Identification of Yeasts from Clinical Samples Using MALDI-TOF MS

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Abstract

Objective: This research aimed to identify the yeasts from the clinical specimens by comparing MALDI-TOF MS using extended direct transfer technique (EDT-MALDI) and the conventional method.

Methods: This study was a retrospective cohort. The samples were the 557 yeasts isolated from the clinical specimens in the Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Thailand by comparing between the EDT-MALDI and the conventional method. The data were analyzed by using McNemar Test. Moreover, the unit cost and the turnaround time of the both methods were calculated. The other data such as age, sex, wards, types of the specimens and the prevalence of the species of yeasts, were collected and analyzed.

Results: The result showed that the EDT-MALDI can identify the yeast isolates into the species level more accurately than the conventional method at 100% and 93.2%, respectively. The conventional method was limited for correctly identification of *Trichosporon asahii*, *Cryptococcus neoformans*, some non-*albicans Candida*, *Magnusiomyces capitatus*, and *Candida rugosa*. However, both methods were not significantly different from each other. Even though the threshold score of the EDT-MALDI was reduced from ≥ 2 to ≥ 1.7, this will increase the acceptable results without effect to specificity, accuracy, and reliability in the species level. The EDT-MALDI was faster than the conventional method for at least 24 hours. Moreover, the unit cost of EDT-MALDI is lower than the conventional method (68 baht/test and 116 baht/test, respectively). In this data, the elderly female admitted to the hospital were the most patients who found yeast in specimens. *Candida albicans* (54.04%) were the most isolated species, followed by *Candida tropicalis* (26.21%).

Conclusion: The EDT-MALDI was a suitable method for the yeast identification in the routine laboratory because it was provided a shorter time of analysis, obtained reliable results and less expensive unit cost.

Keywords: Conventional method, MALDI-TOF MS, Extended direct transfer technique

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บทคัดย่อ

วัตถุประสงค์: เพื่อพิสูจน์แยกชนิดของยีสต์จากสิ่งส่งตรวจทางห้องปฏิบัติการ ด้วยเครื่อง MALDI-TOF MS โดยใช้เทคนิค extended direct transfer (EDT-MALDI) เปรียบเทียบกับวิธี conventional

วิธีดำเนินการวิจัย: การวิจัยนี้เป็นการวิจัยเชิงวิเคราะห์แบบย้อนหลัง โดยใช้ตัวอย่างเป็นยีสต์จำนวน 557 ตัวอย่าง ที่แยกได้จากสิ่งส่งตรวจของผู้ป่วย ในคณะแพทยศาสตร์วชิรพยาบาล ตั้งแต่ เดือน พฤษภาคม 2558 ถึง กรกฎาคม 2558 ศึกษาการพิสูจน์แยกชนิดของยีสต์ด้วยวิธี EDT-MALDI เปรียบเทียบกับวิธี conventional ที่ใช้อยู่เดิม นำมาวิเคราะห์ข้อมูลโดยใช้สถิติ McNemar Test รวมถึงคำนวณราคาต้นทุนต่อหน่วย และระยะเวลารอคอยผล และวิเคราะห์ข้อมูลในปัจจัยด้านอายุ เพศ หอผู้ป่วย ชนิดของสิ่งส่งตรวจ และความชุกของยีสต์ชนิดต่างๆ

ผลการวิจัย: วิธี EDT-MALDI สามารถแยกชนิดของยีสต์ถึงระดับสปีชีส์ได้ 100% ส่วนวิธี conventional ได้ 93.2% เพราะวิธี conventional ไม่สามารถแยก *Trichosporon asahii*, *Cryptococcus neoformans*, non-*albicans Candida* บางชนิด, *Magnusiomyces capitatus* และ *Candida rugosa* ได้อย่างถูกต้อง แต่ทั้งสองวิธีไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ถึงแม้ว่าวิธี EDT-MALDI จะต้องลด threshold จาก ≥ 2 เป็น ≥ 1.7 แต่ไม่กระทบต่อความจำเพาะ ความถูกต้อง และยังเชื่อถือได้ถึงระดับสปีชีส์ วิธี EDT-MALDI ยังช่วยลดเวลาการวิเคราะห์ได้อย่างน้อย 24 ชั่วโมง และมีราคาต่อหน่วยถูกกว่าวิธี conventional คือ 68 และ 116 บาทต่อรายการตรวจ ตามลำดับ จากข้อมูลพบว่า ผู้ป่วยที่มักจะพบยีสต์ในสิ่งส่งตรวจส่วนมากเป็นผู้สูงอายุ เพศหญิงและนอนรักษาตัวในโรงพยาบาล *Candida albicans* (ร้อยละ 54.04) เป็นสปีชีส์ที่พบมากที่สุด รองลงมาคือ *Candida tropicalis* (26.21%)

