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Comparative Evaluation of BD Phoenix and Vitek 2 Systems for Species Identification of Common and Uncommon Pathogenic Yeasts

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The BD Phoenix system was evaluated for species-level identification of yeasts (250 clinical isolates) and compared with the Vitek 2 system, using ribosomal internal transcribed spacer (ITS) sequence analysis as the gold standard. Considering only the species included in each system's database, 96.3% (236/245) and 91.4% (224/245) of the isolates were correctly identified by BD Phoenix and Vitek 2, respectively.

During the last decades, the growing number of vulnerable hosts such as critically ill or otherwise immunocompromised patients—e.g., individuals with advanced HIV infection or cancer who are undergoing transplant—has resulted in ever-increasing diagnoses of fungal infections (1, 2), including those caused by unusual opportunistic yeasts (3). Together with *Candida albicans*, the species most frequently isolated from clinical specimens (4, 5), other yeast species are increasingly recovered from patients with well-documented infections (6–8). They encompass non-*albicans Candida* species, including the rarer species *Candida famata*, *Candida kefyr*, *Candida lipolytica*, *Candida rugosa*, and *Candida utilis*, as well as uncommon species belonging to the genera *Trichosporon*, *Rhodotorula*, *Pichia*, *Malassezia*, and *Saccharomyces* (3).

It was recently observed that some yeast species are highly virulent and show reduced susceptibility to one or several antifungal agents (2, 3, 8–12) and that this has important clinical repercussions, often resulting in therapeutic failures (6, 13–15). Thus, accurate identification of these species is of utmost importance (16), but this goal is difficult to achieve, at least using conventional phenotypic methods (17, 18). In contrast, the newly developed matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) method may offer a highly discriminatory tool for identification of yeast isolates to the species level for *C. albicans* and *Candida glabrata* with unusual phenotypic or biochemical profiles as well (17), but to date its use has been curtailed or confined to large clinical microbiology laboratories (19).

In this study, the performance of the BD Phoenix Yeast ID panel for use with the Phoenix system (Becton, Dickinson Diagnostics, Sparks, MD) was compared to that of the Vitek 2 colorimetric YST card for use with the Vitek 2 system (bioMérieux, Marcy l'Etoile, France) using a large collection of clinical isolates from common and less-common yeast species.

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A total of 250 selected isolates, representing 29 yeast species from seven genera (see Table S1 in the supplemental material), were routinely obtained from mycological cultures of clinical specimens (i.e., blood, cerebrospinal fluid, respiratory tract, stool, and urine) of individual patients hospitalized at the Università Cattolica del Sacro Cuore of Rome (Italy) during the calendar year 2012. Isolate identification was obtained as previously described (20), by means of the DNA sequencing-based method using the ribosomal internal transcribed spacer (ITS) region located between the nuclear 18S and 26S rRNA genes (21), here used as the reference system. The quality control strains *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 2001, *Candida lusitaniae* ATCC 34449, *Candida parapsilosis* ATCC 22019, and *Candida krusei* ATCC 6258 were used throughout the study. Prior to testing, isolates were retrieved from frozen (−70°C) storage and subcultured on Sabouraud dextrose agar (SDA; Kima, Padua, Italy) or Columbia agar with 5% sheep blood (COL5B; bioMérieux) as appropriate for 48 h at 30°C to ensure purity, viability, and sufficient growth. All study isolates were simultaneously tested with the BD Phoenix and the Vitek 2 systems, according to the instructions of their respective manufacturers. Briefly, BD Phoenix Yeast ID panels—which include chromogenic and fluorogenic substrates as well as single-carbon-source substrates—were inoculated with a pure yeast suspension equivalent to a density of 2.0 McFarland, as determined using the BD PhoenixSpec nephelometer. Panels were loaded into the BD Phoenix instrument, incubated at 35°C, monitored every 20 min for up to 16 h, if necessary, and interpreted automatically by the instrument. As the Phoenix system does not report low discrimination or confidence values lower than 90%, results with scores of >90% were considered to represent identifications; otherwise, identification results were considered unacceptable. In the latter case, if a similar result was obtained on repeat testing, the isolate was classified as unidentified by that method. In the meantime, each yeast suspension was adjusted to a McFarland of 2.0 with a Vitek 2 DensiCheck turbidity meter and used to inoculate the colorimetric YST cards con-

