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1 Short-Form Paper

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3 **Accuracy of Yeast Identification from Blood Cultures in 10 University Hospitals**

4 **in Korea: Comparison with the MALDI-TOF-Based VITEK MS system**

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19 Running title: The Accuracy of Blood Yeast Identification

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ABSTRACT

We assessed the accuracy of yeast bloodstream isolate identification performed over a 1-year period at 10 Korean hospitals, using the 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.

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Currently, many clinical microbiology laboratories in 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 Korean hospitals over a 1-year period, in comparison with the new MALDI-TOF-based VITEK MS system (bioMérieux, Marcy L'Étoile, 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, non-duplicate yeast isolates from blood cultures were prospectively collected at 10 university hospitals (A–J) in 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 commercial yeast identification system (the Vitek 2 system, Vitek 2 YST, bioMeriux or API ID 32C, bioMérieux) only, others used one or two commercial identification systems including the Vitek 2 system, API 20C (bioMérieux) or the ATB-Fungus III (bioMérieux), with additional supplemental tests such as germ tube test, or assessment 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 re-identified using the VITEK MS system, as described previously (8, 9). Each isolate were 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 $\geq$ 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%), "bad spectrum", or the strain was

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76 determined to be outside the scope of the database ("no ID") (10). A repeat testing with the
77 VITEK MS was performed when "low discrimination", "bad spectrum", or "no ID" data is
78 obtained. PI and VITEK MS results were compared by Chi square or Fisher's exact test using
79 GraphPad Prism 5 with significance defined as $P < 0.05$.

80 When prior PI results were compared with those obtained using the VITEK MS
81 system, 499 (93.6%) isolates were in agreement at the species level; based upon these
82 findings, these identifications were considered final (7, 8). For the 34 isolates with discordant
83 results, definitive identification was ascertained through sequencing of the D1/D2 domain of
84 the large-subunit rRNA gene using primer pairs NL1 and NL4 (10). Overall, PI and VITEK
85 MS produced similar correct identification rates (96.4% and 96.1%, respectively) across all
86 533 isolates, with misidentification occurring more frequently by PI than VITEK MS (3.4%
87 *vs.* 1.1%, P <0.05) (Table 1). Yeast isolates misidentified by PI included seven isolates of
88 *Candida tropicalis*, three of *Candida albicans*, two of *Pichia fabianii*, one of *Candida*
89 *glabrata*, and single isolates of five rare species. These data show that PI methods had
90 frequently provided inaccurate results for both common and unusual yeast species.

91 VITEK MS correctly identified 96.1% of all yeasts from BSIs. Fifteen isolates (2.8%)
92 were not identified, and six isolates (1.1%) were misidentified. This is comparable to a recent
93 multicenter study evaluating the VITEK MS identification of yeast, which show that 96.1%
94 and 0.6% isolates were either correctly identified or misidentified, respectively (9). The
95 misidentified isolates by the VITEK MS in this study included two isolates of *C. tropicalis*
96 identified as *C. albicans*, one isolate of *C. albicans* identified as *C. glabrata*, one isolate of *C.*
97 *glabrata* identified as *C. tropicalis*, one isolate of *P. fabianii* identified as *Candida boidinii*,
98 and one isolate of *Candida orthopsilosis* identified as *Candida magnoliae*. The
99 misidentification of two rare species was due to improper data base entries, but the reason for

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100 the misidentification of four isolates of common species is not completely understood.
101 However, it may be related to the random error associated with the short extraction method
102 on the plate in the VITEK MS (9), although further data are needed.

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

115 In the present study, the misidentification rate by PI varied considerably between
116 hospitals (0.0%-19.0%, P <0.05) (Table 2). The overall misidentification rate by PI was 2.2%
117 (11/498) for four common *Candida* species including *C. albicans*, *Candida parapsilosis*, *C.*
118 *tropicalis*, and *C. glabrata*, but 20.0% (7/35) for 13 rare species (P <0.001). The most
119 common species misidentified by PI were *C. tropicalis* (n=7), followed by *C. albicans* (n=3),
120 which may have resulted in inappropriate antifungal therapy. Commercial yeast identification
121 systems such as VITEK-2 have been shown to be less satisfactory at identifying *C. tropicalis*
122 (11), while CHROMagar *Candida* has been shown to be useful for identification of *C.*
123 *albicans* and *C. tropicalis* (12). In this study, the misidentification rate for BSI yeasts of *C.*

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124 *albicans* and *C. tropicalis* was 0% (0/75) in three hospitals (hospital B, H, and I) which had
125 used CHROMagar routinely, but 4.1% (10/241) in seven other hospitals. Thus, these data
126 support previous findings that the accuracy of commercial yeast identification systems can be
127 increased if laboratories use supplemental tests (4, 11). In addition, two hospitals (hospitals I
128 and J) which had smaller total number of BSI isolates than other eight hospitals, showed
129 higher misidentification rate (9.1% and 19.0%, respectively), suggesting that the hospitals
130 with higher volumes, thus more experience with phenotypic testing, showed lower
131 misidentification rate.

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

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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	Concordance between phenotypic identification and VITEK MS identification (%)	Prior phenotypic identification at each hospital			VITEK MS identification		
			Correct identification (%)	Mis-identification (%)	No identification[b] (%)	Correct identification (%)	Mis-identification (%)	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]
C. guilliermondii	9	9 (100.0)	9 (100.0)			9 (100.0)		
C. krusei	6	5 (83.3)	6 (100.0)			5 (83.3)		1 (16.7)[d]
C. pelliculosa	5	5 (100.0)	5 (100.0)			5 (100.0)		
C. lusitaniae	3	3 (100.0)	3 (100.0)			3 (100.0)		
C. 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)
C. haemulonii	1	1 (100.0)	1 (100.0)			1 (100.0)		
C. lipolytica	1	1 (100.0)	1 (100.0)			1 (100.0)		
C. orthopsilosis[e]	1	0 (0.0)		1 (100.0)			1 (100.0)	
C. 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]

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[a] Final identification was defined by either 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* isolates) which had been categorized as ‘no identification’ by 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.

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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 methods used for routine identification[b])										Total
	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)	
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 by either matching of results for prior phenotypic identification and VITEK MS, or by D1/D2 sequencing.

[b] Includes V2, VITEK 2 (bioMérieux, Marcy L'Étoile, 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.