of the MICs of the 101 *T. asahii* strains for AMPH-B, 5-FC, FLCZ, MCZ, ITCZ, and VCZ. The clinical isolates showed low susceptibility to amphotericin B, 5-flucytosine, and fluconazole. The most potent compound was voriconazole with a GM MIC of 0.09 µg/mL. The MICs did not differ between major genotypic strains 1 and 3.

#### Discussion

The fungal rRNA gene consists of four subunits (5S, 5.8S, 18S, and 26S) and two spacer regions (ITS, internal transcribed spacer; and IGS). Of them, the partial sequences of 26S and the ITS region have been widely used for fungal identification. The IGS region can be used to investigate intraspecies diversity, since the region shows remarkable diversity compared with the other subunits and the spacer region. Sugita et al. [10] found that there were several genotypes of the *T. asahii* IGS1 region and that there was a geographic substructure among *T. asahii* genotypes.

Of the nine genotypes, both types 1 (44.5%) and 3 (34.7%) were predominant in Thailand, while type 1 strains accounted for 77.0% in Japan. In the United States, types 3 and 5 constituted 61.3 and 38.7%, respectively (Fig. 2). In addition to infections, *T. asahii* is also responsible for the development of summer-type hypersensitivity pneumonitis (SHP) [13]. SHP follows the development of type III or IV allergies by repeated inhalation of *Trichosporon* arthroconidia, which often contaminate home environments during the summer. Interestingly, the major genotype of *T. asahii* obtained from the homes of SHP patients was type 3, not type 1 [2]. Therefore, IGS sequence analysis is a potential epidemiologic tool in trichosporonosis.

We also examined the antifungal drug susceptibility of *T. asahii*. Despite the increased frequency and severity of trichosporonosis, few data on the susceptibility of *T. asahii* to antifungal agents are available for Thailand. *Trichosporon* spp. tend to be susceptible to azole in vitro, and resistant or relatively resistant strains of *T. asahii* have been identified only

**Table 1** In vitro activities of antifungal agents against *T. asahii* isolates obtained from Thai patients

Agent	All isolates (n = 101)								
	GM	MIC <sub>50</sub> /MIC <sub>90</sub>	Range	GM	MIC <sub>50</sub> /MIC <sub>90</sub>	Range	GM	MIC <sub>50</sub> /MIC <sub>90</sub>	Range
Amphotericin B	0.71	0.5/2	0.125 to >16	0.64	0.5/2	0.125 to >16	0.7	0.5/2	0.125 to 16
5-flucytosine	14.2	32/>64	4 to >64	22.63	>64/>64	8 to >64	12.97	8/32	8 to >64
Fluconazole	9.63	8/64	4 to 64	9.77	8/16	4 to 64	10.35	8/16	8 to 32
Miconazole	1.11	1/2	0.5 to 4	0.95	1/2	0.5 to 4	0.89	1/2	0.5 to 2
Itraconazole	0.91	1/1	0.5 to 2	0.93	1/1	0.5 to 1	0.92	1/1	0.5 to 1
Voriconazole	0.09	0.125/0.25	0.06 to 0.25	0.09	0.125/0.125	0.06 to 0.25	0.09	0.125/0.25	0.06 to 0.25

recently [5, 6]. Almost all of the strains in this study were relatively susceptible to all of the drugs tested, except 5-FC. The MICs of the Thai isolates were lower than those reported in other countries. This may be due to clinical circumstances. In Thailand, the first-line treatment for fungal infection is amphotericin B, and azole agents and candin derivative are rarely used. The most potent compound was voriconazole, and there were no resistant strains. The low MICs that we observed for voriconazole concur with previous studies [14, 15], suggesting that voriconazole offers a clinical solution in trichosporonosis when there is no response to the standard treatment.

In conclusion, we elucidated the genotype and antifungal drug susceptibility of *T. asahii* clinical isolates obtained from Thai patients. The genotype distribution of Thai isolates differed from that of other countries, and the *T. asahii* strains had not developed drug resistance. Ongoing collection of this basic information is important, because trichosporonosis due to *T. asahii* is life-threatening.

**Acknowledgments** This study was supported in part by research grants from the Asian and African Science Platform Program of the Japan Society for the Promotion of Science, and a Health Science Research Grant for “Research on Emerging and Re-emerging Infectious Diseases” from the Ministry of Health, Labor, and Welfare of Japan (TS).

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