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TABLE 1 Results of identification by BD Phoenix and Vitek 2 systems for 250 yeast isolates studied

Species (n^a)	No. (%) of isolates in category					
	BD Phoenix			Vitek 2		
	Correct identification	Misidentification	No identification	Correct identification	Misidentification	No identification
Common						
C. albicans (140)	138	1	1	135	4	1
C. glabrata complex (37)	37	0	0	36	1	0
C. parapsilosis complex (21)	19	1	1	19	1	1
C. tropicalis (10)	10	0	0	7	1	2
C. krusei (6)	6	0	0	5	0	1
S. cerevisiae (6)	6	0	0	5	1	0
C. neoformans (4)	4	0	0	4	0	0
Total common (224)	220 (98.2)	2 (0.9)	2 (0.9)	211 (94.2)	8 (3.6)	5 (2.2)
Rare						
B. capitatus (2)	2	0	0	0	2	0
C. dubliniensis (1)	1	0	0	1	0	0
C. guilliermondii (2)	2	0	0	1	1	0
C. kefyr (2)	1	1	0	1	1	0
C. lambica (1)	0	1	0	1	0	0
C. lipolytica (1)	0	1	0	1	0	0
C. lusitaniae (3)	3	0	0	2	1	0
C. norvegensis (2)	2	0	0	1	1	0
C. pararugosa (1)	0	1	0	0	1	0
C. utilis (1)	0	1	0	1	0	0
P. caribbica[b] (1)	0	1	0	0	1	0
P. fabianii[b] (1)	0	0	1	0	1	0
P. onychis[b] (1)	0	1	0	0	0	1
P. manshurica[b] (2)	0	0	2	0	2	0
R. mucilaginosa (2)	2	0	0	1	1	0
T. asahii (1)	1	0	0	1	0	0
T. inkin (1)	1	0	0	1	0	0
T. mucoides (1)	1	0	0	1	0	0
Total rare						
All (26)	16 (61.5)	7 (26.9)	3 (11.5)	13 (50.0)	12 (46.1)	1 (3.9)
Only those included in the database (21)	16 (76.2)	5 (23.8)	0 (0.0)	13 (61.9)	8 (38.1)	0 (0.0)
Total						
All (250)	236 (94.4)	9 (3.6)	5 (2.0)	224 (89.6)	20 (8.0)	6 (2.4)
Only those included in the database (245)	236 (96.3)	7 (2.8)	2 (0.8)	224 (91.4)	16 (6.5)	5 (2.0)

[a] *n*, no. of isolates tested.
[b] Species not included in each system's database.

taining the biochemical substrates. Cards were incubated for 18 h at 35°C and read every 15 min, and the final readings—as interpreted by the Vitek 2 instrument using the established algorithm for yeast identification—indicated a result of excellent identification, very good identification, good identification, acceptable identification, or low discrimination. In the last-named case, isolates were assessed for the presence or absence of well-formed pseudohyphae on cornmeal-Tween 80 agar or the growth at 42 to 45°C, which was required as supplemental testing for a definitive identification. Whenever identification was not obtained, the test was repeated. For each isolate, results from the two systems were categorized as follows: (i) correct identification, in which the isolate was properly assigned to a given species or, for the Vitek 2 system only, identified with low discrimination but resolved by supplemental tests; (ii) misidentification, in which the species identified with BD Phoenix Yeast ID panel or Vitek 2 colorimetric YST card was different from that identified by the reference system; and (iii) no identification, in which the isolate did not produce any result. Isolates identified by the Vitek 2 system with a low level of discrimination—either identification to the genus level or a low level of discrimination between two or more species—and not resolved by supplemental tests were considered misidentified.