สรุป: วิธี EDT-MALDI เหมาะสมที่จะนำมาใช้พิสูจน์ยีสต์ทางห้องปฏิบัติการ เพราะช่วยลดขั้นตอนการสกัดที่ยุ่งยาก ทำให้พิสูจน์ชนิดของเชื้อได้เร็วขึ้น ผลที่ได้น่าเชื่อถือและต้นทุนที่ถูกกว่า

คำสำคัญ: วิธี conventional, วิธี EDT-MALDI, เทคนิค extended direct transfer

Introduction

The infectious diseases are caused by many organisms such as bacteria, virus, fungi, and parasites. The infections are important causes of morbidity and mortality. Moreover, the cost of treatments is significantly increased every year. The most infections commonly caused by bacterial and fungal pathogens. Currently, fungi are one of the important roles in infectious diseases. The most common fungal diseases are caused by opportunistic fungi yeast such as candidiasis and cryptococcosis which have high mortality rate[1-3]. The yeast identification is a crucial method in clinical treatment for appropriate selection antifungal to treat the fungal infections, so the accurate and fast results need to be maintained[2, 4-7].

Generally, the conventional method used for identification of yeast into the species level by using the combination result from biochemical tests such as morphology observation, germ tube production, chlamydoconidia production, carbon assimilation tests, nitrogen assimilation tests, and sugar fermentation tests. Although this method is less expensive, the test step is tedious, time-consuming, and incorrectly identified in some non-*albicans Candida*[8-14]. Moreover, the false positive or false negative results can occur[8, 15]. Thus, the other test procedure which is faster than the conventional method should be used instead. The chromogenic media is used to separate yeasts from direct specimens or mixed-yeast cultures by generate the specific pigment of each species. However, it is limited used in some species such as *Candida albicans, Candida glabrata, Candida krusei,* and *Candida tropicalis*. Other than these species, they will produce the same color of colonies and cannot be correctly separated into species level[11, 12, 14, 16].

At present, the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is used to identify both bacteria and yeasts[17]. The use of MALDI-TOF MS to identify species of yeast provides simpler test procedure, more convenience, higher accuracy and more reliable result than the conventional method. Also, the use of MALDI-TOF MS does not require both biochemical test preparation and quality control, resulting in the increment of efficiency of the laboratory. These advantages of species classification of yeast are leading to potential enhancement of treatment: it is effective for physician to select appropriate drug for treatment[8, 10-13, 18-22]. However, the yeast identification with MALDI-TOF MS was recommended to perform the yeast extraction process with formic acid tube extraction before analysis[7, 23, 24] because of the thick and strong cell wall of yeasts[8, 25]. The result of yeast identification by MALDI-TOF MS depends on the quality of spectrum compared to the reference spectrum in a library. Its performance can distinguish species of yeast which has closely related species or subspecies such as *Candida parapsilosis/orthopsilosis/metapsilosis* or *Candida albicans/dubliniensis*, while biochemical tests cannot[8, 11, 13, 18, 25]. However, the standard yeast extraction of MALDI-TOF MS was contained many steps which are not appropriate for yeast identification in routine laboratory. To make it suitable for routine laboratory, the extended direct transfer technique (EDT-MALDI) had been developed[8, 12, 19, 25]. This technique is partial yeast extraction.

Normally, the determined threshold of identification with MALDI-TOF MS was accepted at score cut off more than 2.0 for accepting the result of both genus and species level. Although the most result from the EDT-MALDI shows a significantly lower score of identification than standard extraction and acceptable threshold (≤ 2.0), the identification results are also correct. The unreliable result was the identification in the cutoff below 1.7[22, 26]. The study of Vlek and staff found that the cutoff at 1.7 is the most suitable threshold for identifying yeast by MALDI-TOF MS regardless of the method used among the direct transfer, the EDT-MALDI, and formic acid tube extraction[10, 12, 20]. While Stevenson *et al.* and Dhiman *et al.*[10, 21] support that the reducing cutoff threshold to 1.8, the identification rate was increased equivalent to standard extraction without affect to accuracy and also trust 100% in species level[8, 19, 22, 24]. Constanza GT *et al.* use

