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Accuracy of Species-Level Identification of Yeast Isolates from Blood Cultures from 10 University Hospitals in South Korea by Use of the Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry-Based Vitek MS System

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We assessed the accuracy of yeast bloodstream isolate identification performed over a 1-year period at 10 South Korean hospitals, using the matrix-assisted laser desorption ionization–time of flight (MALDI-TOF)-based Vitek MS system. The overall phenotypic misidentification rate was 3.4% (18/533), with considerable variation between hospitals (0.0% to 19.0%), compared to 1.1% (6/533) for the Vitek MS system.

Bloodstream infections (BSIs) caused by yeasts, especially *Candida* species, are associated with a poor prognosis, though attributable mortality can be limited by prompt, appropriate administration of antifungal therapy (1). Rapid and accurate identification of bloodstream isolates can aid in selection of empirical antifungal therapy based upon general predictable resistance profiles to antifungal agents (2). The Clinical and Laboratory Standards Institute (CLSI) recently proposed new species-specific breakpoints for antifungal agents (3), highlighting the importance of accurate species identification in the clinical laboratory. While misidentification of yeast species can have profound effects on the interpretation of antifungal data and the appropriateness of therapeutic decisions (2–4), the accuracy of clinical identifications of yeast BSIs has not been assessed in a multicenter study.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has recently been developed as a fast, easy-to-use, cost-effective method for yeast identification (5–8) and may supplant traditional methods of pathogen identification. Currently, many clinical microbiology laboratories in South Korea continue to use phenotypic methods for routine identification of yeasts. In this study, we assessed the accuracy of phenotypic identification (PI) of yeast BSIs at 10 South Korean hospitals over a 1-year period, in comparison with the new MALDI-TOF-based Vitek MS system (bioMérieux, Marcy l'Etoile, France). To our knowledge, this represents the first nationwide multicenter study of the accuracy of yeast BSI identifications, as determined using routine clinical practices.

From January to December 2011, nonduplicate yeast isolates from blood cultures were prospectively collected at 10 university hospitals (A to J) in South Korea. All isolates were identified by the participating institutions using routine phenotypic methods; however, the procedures used varied between hospitals. While some hospitals used one of the commercial yeast identification systems (the Vitek 2 system or Vitek 2 YST [bioMérieux] or API ID 32C [bioMérieux]) only, others used one or two commercial identification systems, including the Vitek 2 system, the API 20C (bioMérieux), or the ATB-Fungus III (bioMérieux), with additional supplemental tests such as the germ tube test or assessment of the isolate on cornmeal agar or CHROMagar *Candida*. In total, 533 isolates were submitted to Chonnam National University Hospital for further MALDI-TOF-based identification, along with the PI results obtained at each hospital.

All isolates were reidentified using the Vitek MS system, as described previously (8, 9). Each isolate was prepared by a direct on-plate extraction method using 70% formic acid. Spectra were analyzed, and identifications were calculated automatically by the advanced spectrum classifier algorithm provided by the manufacturer. A confidence value of ≥60 with the unique spectrum of a single organism indicated good species-level identification. If no unique identification pattern was found, a list of possible organisms was given as "low discrimination" (confidence value of <60%) or "bad spectrum," or the strain was determined to be outside the scope of the database ("no ID") (10). A repeat testing with the Vitek MS was performed when "low discrimination," "bad spectrum," or "no ID" data were obtained. PI and Vitek MS results were compared by chi-square or Fisher's exact test using GraphPad Prism 5 with significance defined as $P < 0.05$.

When prior PI results were compared with those obtained using the Vitek MS system, 499 (93.6%) isolates were in agreement at the species level; based upon these findings, these identifications were considered final (7, 8). For the 34 isolates with discordant results, definitive identification was ascertained through sequencing of the D1/D2 domain of the large-subunit rRNA gene using

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TABLE 1 Species identification by routine phenotypic methods at 10 hospitals during a 1-year period and those obtained by Vitek MS for 533 bloodstream yeast isolates, compared to the final identification