Table 1 shows the species identifications by two test systems, BD Phoenix and Vitek 2, for the 250 yeast isolates studied, 224 of which represent species most frequently encountered in the clinical setting such as *C. albicans*, *C. glabrata*, *C. parapsilosis* complex, and *C. tropicalis*, as well as species relatively less frequently seen such as *C. krusei*, *Cryptococcus neoformans*, and *Saccharomyces*

TABLE 2 Results for 24 yeast isolates misidentified with the BD Phoenix or Vitek 2 system[a]

Species (no. of misidentified isolates)	Identification result by the method:	
	BD Phoenix	Vitek 2
B. capitatus (2)	*B. capitatus*	*C. lipolytica*
	B. capitatus	*C. lipolytica*
C. albicans (4)	*C. parapsilosis* complex	*C. parapsilosis* complex
	C. albicans	*C. famata*
	C. albicans	*C. famata*
	C. albicans	*C. lusitaniae*
C. glabrata (1)	*C. glabrata* complex	*C. albicans*
C. guilliermondii (1)	*C. guilliermondii*	*C. famata*
C. kefyr (1)	*S. cerevisiae*	*C. sphaerica*
C. lambica (1)	*C. firmentaria*	*C. lambica*
C. lipolytica (1)	*B. capitatus*	*C. lipolytica*
C. lusitaniae (1)	*C. lusitaniae*	*C. famata*
C. norvegensis (1)	*C. norvegenisis*	*C. incospicua*
C. orthopsilosis (1)	*S. cerevisiae*	*C. famata*
C. pararugosa (1)	*C. apicola*	*C. lipolytica*
C. tropicalis (1)	*C. tropicalis*	*C. parapsilosis* complex
C. utilis (1)	*S. cerevisiae*	*C. utilis*
P. caribbica (1)	*C. guilliermondii*	*C. famata*
P. fabianii (1)	None	*C. utilis*
P. manshurica (2)	None	*C. incospicua*
	None	*C. krusei*
P. onychis (1)	*C. kefyr*	None
R. mucilaginosa (1)	*R. mucilaginosa*	*Cryptococcus uniguttulatus*
S. cerevisiae (1)	*S. cerevisiae*	*C. sphaerica*

[a] The isolates were identified as *Blastoschizomyces capitatus* (2 isolates), *Candida* species (15 isolates), *Pichia* species (5 isolates), *Rhodotorula mucilaginosa* (1 isolate), and *Saccharomyces cerevisiae* (1 isolate), using the ITS sequencing reference method.

cerevisiae (see Table S1 in the supplemental material). Except for 2 *C. albicans* isolates (1 misidentified with *C. parapsilosis* complex and 1 unidentified) and 2 *C. parapsilosis* complex isolates (1 [*C. orthopsilosis*] misidentified with *S. cerevisiae* and 1 [*C. parapsilosis sensu stricto*] unidentified), all 220 of the 224 (98.2%) isolates were correctly identified by the BD Phoenix system. Conversely, the comparator Vitek 2 system resulted in 94.2% (211/224) of isolates correctly identified, 3.6% (8/224) misidentified, and 2.2% (5/224) not identified; all mistaken results were from 7 isolates of *Candida* species (including the isolate of *C. orthopsilosis* and 1 isolate of *C. albicans*, both misidentified by the BD Phoenix) and 1 *S. cerevisiae* isolate, as well; none of the results with no identification were from *S. cerevisiae* or *C. neoformans* isolates (see also Table 2).

Besides the results with respect to identification of *C. albicans* and other common non-*C. albicans* species, the ability of each of the systems shown here to accurately identify medically relevant non-*Candida* yeasts such as *Cryptococcus* or *Saccharomyces* (3) is notable *per se* but also in relation to recent studies aimed at evaluating the Vitek 2 and BD Phoenix systems in a face-to-face comparison or in comparisons of each with other commercial yeast identification systems (20, 22–24). In one BD Phoenix evaluation, 11 *Cryptococcus* isolates, including 3 *Cryptococcus gattii* isolates, were all initially identified as *C. neoformans*, but a subsequent analysis using upgraded BD Phoenix/Epicenter software led to assignment of only 8 *C. neoformans* isolates to the correct species designation, whereas the 3 *C. gattii* isolates were reported as *C. neoformans* along with the suggestion of performing additional tests to rule out *C. gattii* (23). In another study—in which 102 clinical isolates from 92 *Candida* species and 10 *C. neoformans* isolates were tested—all but 1 of the cryptococcal isolates were correctly identified to the species level by the BD Phoenix system, whereas 2 of the 10 isolates produced a low-discrimination result with the Vitek 2 system (24). Unfortunately, we were not able to include *C. gattii* isolates in the present study and, consequently, are unable to give any testing result by both the systems for this emerging cryptococcal species (3).