EDT-MALDI and formic acid tube extraction with a cutoff value of 1.7 to correctly identify at species level with a 94% sensitivity and 96% specificity[27]. While Wang *et al.* use EDT-MALDI and cutoff 1.7, the results were 94.3% identical results with acceptable confidence values, 98.8% accuracy rate for overall identification of yeast isolates, and poorly 41.8% in the identification of *Cryptococcus* species because of the organism's carbohydrate-rich cell walls, making protein extraction more difficult with the direct on-plate testing method[28]. However the cutoff value of 1.7 have insufficient evidence (low number of isolates of each species) to support the use of this cutoff to correctly identify other yeast species. From the previous studies, Erin *et al.* and Mather *et al.* using EDT-MALDI and cutoff 1.7 with the other organisms; aerobic Gram-positive organisms and Mycobacterium, the results were 92.2% and 94.9% correctly identification in species level, respectively[29, 30].

MALDI-TOF MS (MALDI BioTyper) has been used in Microbiology Laboratory Unit, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand for use in bacterial identification. To empower using this instrument, the application for yeast identification would be done. Due to the complicated processes of yeast extraction, which is not suitable in routine laboratory; both the factors of high workload and limited turnaround time, the EDT-MALDI shall be applied. The EDT-MALDI, which reduces tedious steps and turnaround time, was selected to identify yeast with MALDI-TOF MS because this method can be used along with bacterial identification. This research aims to identify yeast isolated from patients using the EDT-MALDI compared to the conventional method and to observe the prevalence of yeasts in the hospital, thereby the comparison of unit cost and turnaround time of both methods were calculated.

Methods

Samples

This study was retrospective cohort. A total of 557 clinical yeast isolates were collected from 522 clinical patients specimens at Microbiological Laboratory Unit, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand (since May to July 2015). The types of clinical specimens infected with yeasts came from urine (n = 236, 45.2%), sputum (n = 204, 39.1%), stool (n = 28, 5.4%), hemoculture (n = 18, 3.4%), wound/pus (n = 11, 2.1%), tissue (n = 6, 1.1%), cervix (n = 5, 1.0%), vagina (n = 4, 0.8%), bile (n = 2, 0.4%), peritoneal dialysis (n = 2, 0.4%), CSF (n = 2, 0.4%), joint (n = 1, 0.2%), tip (n = 1, 0.2%), abdomen fluid (n = 1, 0.2%) and bronchial washing (n = 1, 0.2%). The following clinical information of patients was recorded from the hospital information system: ward, sex and age. The reference strains of yeasts came from National Institute of Health of Thailand consisting of *Candida albicans* DMST 5315**,** *Candida tropicalis* DMST 15495*, Candida glabrata* DMST 46683*, Candida parapsilosis* DMST 15315*, Candida krusei* DMST 15317 and *Cryptococcus neoformans* DMST 15319.

Yeast identification

All clinical yeast isolates from stock were recovered and subcultured on the blood agar plate containing 5% sheep blood. The plates were incubated in ambient air at 37°C for 24-48 hours. Then, the pure isolation of yeast colonies were identified the phenotype into species level using the EDT-MALDI in comparison to the conventional method.

Identification of the phenotype of yeasts with conventional method

The colony morphology was observed, dyed with gram stain and determined characteristics under a microscope. If suspected *Cryptococcus* spp., the india ink preparation was performed. The combination of biochemical tests such as germ tube production, chlamydoconidia production,

carbon assimilation tests, nitrogen assimilation tests, and sugar fermentation tests, were differently done. Moreover, the species of *Candida* were separated according to the color of the colony on CHROMagar *Candida*.

Identification of the phenotype of yeasts using MALDI-TOF MS with extended direct transfer technique (EDT-MALDI)

The isolation of the yeast colony was smeared and dried on the MALDI target plate. Yeasts reference strains were used as a control and bacterial test standard (BTS) was used as a calibrator. The partial extraction was performed by using 1 µL of 70% formic acid that was covered onto all smeared channels, except the BTS channel, and then, dried at room temperature. After that, 1 µL of Matrix HCCA was dropped onto all smeared channels, then dried at room temperature and analyzed by MALDI BioTyper 3.1.

The spectrum was measured by flexControl 3.3 software. The data was processed using MALDI BioTyper 3.1 program by comparing the spectrum result to the library. The results were interpreted as color and score following; green color means that the results can report and accept at species level which a score range is 2.00 – 3.00, yellow color means that the results can report and accept only genus level which a score range is 1.700 – 1.999, and red color means unidentified by MALDI or did not have database in library which a score range is 0.00 – 1.699 that had to be repeated.

