Characterization of *Trichosporon spp.* Isolates from Blood Stream and Central Nervous System Infections in A Tertiary Hospital in Singapore

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Introduction

The lack of accurate identification and susceptibility data on *Trichosporon spp.* limit our understanding and treatment of this emerging pathogen.
Although molecular methods are the most accurate for identification of *Trichosporon spp.*, they are costly and not suitable for most routine laboratories. The commercially available biochemical test kit, bioMérieux API 20C AUX and semi-automated test with the matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) are commonly used tests for identification of *Trichosporon spp.* in many routine laboratories.
In this study, we attempt to correlate API, MALDI-TOF, internal transcribed spacer (ITS), D1/D2 sequencing results as well as evaluate MIC values for *Trichosporon spp.* isolates from blood and central nervous system specimens.

Methods

We performed a retrospective review of blood stream (BS) or central nervous system (CNS) infections with *Trichosporon spp.*. Archived isolates were subcultured onto Sabouraud dextrose agar. Colonies were identified using bioMérieux API 20C AUX, Bruker MALDI Biotyper, sequencing of ITS region and D1/D2 region of the 28S ribosomal subunit for correlation. Susceptibility testing was performed using Sensititre YeastOne Y010 broth dilution panel. Controls were performed for API 20C AUX, Bruker Biotyper and Sensititre YeastOne YO10 as per manufacturers' recommendation.

Results

- There were 14 cases of BS or CNS infections with archived isolates available for further characterization. Two *Trichosporon Inkin (T. inkin)* and 12 *Trichosopron asahii (T. asahii)* isolates were identified by ITS and D1/D2 sequencing; which were concordant with MALDI-TOF. In contrast, 1 *T. asahii* isolate was identified as *T. inkin* on API 20UX. See Table 1.
- The MIC values to the *Trichosporon* isolated are as presented in Table 2.
- Applying Clinical and Laboratory Standards Institute (CLSI) breakpoints for *Candida albicans, T. inkin* were susceptible to both fluconazole and voriconazole; *T. asahii* were resistant to fluconazole and demonstrated variable susceptibility to voriconazole.
- There are no existing CLSI amphotericin B breakpoints for either *Candida spp* or *Trichosporon spp.* Based on the epidemiological cut off value of 2mcg/mL, proposed by Pfaller et al, only 5 of 12 *T. asahii* tested were considered susceptible (1).
- Most patients were critically ill in the intensive care unit and on ventilatory support (71.4%), with central lines (78.6%) and had underlying hematological malignancy (71.4%). Inpatient mortality rate was 78.6%. See Table 3.

Table 1: *Trichosporon spp.* identification using API, MALDI-TOF, ITS, D1/D2 Sequence

Isolate No	API 20C AUX	Bruker MALDI Biotyper	ITS	D1/D2 region of Large Ribosomal Subunit
1	*T. asahii*	*T. asahii*	*Trichosporon spp*[1]	*T. asahii*
2	*T. inkin*	*T. asahii*	*T. asahii*	*T. asahii*
3	*T. asahii*	*T. asahii*	*T. asahii*	*T. asahii*
4	*T. asahii*	*T. asahii*	*T. asahii*	*T. asahii*
5	*T. asahii*	*T. asahii*	*T. asahii*	*T. asahii*
6	*T. asahii*	*T. asahii*	*T. asahii*	*T. asahii*
7	*T. asahii*	*T. asahii*	*T. asahii*	*T. asahii*
8	*T. asahii*	*T. asahii*	*T. asahii*	*Trichosporon spp*[3]
9	*T. asahii*	*T. asahii*	*T. asahii*	*T. asahii*
10	*T. asahii*	*T. asahii*	*T. asahii*	*T. asahii*
11	*T. asahii*	*T. asahii*	*T. asahii*	*Trichosporon spp*[4]
12	*T. asahii*	*T. asahii*	*Trichosporon spp*[1]	*T. asahii*
13	*T. inkin*	*T. inkin*	*Trichosporon spp*[2]	*T. inkin*
14	*T. inkin*	*T. inkin*	*Trichosporon spp*[2]	*T. inkin*

Foot note:
[1.] 99% similarity to *T. asahii, T. japonicum and T. insecticum,*
[2.] 99% similarity to *T. inkin, T. japonicum* and *T. insecticum,*
[3.] 99% similarity to *T. asteriodes, T. asahii, T. japonicum* and *T. insecticum*
[4.] *99% similarity to T. asahii and T. japonicum.*

Discussion

- In this series, *T. asahii* and *T. inkin* are associated with blood stream and central nervous system infections, *T. asahii* is more common than *T. inkin.*
- The limitations of biochemical identification are again highlighted in this series. The API 20C AUX database identifies 3 common and medically important *Trichosporon spp., T. asahii, T. inkin and T. mucoides*. Even within the limits of the database, 1 *T. asahii* isolate was misidentified as *T. inkin*. The MALDI-TOF on the other hand, is able to identify the *T. asahii* and *T. inkin* accurately in this study. The MALDI-TOF appears to be a valuable tool for rapid and accurate identification of *Trichosporon* isolates at the species level (2). With time, as their *Trichosporon spp.* database expands, the MALDI-TOF is expected to be the mainstay for routine laboratory identification of *Trichosporon* isolates.
- ITS or D1/D2 are useful molecular tools for accurate *Trichosporon spp.* identification. However, when used in isolation, they may not provide enough resolution for certain *Trichosporon spp.* which are very closely related (3, 4). An alternative more accurate and discriminatory tool is sequence-based identification using IGS1 of rDNA (IGS1) (5).
- Anti-fungal susceptibilities of different strains of *Trichosporon spp.* are not homogenous (6). *T. inkin* in this series has lower MIC to the azoles.
- This highlights the importance of accurate identification of fungal pathogens for the administration of appropriate anti-fungal therapy to optimize treatment outcomes.