Final identification[a]	No. of isolates tested	No. of isolates (%)						
		Concordance between phenotypic identification and Vitek MS identification	Prior phenotypic identification at each hospital			Vitek MS identification		
			Correct identification	Misidentification	No identification[b]	Correct identification	Misidentification	No identification[c] (%)
C. albicans	210	200 (95.2)	206 (98.1)	3 (1.4)	1 (0.5)	203 (96.7)	1 (0.5)	6 (2.9)[d]
C. parapsilosis	111	109 (98.2)	111 (100.0)			109 (98.2)		2 (1.8)[d]
C. tropicalis	96	84 (87.5)	89 (92.7)	7 (7.3)		91 (94.8)	2 (2.1)	3 (3.1)[d]
C. glabrata	81	79 (97.5)	80 (98.8)	1 (1.2)		79 (97.5)	1 (1.2)	1 (1.2)[d]
Candida guilliermondii	9	9 (100.0)	9 (100.0)			9 (100.0)		
Candida krusei	6	5 (83.3)	6 (100.0)			5 (83.3)		1 (16.7)[d]
Candida pelliculosa	5	5 (100.0)	5 (100.0)			5 (100.0)		
Candida lusitaniae	3	3 (100.0)	3 (100.0)			3 (100.0)		
Candida intermedia	2	2 (100.0)	2 (100.0)			2 (100.0)		
Saccharomyces cerevisiae	2	1 (50.0)	1 (50.0)	1 (50.0)		2 (100.0)		
Pichia fabianii[e]	2	0 (0.0)		2 (100.0)			1 (50.0)	1 (50.0)
Candida haemulonii	1	1 (100.0)	1 (100.0)			1 (100.0)		
Candida lipolytica	1	1 (100.0)	1 (100.0)			1 (100.0)		
C. orthopsilosis[e]	1	0 (0.0)		1 (100.0)			1 (100.0)	
Candida melibiosica	1	0 (0.0)		1 (100.0)		1 (100.0)		
Kodamaea ohmeri	1	0 (0.0)		1 (100.0)		1 (100.0)		
Lodderomyces elongisporus[e]	1	0 (0.0)		1 (100.0)				1 (100.0)
Total	533	499 (93.6)	514 (96.4)	18 (3.4)[f]	1 (0.2)	512 (96.1)	6 (1.1)[f]	15 (2.8)[d]

[a] Final identification was defined either by matching of results for prior phenotypic identification and Vitek MS or by D1/D2 sequencing.
[b] Includes identification to the genus level only (one isolate).
[c] Includes the "low discrimination," "bad spectrum," or "no identification" results.
[d] Thirteen isolates (six *C. albicans*, two *C. parapsilosis*, three *C. tropicalis*, one *C. glabrata*, and one *C. krusei* isolate) which had been categorized as "no identification" by the initial test were correctly identified after repeating the test with the Vitek MS.
[e] Not included in the database of Vitek MS.
[f] $P < 0.05$, prior phenotypic identification versus Vitek MS identification.

primer pairs NL1 and NL4 (11). Overall, PI and Vitek MS produced similar correct identification rates (96.4% and 96.1%, respectively) across all 533 isolates, with misidentification occurring more frequently by PI than by Vitek MS (3.4% versus 1.1%, $P < 0.05$) (Table 1). Yeast isolates misidentified by PI included seven isolates of *Candida tropicalis*, three of *Candida albicans*, two of *Pichia fabianii*, one of *Candida glabrata*, and single isolates of five rare species. These data show that PI methods had frequently provided inaccurate results for both common and unusual yeast species.

Vitek MS correctly identified 96.1% of all yeasts from BSIs. Fifteen isolates (2.8%) were not identified, and six isolates (1.1%) were misidentified. This is comparable to a recent multicenter study evaluating the Vitek MS identification of yeasts, which showed that 96.1% and 0.6% isolates were either correctly identified or misidentified, respectively (9). The isolates misidentified by the Vitek MS in this study included two isolates of *C. tropicalis* identified as *C. albicans*, one isolate of *C. albicans* identified as *C. glabrata*, one isolate of *C. glabrata* identified as *C. tropicalis*, one isolate of *P. fabianii* identified as *Candida boidinii*, and one isolate of *Candida orthopsilosis* identified as *Candida magnoliae*. The misidentification of two rare species was due to improper database entries, but the reason for the misidentification of four isolates of common species is not completely understood. However, it may be related to the random error associated with the short extraction method on the plate in the Vitek MS (9), although further data are needed.