Confirmation test

The uncorrelated results from both methods were confirmed by DNA sequencing (Sanger sequencing) at the Faculty of Allied Health Sciences, Thammasat University Rangsit campus. The sequences were compared to the reference data available at the Genbank database using the BLAST program to determine the species of yeast identification.

Data analysis

The results of yeast identification tested by the EDT-MALDI and the conventional method were compared, analyzed and presented into 2 parts of the following; the first is quantitative data including the ability of yeast identification, between the EDT-MALDI and the conventional method. The result was calculated by using McNemar Test, which was considered statistically significant when p-value <0.05. Also, the patient's age was presented by volume (%). The second is qualitative data including sex, ward, types of specimen, and the prevalence of yeast in each species was presented by percentage (%).

Calculation of unit cost and turnaround time

The unit cost and turnaround time of both methods were calculated and compared.

Results

The ability of yeast identification between the MALDI-TOF MS with extended direct transfer technique (EDT-MALDI) and conventional method

A total of 557 clinical yeast isolates were separated from 522 various clinical specimens of patients from May to July 2015. The results show that the conventional method can identify yeast isolates into species-level at 520 isolates (93.4%), which consists of *C. albicans* (301), *C. tropicalis* (147), *C. glabrata* (57), *C. dubliniensis* (7) and *C. krusei* (8). Meanwhile, the identification of yeast isolate into genus level was reported at 28 isolates (5.0%), which consists of *Trichosporon* spp. (20) and Encapsulated Budding Yeast suspected *Cryptococcus* spp. (8). However, this method report as "yeasts not *C. albicans* and *Cryptococcus* spp." at 8 isolates (1.4%), and unidentified was 1 isolate (0.2%) (see Table 1).

Meanwhile, the results of the EDT-MALDI report the identification of yeast isolate into species-level at 557 isolates (100.0%), which consist of *C. albicans* (301), *C. tropicalis* (146), *C. glabrata* (58), *C. dubliniensis* (7), *C. krusei* (8), *Trichosporon asahii* (20), *C. neoformans* (8), *Candid intermedia* (1), *C. metapsilosis* (2), *C. parapsilosis* (3), *C. orthopsilosis* (1), *Candida rugosa* (1), and *Magnusiomyces capitatus* (1) (see Table 1).

Table 1:

Comparison of yeast identification results from the conventional method and MALDI-TOF MS with Extended direct transfer technique in clinical yeast isolates

Clinical yeast isolates	Number of isolates	
	Conventional method	MALDI-TOF MS with Extended direct transfer technique
Species-level		
Candida albicans	301	301
Candida tropicalis	147[a]	146
Candida glabrata	57[b]	58
Candida dubliniensis	7	7
Candida krusei	8	8
Trichosporon asahii	0	20
Cryptococcus neoformans	0	8
Candida intermedia	0	1[c]
Candida metapsilosis	0	2[c]
Candida parapsilosis	0	3[c]
Candida orthopsilosis	0	1[c]
Candida rugosa	0	1[a]
Magnusiomyces capitatus	0	1[d]
Genus level		
Trichosporon spp.	20	0
Encapsulated Budding Yeast suspected *Cryptococcus* spp.	8	0
"Yeasts not *Candida albicans* and *Cryptococcus* spp."		
Yeasts not *Candida albicans* and *Cryptococcus* spp.	8[b]	0
Unidentified		
Unidentified	1[d]	0
Total (557)	**520/557 (93.4%)**	**557/557 (100%)**
Misidentified	1[a]	0
Total of species level	**519/557 (93.2%)**	557/557 (100%)

a; One isolate was reported as *C. tropicalis* by the conventional method while using MALDI-TOF MS with extended direct transfer technique was reported as *C. rugosa*.
b; One isolate was reported as yeasts not *C. albicans* and *Cryptococcus* spp. by the conventional method while using MALDI-TOF MS with extended direct transfer technique was reported as *C. glabrata*.
c; The conventional method was reported as yeasts not *C. albicans* and *Cryptococcus* spp.
d; The conventional method was unidentified while using MALDI-TOF MS with extended direct transfer technique was reported as *M. capitatus*.