Table 2: Antifungal susceptibility by Sensititre YeastOne YO10 Panel

Antifungal agent	MIC (mg/ml) *T. asahii*		MIC (mg/ml) *T. inkin*
	range	MIC90	range
Anidulafungin	>8	NA	>8
Caspofungin	>8	NA	>8
Micafungin	>8	NA	>8
Itraconazole	0.12 – 0.5	0.5	0.25
Fluconazole	4 – 16	16	1
Voriconazole	0.06 – 0.5	0.5	0.06 – 0.12
Posaconazole	0.25 – 1.0	1	0.25
Amphotericin B	≤0.12 – 8	4	0.5 – 2

Table 3: Microbiological and clinical data

No	*Trichosporon spp.* isolated	Site	Age	Underlying disease	CVC	Ventilator	Antifungal therapy	Inpatient Mortality	*Trichosporon* identified prior to demise
1	*T. inkin*	Blood	70	BPDCN	Yes	Yes	Voriconazole →Ambisome	Death	Yes
2	*T. asahii*	Blood	63	Burkitt's lymphoma	Yes	Yes	Voriconazole	Death	Yes
3	*T. asahii*	Blood	54	AITL	Yes	No	Caspofungin →Ambisome	Death	No
4	*T. asahii*	Blood	33	SAA	Yes	Yes	Caspofungin	Death	No
5	*T. asahii*	Blood	37	NK cell leukemia	Yes	Yes	Caspofungin	Death	No
6	*T. asahii*	Blood	66	AML	Yes	Yes	Voriconazole	Death	Yes
7	*T. asahii*	Blood	21	ALL	Yes	No	Caspofungin	Death	No
8	*T. asahii*	Blood	34	DM	No	No	Fluconazole	Alive	-
9	*T. asahii*	Blood	66	AML	Yes	Yes	Caspofungin	Death	No
10	*T. asahii*	Blood	16	PTB	Yes	Yes	Caspofungin →Ambisome	Death	No
11	*T. inkin*	Blood	50	Aortic dissection	No	Yes	Ambisome	Death	Yes
12	*T. asahii*	Blood	80	TENS	Yes	Yes	Voriconazole	Death	Yes
13	*T. asahii*	CSF	62	DM	No	Yes	Fluconazole	Alive	No
14	*T. asahii*	Blood	57	AML	Yes	No	Voriconazole	Alive	-

BPDCN: Blastic plasmacytoid dendritic cell neoplasm, AITL: Angioimmunoblastic T cell lymphoma, SAA: severe aplastic anemia, AML: Acute myeloid leukemia, ALL: Acute lymphoblastic leukemia, DM: Diabetes mellitus, TENS: Toxic epidermal necrolysis, CVP: central venous catheter. PTB: pulmonary tuberculosis

Conclusions

Identification of *T. asahii* and *T. inkin* by MALDI-TOF, ITS and D1/D2 sequencing appear to be concordant; discrepancies with API 20C AUX may occur. *T. inkin* has low MIC to the azoles; *T. asahii* susceptibility is more variable. Voriconazole appears to the drug of choice for *T. asahii*. Based on MIC values, fluconazole may be used for *T. inkin.*

References:
1.Pfaller MA, Espinel-Ingroff A, Canton E, Castanheira M, Cuenca-Estrella M, Diekema DJ, et al. Wild-type MIC distributions and epidemiological cutoff values for amphotericin B, flucytosine, and itraconazole and Candida spp. as determined by CLSI broth microdilution. J Clin Microbiol. 2012;50(6):2040-6.
2.de Almeida Junior JN, Figueiredo DS, Toubas D, Del Negro GM, Motta AL, Rossi F, et al. Usefulness of matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry for identifying clinical *Trichosporon* isolates. Clin Microbiol Infect. 2014;20(8):784-90.
3.Kurtzman CP, Robnett CJ. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large-subunit (26S) ribosomal DNA gene. J Clin Microbiol. 1997;35(5):1216-23.
4.White T J BT, Lee S, Taylor J W. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. . In: Innis M A GDH, Sninsky J J, White T J, editor. PCR protoccols: a guide to methods and applications. New York, N.Y.: Academic Press, Inc; 1990. p. 315 – 22.
5.Sugita T, Nakajima M, Ikeda R, Matsushima T, Shinoda T. Sequence analysis of the ribosomal DNA intergenic spacer 1 regions of *Trichosporon* species. J Clin Microbiol. 2002;40(5):1826-30.
6.Arabatzis M, Abel P, Kanellopoulou M, Adamou D, Alexandrou-Athanasoulis H, Stathi A, et al. Sequence-based identification, genotyping and EUCAST antifungal susceptibilities of *Trichosporon* clinical isolates from Greece. Clin Microbiol Infect. 2014;20(8):777-83.