Many users of the MALDI-TOF technology in microbial identification build in an automatic repeat testing with extraction or lysis when poor discriminatory or spectral data are obtained. Usually, this resolves the vast majority of discrepancies, especially with the more common yeasts or bacteria (10). In our study, Vitek MS was unable to identify ("low discrimination," "bad spectrum," or "no ID") 2.8% of all samples (15 isolates). Of these 15 isolates, two belonging to two species (*P. fabianii* and *Lodderomyces elongisporus*) were not included in the database of the Vitek MS or Vitek 2 (bioMérieux) system. However, the remaining 13 were correctly identified by the repeat testing using Vitek MS, and the overall number of correctly identified *Candida* isolates increased from 512 (96.1%) to 525 (98.5%). As all isolates inconclusively identified by the primary testing method are routinely retested using other methods, these findings highlight the advantage of MALDI-TOF-based systems over conventional PI, as retesting is superior to misidentification (6–8).

In the present study, the misidentification rate by PI varied considerably between hospitals (0.0% to 19.0%, $P < 0.05$) (Table 2). The overall misidentification rate by PI was 2.2% (11/498) for four common *Candida* species, including *C. albicans*, *Candida parapsilosis*, *C. tropicalis*, and *C. glabrata*, but 20.0% (7/35) for 13 rare species ($P < 0.001$). The most common species misidentified

TABLE 2 Misidentification rates for yeast bloodstream isolates among 10 hospitals according to the phenotypic method used

Final identification[a]	No. (%) of isolates misidentified/tested at each hospital (main method[s] used for routine identification[b]):										
	A (V2)	B (V2, Ch)	C (Gt, AT)	D (V2)	E (V2)	F (32C)	G (V2)	H (V2, Ch)	I (V2, Ch)	J (Gt, V2)	Total
Common four species	3/94	0/77	2/74	1/57	1/45	0/40	1/37	0/35	1/20	2/19	11/498 (2.2)[c]
C. albicans	1/50	0/30	1/27	0/23	0/16	0/18	1/12	0/17	0/8	0/9	3/210 (1.9)
C. parapsilosis	0/10	0/29	0/10	0/18	0/8	0/11	0/10	0/7	0/6	0/2	0/111 (0.0)
C. tropicalis	2/24	0/13	1/13	1/12	1/8	0/3	0/9	0/3	0/4	2/7	7/96 (7.3)
C. glabrata	0/10	0/5	0/24	0/4	0/13	0/8	0/6	0/8	1/2	0/1	1/81 (1.2)
Other 13 species	0/0	2/8	0/2	0/6	0/5	2/7	0/2	0/1	1/2	2/2	7/35 (20.0)[c]
Total	3/94 (3.2)	2/85 (2.4)	2/76 (2.6)	1/63 (1.6)	1/50 (2.0)	2/47 (4.3)	1/39 (2.6)	0/36 (0.0)	2/22 (9.1)	4/21 (19.0)	18/533 (3.4)

[a] Final identification was defined either by matching of results for prior phenotypic identification and Vitek MS or by D1/D2 sequencing.
[b] V2, Vitek 2 (bioMérieux, Marcy l'Etoile, France); Ch, CHROMagar *Candida*; AT, ATB-Fungus III (bioMérieux); Gt, germ tube test; 32C, API ID 32C (bioMérieux).
[c] $P < 0.001$, common four species versus other 13 species.

by PI were *C. tropicalis* ($n = 7$) and then *C. albicans* ($n = 3$), which may have resulted in inappropriate antifungal therapy. Commercial yeast identification systems such as Vitek 2 have been shown to be less satisfactory at identifying *C. tropicalis* (12), while CHROMagar *Candida* has been shown to be useful for identification of *C. albicans* and *C. tropicalis* (13). In this study, the misidentification rate for BSI yeasts of *C. albicans* and *C. tropicalis* was 0% (0/75) in three hospitals (hospitals B, H, and I) which had used CHROMagar routinely but 4.1% (10/241) in seven other hospitals. Thus, these data support previous findings that the accuracy of commercial yeast identification systems can be increased if laboratories use supplemental tests (4, 12). In addition, two hospitals (hospitals I and J), which had smaller total numbers of BSI isolates than did the other eight hospitals, showed higher misidentification rates (9.1% and 19.0%, respectively), suggesting that the hospitals with higher volumes and thus more experience with phenotypic testing showed lower misidentification rates.