The 38 discrepancy results between both methods shown in Table 1 were confirmed with DNA sequencing (Sanger sequencing). The retrieved sequence files were edited and subjected to pairwise alignment using BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Edited sequences were compared with existing sequences in GenBank using BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The generated nucleotide sequences were compared to the deposited sequence in GenBank. All of the sequences were accepted when the percent identity or homology with other entires in the databases was over than 97%. The DNA sequencing confirmed that all of the 20 isolates of *Trichosporon* spp., and all of the 8 isolates of Encapsulated Budding Yeast suspected *Cryptococcus* spp., which reported by the conventional method, were *T. asahii,* and *C. neoformans*, respectively (see Table 2). Moreover, the 8 isolates of yeasts not *C. albicans* and *Cryptococcus* spp. reported by the conventional method were identified into *C. intermedia* (1), *C. metapsilosis* (2), *C. parapsilosis* (3), *C. orthopsilosis* (1), and *C. glabrata* (1). An unidentified isolate reported by the conventional method was identified as *M. capitatus*. Meanwhile, one of *C. tropicalis* reported by the conventional method, the DNA sequencing reported as *C. rugosa*. Besides, the results from the EDT-MALDI were confirmed and received the same result as the DNA sequencing report (see Table 2).

Although the results from the EDT-MALDI were correct, the acceptable results to species level are only 65.9% (threshold score ≥ 2.0). The acceptable results can increase by reducing the threshold score range. Therefore, the reduction in threshold score range to ≥ 1.90, ≥ 1.80 and ≥ 1.70, will increase the identification rate into species level to 87.1%, 97.9%, and 100%, respectively (Table 3).

Assessment and comparison yeast identification results between both methods using statistical analysis SPSS 15.0 Program

After confirming test results by the DNA sequencing, the conventional method and the EDT-MALDI have the potential to identify yeast isolation into species-level at 519 isolates (93.2%) and 557 isolates (100%), respectively. However, there was no statistical difference between both methods (*p*-value > 0.05).

Table 2:

Thirty-eight isolates of the discrepancy results between the conventional method and the MALDI-TOF MS using extended direct transfer technique confirm with DNA sequencing (Sanger sequencing)

Conventional method	No.	DNA sequencing (Sanger sequencing)	No.	Extended direct transfer technique	No.
Trichosporon spp.	20	*Trichosporon asahii*	20	*Trichosporon asahii*	20
Encapsulated Budding Yeast suspected *Cryptococcus* spp.	8	*Cryptococcus neoformans*	8	*Cryptococcus neoformans*	8
Yeasts not *Candida albicans* and *Cryptococcus* spp.	8	*Candida glabrata*	1	*Candida glabrata*	1
		Candida intermedia	1	*Candida intermedia*	1
		Candida metapsilosis	2	*Candida metapsilosis*	2
		Candida parapsilosis	3	*Candida parapsilosis*	3
		Candida orthopsilosis	1	*Candida orthopsilosis*	1
Unidentified	1	*Magnusiomyces capitatus*	1	*Magnusiomyces capitatus*	1
Misidentified	1	Candida rugosa	1	Candida rugosa	1
Total	**38**		**38**		**38**

Table 3:

Interpretation results analyzed by using MALDI-TOF MS with extended direct transfer technique into range score

Color	Range Score	The acceptance level	No.	%	Total	Total%
Red	0.00 – 1.699	Unidentified/No database in the library have to analyzed again	0	0	0	0
Yellow	1.700 – 1.799	Acceptable to genus level	12	2.2	190	34.1
	1.800 – 1.899		60	10.8		
	1.900 – 1.999		118	21.2		
Green	2.00 – 3.00	Acceptable to species level	367	65.9	367	65.9

Distribution of patients found yeasts in specimen by age and sex

As shown in Table 4, the specimens collected from female were found yeast rather than male. There were 177 (33.9%) male and 345 (66.1%) female clinical patients, ranging in age from 0 day to >90 years, among 522 patients who found yeasts in specimen. The number of patients suspected to be infected yeasts was the highest among those 81 to 90 years old (151 patients, 28.9%) following by 71 to 80 years old (145 patients, 27.8%) and 61 to 70 years old (76 patients, 14.6%), respectively.

Distribution of patients found yeasts in specimen by wards

Female Medicine, are the ward that mostly found yeast in the specimens (131 patients, 25.09%), following by OPD (47 patients, 9.00%).