A potential drawback of BD Phoenix, as well as of the Vitek 2 system, concerns the inability to discriminate cryptic or closely related species within the *C. glabrata* and *C. parapsilosis* complexes. Although *C. parapsilosis sensu stricto* is responsible for the vast majority of human diseases associated with the three *Candida* species of the "*psilosis*" group, it is known that *C. orthopsilosis* and *C. metapsilosis* show antifungal susceptibility profiles different from that of *C. parapsilosis* that could affect therapeutic choices (25). To date, no commercial systems are currently available for this purpose (26, 27), but the low rates of antifungal resistance in fungemias caused by *C. orthopsilosis* and *C. metapsilosis* strains (9, 12) may counterbalance this lack. Of note, no isolation of *Candida nivariensis*—two isolates were studied here—and *Candida bracarensis* was reported in those survey studies (9, 12), in spite of the clinical, but moderate, interest elicited with respect to these *C. glabrata*-related species due to the propensity of some isolates to exhibit antifungal resistance (28).

With regard to the rare yeast species presently studied, all 26 isolates were encompassed in five genera of *Candida*, *Blastoschizomyces*, *Pichia*, *Rhodotorula*, and *Trichosporon*, thus resulting in 1 to 2 isolates per species (see Table S1 in the supplemental material). Of these isolates, the BD Phoenix system correctly identified 16 (61.5%), while 13 (50.0%) showed correct identification results by the Vitek 2 system (Table 1). Compared to that of Vitek 2, better performance of BD Phoenix was noted with *Blastoschizomyces capitatus* (2 isolates versus 0 isolates), *Candida guilliermondii* (2 isolates versus 1 isolate), *C. lusitaniae* (3 isolates versus 2 isolates), *Candida norvegensis* (2 isolates versus 1 isolate), and *Rhodotorula mucilaginosa* (2 isolates versus 1 isolate). In contrast, the three isolates of *Candida lambica*, *C. lipolytica*, and *C. utilis* were all identified by Vitek 2 and misidentified by BD Phoenix (Table 2). Also, isolates belonging to the rare species (i.e., from the genus *Pichia*) not included in each system's database were either misidentified or not identified; thus, it is remarkable that these isolates are mostly not identified rather than misidentified by the systems; such results were more frequently the case with the BD Phoenix system (3 isolates not identified versus 2 isolates misidentified) than with the Vitek 2 system (1 isolate not identified versus 4 isolates misidentified) (Tables 1 and 2).

By excluding the *Pichia* species (*P. caribbica*, *P. fabianii*, *P. manshurica*, *P. onychis*) that were predictably not identifiable by each system tested, correct identification rates for rare species increased to 76.2% and 61.9% for BD Phoenix and Vitek 2, respectively. Unfortunately, it was difficult to compare these findings with those from previous studies performed with the BD Phoenix system (23, 24) since, in one case (23), the 8 *Trichosporon* and 2 *Rhodotorula* isolates were evaluated only to the genus level; in the other case (24), no clinical isolates from species other than *Candida* and *C. neoformans* were studied, whereas the spectrum of

TABLE 3 Turnaround times for 224 common yeast isolates identified by BD Phoenix according to the species[a]

Species (no. of isolates)[b]	Time to result (h)	
	Mean ± SD	Range
C. albicans (140)	5.43 ± 2.13	4.09–15.47
C. glabrata complex (37)	8.90 ± 3.14	4.11–15.44
C. *parapsilosis* complex (21)	7.74 ± 3.18	4.22–15.42
C. tropicalis (10)	6.84 ± 4.68	4.11–15.45
C. krusei (6)	13.53 ± 4.50	4.33–15.43
S. cerevisiae (6)	4.28 ± 0.09	4.16–4.39
C. neoformans (4)	15.38 ± 0.05	15.35–15.46

[a] For all isolates, the Vitek 2 system provided an identification result at 18.00 h.
[b] Isolates from the *C. glabrata* complex included two *C. nivariensis* strains, whereas those from the *C. parapsilosis* complex belonged to *C. parapsilosis sensu stricto* (16 isolates), *C. metapsilosis* (three isolates), and *C. orthopsilosis* (two isolates).