Clinical laboratories play a key role in the accurate identification of yeast BSIs. Our findings show that misidentification rates by PI for yeasts from BSIs at 10 South Korean hospitals during a 1-year period were higher than those obtained using Vitek MS and varied considerably among hospitals. We believe that Vitek MS represents a robust tool to reduce the rate of misidentification seen with conventional PI methods. This method offers faster and more reliable identification of yeast isolates, allowing for prompt and appropriate antifungal therapy for fungemic patients.

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REFERENCES

1. **Fernandez J, Erstad BL, Petty W, Nix DE.** 2009. Time to positive culture and identification for *Candida* blood stream infections. Diagn. Microbiol. Infect. Dis. **64:**402–407.
2. **Pappas PG, Kauffman CA, Andes D, Benjamin DK, Jr, Calandra TF, Edwards JE, Jr, Filler SG, Fisher JF, Kullberg BJ, Ostrosky-Zeichner L, Reboli AC, Rex JH, Walsh TJ, Sobel JD.** 2009. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin. Infect. Dis. **48:**503–535.
3. **Pfaller MA, Andes D, Diekema DJ, Espinel-Ingroff A, Sheehan D.** 2010. Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and *Candida*: time for harmonization of CLSI and EUCAST broth microdilution methods. Drug Resist. Updat. **13:**180–195.
4. **Borman AM, Szekely A, Palmer MD, Johnson EM.** 2012. Assessment of accuracy of identification of pathogenic yeasts in microbiology laboratories in the United Kingdom. J. Clin. Microbiol. **50:**2639–2644.
5. **Stevenson LG, Drake SK, Shea YR, Zelazny AM, Murray PR.** 2010. Evaluation of matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of clinically important yeast species. J. Clin. Microbiol. **48:**3482–3486.
6. **Marklein G, Josten M, Klanke U, Muller E, Horre R, Maier T, Wenzel T, Kostrzewa M, Bierbaum G, Hoerauf A, Sahl HG.** 2009. Matrix-assisted laser desorption ionization–time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J. Clin. Microbiol. **47:**2912–2917.
7. **Bader O, Weig M, Taverne-Ghadwal L, Lugert R, Gross U, Kuhns M.** 2011. Improved clinical laboratory identification of human pathogenic yeasts by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin. Microbiol. Infect. **17:**1359–1365.
8. **Iriart X, Lavergne RA, Fillaux J, Valentin A, Magnaval JF, Berry A, Cassaing S.** 2012. Routine identification of medical fungi by the new Vitek MS matrix-assisted laser desorption ionization–time of flight system with a new time-effective strategy. J. Clin. Microbiol. **50:**2107–2110.
9. **Westblade LF, Jennemann R, Branda JA, Bythrow M, Ferraro MJ, Garner OB, Ginocchio CC, Lewinski MA, Manji R, Mochon AB, Procop GW, Richter SS, Rychert JA, Sercia L, Burnham CA.** 8 May 2013, posting date. Multi-center study evaluating the VITEK MS for identification of medically important yeasts. J. Clin. Microbiol. doi:10.1128/JCM.00680-13.
10. **Dubois D, Grare M, Prere MF, Segonds C, Marty N, Oswald E.** 2012. Performances of the Vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry system for rapid identification of bacteria in routine clinical microbiology. J. Clin. Microbiol. **50:**2568–2576.
11. **Kim MN, Shin JH, Sung H, Lee K, Kim EC, Ryoo N, Lee JS, Jung SI, Park KH, Kee SJ, Kim SH, Shin MG, Suh SP, Ryang DW.** 2009. *Candida haemulonii* and closely related species at 5 university hospitals in Korea: identification, antifungal susceptibility, and clinical features. Clin. Infect. Dis. **48:**e57–e61. doi:10.1086/597108.
12. **Valenza G, Strasen J, Schafer F, Frosch M, Kurzai O, Abele-Horn M.** 2008. Evaluation of new colorimetric Vitek 2 yeast identification card by use of different source media. J. Clin. Microbiol. **46:**3784–3787.
13. **Horvath LL, Hospenthal DR, Murray CK, Dooley DP.** 2003. Direct isolation of *Candida* spp. from blood cultures on the chromogenic medium CHROMagar Candida. J. Clin. Microbiol. **41:**2629–2632.