Table 4:

The distribution of yeast isolates by age and sex during May - July 2015

Sex	Hospitalized patients (n=522)			Total (%)
	Age range	Male (n=177)	Female (n=345)	
Age group	<1	5	2	7 (1.3)
	1-10	4	5	9 (1.7)
	11-20	2	4	6 (1.2)
	21-30	1	5	6 (1.2)
	31-40	2	13	15 (2.9)
	41-50	5	18	23 (4.4)
	51-60	27	43	70 (13.4)
	61-70	27	49	76 (14.6)
	71-80	**62**	83	145 (27.8)
	81-90	36	**115**	**151 (28.9)**
	>90	6	6	12 (2.3)
	No data	0	2	2 (0.4)
	Total (%)	**177 (33.9)**	**345 (66.1)**	**522 (100.0)**

Distribution of yeast and yeast-like fungal isolates

A total of 557 clinical yeast isolates were separated from 522 various clinical specimens of patients from May to July 2015. *Candida* spp. and non-*Candida* spp. were identified in 528 and 29 isolates, respectively. The isolation frequency of *C. albicans* (n=301, 54.0%) is the highest in *Candida* spp. *T. asahii* (n=20, 3.6%) is the highest in non-*Candida* spp. as shown in (see Table 5). The distribution of species as follows: *C. albicans*, 301 (54.0%) isolates; *C. tropicalis,* 146 (26.2%) isolates; *C. glabrata,* 58 (10.4%) isolates; *T. asahii*, 20 (3.6%) isolates; *C. krusei*, and *C. neoformans,* 8 (1.4%) isolates each; *C. dubliniensis*, 7 (1.3%) isolates; *C. parapsilosis*, 3 (0.5%) isolates; *C. metapsilosis*, 2 (0.4%) isolates; *C. orthopsilosis, C. rugosa, C. intermedia,* and *M. capitatus*, 1 (0.2%) isolate each.

Characterization of patients, clinical samples, types of specimens and isolation frequency

Five-hundred and twenty-two clinical samples from May to July 2015 were yeast positive. The features of patients and the isolation frequency of clinical isolates according to age, sex, and type of specimen has been identified as the elderly female patients who were admitted in the hospital, whereas the prevalence of types of specimens was higher in urine (n = 236, 45.2%) and sputum (n = 204, 39.1%). Among 557 of yeast isolates, *C. albicans* was most frequently isolated in almost all clinical specimens; especially urine and sputum, except hemoculture and some specimens from sterile site which was often found non-*albicans* species. Non-*Candida* species was slightly found from clinical specimens. Urine was the most common clinical specimens for the isolation of Non-*Candida* spp. (20 isolates); all of them were *T. asahii*, followed by hemoculture (6 isolates); all of them were *C. neoformans*, CSF (2 isolates); all of them were *C. neoformans* and sputum (1 isolate); it was *M. capitatus.* Furthermore, the distribution of co-infection, found yeast isolates more than one species in the same specimens, were often isolated from sputum, urine, wound/pus, and bile, respectively.

Table 5: the distribution of yeast isolates by clinical sample type during May - July 2015

Isolates	(n=557)	*Candida* species n (%)									Non-*Candida* species n (%)			Total (%)
Sample type	(n=522)	*albicans*	*tropicalis*	*glabrata*	*metapsilosis*	*parapsilosis*	*krusei*	*dubliniensis*	*orthopsilosis*	*rugosa*	*intermedia*	*C. neoformans*	*M. capitatus*	*T. asahii*	
Hemoculture	18 (3.4)	1 (5.6)	10 (55.6)	1 (5.6)	-	-	-	-	-	-	-	6 (33.3)	-	-	18 (3.2)
Wound/Pus	11 (2.1)	7 (58.3)	3 (25.0)	1 (8.3)	-	1 (8.3)	-	-	-	-	-	-	-	-	12 (2.2)
Tissue	6 (1.1)	6 (100.0)	-	-	-	-	-	-	-	-	-	-	-	-	6 (1.1)
Joint	1 (0.2)	1 (100.0)	-	-	-	-	-	-	-	-	-	-	-	-	1 (0.2)
Bile	2 (0.4)	1 (33.3)	1 (33.3)	1 (33.3)	-	-	-	-	-	-	-	-	-	-	3 (0.5)
Peritoneal Dialysis	2 (0.4)	-	-	-	2 (100.0)	-	-	-	-	-	-	-	-	-	2 (0.4)
CSF	2 (0.4)	-	-	-	-	-	-	-	-	-	-	2 (100.0)	-	-	2 (0.4)
Tip	1 (0.2)	-	-	1 (100.0)	-	-	-	-	-	-	-	-	-	-	1 (0.2)
Abdomen	1 (0.2)	1 (100.0)	-	-	-	-	-	-	-	-	-	-	-	-	1 (0.2)
Sputum	204 (39.1)	152 (67.6)	57 (25.3)	2 (0.9)	-	1 (0.4)	4 (1.8)	6 (2.7)	1 (0.4)	1 (0.4)	-	-	1 (0.4)	-	**225 (40.4)**
Bronchial	1 (0.2)	1 (100.0)	-	-	-	-	-	-	-	-	-	-	-	-	1 (0.2)
Urine	236 (45.2)	111 (44.8)	62 (25.0)	49 (19.8)	-	1 (0.4)	4 (1.6)	-	-	-	1 (0.4)	-	-	20 (8.1)	**248 (44.5)**
Stool	28 (5.4)	15 (53.6)	12 (42.9)	-	-	-	-	1 (3.6)	-	-	-	-	-	-	28 (5.0)
Cervix	5 (1.0)	3 (60.0)	1 (20.0)	1 (20.0)	-	-	-	-	-	-	-	-	-	-	5 (0.9)
Vagina	4 (0.8)	2 (50.0)	-	2 (50.0)	-	-	-	-	-	-	-	-	-	-	4 (0.7)
Total (%)	**522 (100.0)**	**301 (54.0)**	**146 (26.2)**	58 (10.4)	2 (0.4)	3 (0.5)	8 (1.4)	7 (1.3)	1 (0.2)	1 (0.2)	1 (0.2)	8 (1.4)	1 (0.2)	20 (3.6)	557 (100.0)