uncommon and rarer yeast species—distributed over 42 challenge isolates supplied from Becton Dickinson—greatly differed from that in our study. Also, the percentage of rare yeast isolates for which Vitek 2 displayed a correct identification result in the present study was lower than that reported by Meletiadis et al. (22). This discrepancy (61.9% in our study versus 82.0% in reference 22) can be attributed to differences in the types and numbers of species examined, and it is in agreement with our previous findings (20). In fact, considering the nine taxonomically identical, but numerically different, yeast species analyzed in our two studies (this study and reference 20), Vitek 2 here showed a decreased ability to identify isolates within a subset of five species (*C. guilliermondii*, *C. kefyr*, *C. lusitaniae*, *C. norvegensis*, *Geotrichum capitatum*), but its performance was superior or equal with isolates of the species *C. lipolytica*, *C. utilis*, *Trichosporon asahii*, and *Trichosporon mucoides*.

In view of the potential clinical impact that an erroneous identification result would have, a straightforward appraisal of how the BD Phoenix works with uncommon yeast species implies the need for multicenter studies which examine a large number of the isolates for each rare species of yeast. Castanheira et al. (18) in reexamining by means of DNA sequencing methods 53 isolates collected during the SENTRY and ARTEMIS surveillance programs found that isolates of *C. guilliermondii*, *Candida fermentati*, *C. lusitaniae*, and *Candida intermedia* and other species had been all misidentified as *C. famata*. Not surprisingly, the yeast identification methods mainly used in the submitting laboratories had been Vitek 2 (60% of the isolates), MicroScan (8% of the isolates), API (8% of the isolates), and AuxaColor (4% of the isolates).

As early diagnosis and/or treatment of fungal infections is crucial to improve patient outcomes (29, 30), a higher diagnostic precision with respect to identification to the fungal species level needs to be accomplished at significantly reduced turnaround times (31). Final BD Phoenix results are generally available in 4 to 12 h, while those determined by the Vitek 2 system are not readable in less than 18 h (Table 3), thus implying a time savings of 6 to 14 h when BD Phoenix is used instead of Vitek 2. In the present study, the turnaround times (in hours) to identify 250 yeast isolates by BD Phoenix ranged from 4.09 to 15.47, with a mean time of 6.90 (see Table S2 in the supplemental material). Expectedly, for the most common species, excluding *C. albicans*, the mean time to result was shorter for *C. tropicalis* isolates (6.84 h) and longer for *C. krusei* isolates (13.53 h), but it is noteworthy that a minimum of ~4.1 h, on average, was sufficient to provide a final BD Phoenix identification result, regardless of whether the isolate was identified as *C. albicans*, *C. glabrata* complex, *C. parapsilosis* complex, *C. krusei*, or *S. cerevisiae* (Table 3). In contrast, long turnaround times (~11 to ~15 h)—but still below the 18-h threshold seen with Vitek 2—were needed to identify rarer species of *Candida* (i.e., *C. lusitaniae*, *C. dubliniensis*, *C. lipolytica*, *C. lambica*, *C. norvegensis*, and *C. nivariensis*), *Pichia*, or *Rhodotorula*; advantageously, short turnaround times were observed for identification of *Trichosporon* species (~4 to ~8 h) or *Blastoschizomyces capitatus* (mean, 9.83 h) that were quite far from those observed for *S. cerevisiae* (mean, 4.28 h) or *C. neoformans* (mean, 15.38 h) (see Table S2).

In conclusion, both the BD Phoenix and the Vitek 2 identification systems performed well with clinical yeast species, providing a reliable and timely—particularly for BD Phoenix—means to correctly identify, respectively, 96.3% and 91.4% of the isolates evaluated in our study. The performance of BD Phoenix was superior that of the Vitek 2 system, including its performance with those yeast species that are rarely isolated in the clinical setting, although it was not completely satisfactory. New and very promising molecular phenotypic methods such as MALDI-TOF MS are ready to use, but many clinical microbiology laboratories still rely on instruments such as BD Phoenix and Vitek 2 for the routine identification of bacterial and fungal isolates, and it is reasonable to expect a continuing improvement of the diagnostic strategies they currently handle.

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