Unit cost and turnaround time

The unit cost of the EDT-MALDI is approximately 68 baht/test while the conventional method is approximately 116 baht/test. Three cost calculations were analyzed in this study: Labor Cost (LC), Material Cost (MC), and Capital Cost (CC) that included technologist time, all material and reagent cost, and maintenance costs. After receiving a specimen, the turnaround time of the EDT-MALDI is 24-48 hours while the turnaround time of the conventional method is 48-72 hours.

Discussion

In this study, the assessment of the yeast identification between using the EDT-MALDI and the conventional method is not statistically significant difference. However, the EDT-MALDI has higher efficiency for identifying yeasts to species level more than the conventional method; the identification rate was 100% and 93.2%, respectively. So, the yeast identification using the EDT-MALDI is suitable to use in the laboratory because the routinely conventional method may be misidentified for many reasons. The first reason is the unclearness of colony color observation on CHROMagar *Candida* such as; *C. dubliniensis*, *C. glabrata*, *C. rugosa* and *M. capitatus*. Although some studies such as Kirkpatrick *et al.* and Pfaller *et al.*, support the reliability of the use of CHROMagar *Candida* for identifying *C. dubliniensis* and *C. glabrata*, some researcher disagree because the color of *C. dubliniensis* is difficult to distinguish from *C. albicans*[31]. Moreover, this method must using *C. albicans* as a control simultaneously, or incubating more than 72 hours[32], or using yeasts from primary specimens, otherwise, it can be affected by collected condition and subculture steps. As shown in the study of Eraso E *et al.*, they were used *C. albicans* from stock, found that colony color was changed from green to pink, then it misidentified to non-*C. albicans*. Also, *C. glabrata* is difficult to identify because of the color of the colony similar to the non-*C. albicans*[33]. They clarify one isolate of *C. glabrata* that cannot identify due to showing a white colony.

The second reason is the non-specific reporting isolates or report as yeasts not *C. albicans* and *Cryptococcus* spp. group, such as; *C. intermedia, C. metapsilosis, C. parapsilosis* and *C. orthopsilosis*. From Esposto MC *et al.*, the others non-*albicans Candida* such as *C. parapsilosis, Candida nivariensis* and *Candida bracarensis* could not identify on CHROMagar *Candida* because they could produce many colors and many morphology of colonies, but most of them are white[34]. The third reason is the misidentified from the limitation of test such as *C. rugosa*. This issue as a result of the colony characteristics of *C. rugosa* on CHROMagar *Candida* is similar to *C. krusei*, except the colony color that produced the blue color similar to *C. tropicalis* and *Trichosporon* spp. The colony morphology observation on CHROMagar *Candida* should be aware to prevent the mistake of interpretation[32]. The last reason is the inability to identify into the genus level such as *M. capitatus* and species-level such as; *Trichosporon* spp. and Encapsulated Budding Yeast suspected *Cryptococcus* spp. These reasons are caused by the limitation of the biochemical tests, which differently use in each laboratory, cover all of the yeast isolation that would like to identify or not. Also, the false positive or false negative results should be suspected.

Whereas using the EDT-MALDI is better. Other than simpler test procedure, more convenience, time-consuming, higher accuracy and more reliable result, it can correctly separate *C. dubliniensis* from *C. albicans*, and the others that closely related species. While currently the conventional method was provided the wrong results because the phylogenetic of them are very closer[21]. Meanwhile, the turnaround time is quicker than the conventional method at least 24 hours that useful for physicians to decide suitable antifungal drugs leading to improve potential treatment for patients. Although the price of MALDI-TOF MS instrument is expensive, the solution is not. In the long term use, it will give more high effectiveness of identification. The unit cost of EDT-MALDI was 68 baht whereas 116 baht from the conventional method.

However, the suitable cutoff of the threshold score range is one consideration to using EDT-MALDI. In this study, the reduction of threshold will increase the identification rate and the reliability rate in the same way as the study of Stevenson *et al.* and Dhiman *et al*[10, 21]. At cutoff score ≥ 1.7, the results of yeast identification are still 100% corrected in the species level. So, our study is following Vlek *et al.* which determine cutoff at 1.7 is the most suitable for identify yeasts by MALDI-TOF MS[20] because misidentified cutoff is score below 1.7[12]. This cutoff is the suitable threshold for decision acceptance for the results of yeast identification when using the EDT-MALDI[8, 19, 22, 24]. We should adjust the threshold according to the identification method for increasing the identification rate without effect to specificity, accuracy, and reliability in species-level.

In the experiment, 24 isolates (4.31%) using EDT-MALDI were repeated for identification twice or more which consists of *C. tropicalis* 9 isolates, *C. albicans* 5 isolates, *C. glabrata*, *C. neoformans* and *T. asahii* 3 isolates each, *C. orthopsilosis* 1 isolate. Due to the limitations of the EDT-MALDI depend on many factors such as the quality of spectrum, the thickness of organisms and smear, the quality of HCCA Matrix concentration, including the quality and the number of databases in the library. The EDT-MALDI can identify difficult organisms and closely related organisms, which prevent the mistake of identification. However, Gorton *et al*. suggested that the EDT-MALDI should do duplicate[10, 12, 20].

The part of an epidemiological study which is one of the benefits of using MALDI-TOF MS, when analyzing data in the aspect of age, sex, and wards, Female Medicine are the ward that mostly found yeast in the specimens because the patients who mostly found yeast in specimens were elderly female and admitted in the hospital. The most common specimens infected with yeasts in this study are urine. One reason is female anatomy which has short urethra and near the vagina and anus, causing risk factors for urinary tract infection[35]. Besides that, female who in the reproductive period commonly have yeast colonized or have yeast group *Candida* infection in the reproductive system[36].

Limitations

Limitations of our study include the sampling period only three months and all of isolates analyzed from only one hospital.

Conclusion

This study shows the prevalence of yeasts in each species that isolate from all types of specimens for monitoring the outbreak of yeasts in the Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand. Urine is the most common specimens found yeast, followed by sputum. The frequency of yeast species found in various specimens is *C. albicans* 54.04% (301/557), and *C. tropicalis* 26.21% (146/557 respectively, which were consistent with several studies such as Sumitra *et al*. that found *C. albicans* 52%, followed by *C. tropicalis* that is the most common of non *albicans* species[14]. Our study, urine is also found *C. glabrata* and *T. asahii* in third and fourth rank, respectively. From Mattede *et al*., urine was found the high prevalence of *Trichosporon* spp. in male (65%), age > 70 years (55%), ICU patients who associated with urinary catheter or use broad-spectrum antibiotics[35]. Likewise, our study found a high prevalence of *Trichosporon* spp. in males (10/177 patients, 5.65%), and females (10/345 patients, 2.90%). Hemoculture, first rank is *C. tropicalis* followed by *C. neoformans* same as some specimens from sterile site that found different species spreading such as peritoneal dialysis, CSF and catheter tip; especially in CSF which the outbreak is *C. neoformans*. Also, the types of specimens such as wound/pus, bile, sputum, urine, especially sputum and urine are often found co-infection. However, the situation of yeast infection prevalence in this study limited in specimens that sent to investigate in the microbiology laboratory only does not cover every patient in the hospital. The results could not be extended to the general population because the

study only in the Faculty of Medicine Vajira Hospital, Navamindradhiraj University.

Conflict of interest

The authors have not declared any conflict of interests.

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