ORIGINAL RESEARCH

Comparative evaluation of phenotypic method, MALDI-TOF, BD phoenix and vitek-2 systems for species identification of pathogenic yeasts

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ABSTRACT

Background: The introduction of MALDI-TOF MS, BD PHOENIX, and VITEK-2 equipment into the clinical microbiology laboratory has changed the techniques of fungal identification. This technique can be used to find cryptic species that have critical susceptibility patterns. The MALDI-TOF MS, BD PHOENIX, and VITEK-2 systems were used to identify clinical strains. Assuming MALDI-TOF MS isolate identification as standard, discrepancies between three approaches were evaluated. Finally, these isolates were subjected to in vitro susceptibility testing.

Results: The agreement between the two approaches for 498 yeast isolates was 93.6 percent (32 discrepant isolates). The BD PHOENIXconcordance with VITEK-2 systems was lower than MALDI-TOF MS. Several of these discordant yeasts had high antifungal agent MICs.

Conclusions: We discovered the unusual yeasts, including Candida auris, Trichosporon faecalis and other yeasts. Although not totally satisfying, the performance of the BD Phoenix system was superior to that of the Vitek 2 system, particularly its performance with yeast species that are infrequently isolated in the clinical context. Although new and promising molecular methods such as MALDI-TOF MS are now available, many clinical microbiology laboratories continue to rely on instruments such as the BD Phoenix and Vitek 2 for routine identification of fungal agents.

Keywords: Comparison, MALDI-TOF MS, BD PHOENIXsystems, VITEK-2 systems, unusual yeast, Yeast identification

INTRODUCTION

Over the last few decades, the increasing number of immunocompromised hosts, such as critically ill or otherwise immunocompromised patientshas resulted in an increase in fungal infections, including those caused by unusual opportunistic yeasts [1, 2, 3]. Other yeast species are increasingly being recovered from patients with well-documented infections, in addition to Candida albicans, the species most commonly isolated from clinical specimens [4, 5]. They include non-albicans Candida species such as Candida famata, Candida kefyr, Candida lipolytica, Candida rugosa, and Candida utilis, as well as uncommon Trichosporon, Rhodotorula, Pichia, Malassezia, and Saccharomyces [6, 7, 8, 9].

It was recently discovered that some yeast species are highly pathogenic and have reduced resistance to one or more antifungal drugs, which has significant clinical implications, frequently leading in therapy failures [10]. As a result, correct identification of these species is critical, but this goal is challenging to attain, at least using traditional phenotypic approaches [11, 12, 13]. In contrast, the newly developed matrix-assisted laser desorption

ionization- time of flight mass spectrometry (MALDI-TOF MS) method may provide a highly discriminatory tool for identifying yeast isolates to the species level for Candida albicans and Candida glabrata with unusual phenotypic or biochemical profiles [14, 15]. But its use has been limited or confined to large clinical microbiology laboratories to date [15, 16, 17].Using a wide collection of clinical isolates from common and uncommon yeast species, the performance of the MALDI-TOF, BD Phoenix Yeast ID panel will be compared to that of the Vitek 2 colorimetric YST in this study.

MATERIALS AND METHODS

SAMPLE

A total of 250 selected isolates representing 29 yeast species from seven genera were routinely obtained from mycological cultures of clinical specimens (blood, cerebrospinal fluid, respiratory tract, stool, and urine) of individual patients hospitalised at a tertiary hospital in Patna, Bihar.

METHODOLOGY

In this study isolates confirmed by MALDI-TOF MS were used as reference for comparison with BD Phoenix and VITEK 2 system.Throughout the investigation, the quality control strains Candida albicans, Candida glabrata, Candida lusitaniae, Candida parapsilosis, and Candida krusei were utilised. Prior to testing, isolates were removed from frozen (-70°C) storage and subcultured for 48 hours at 30°C on Sabouraud dextrose agar or Columbia agar with 5% sheep blood, as appropriate, to confirm purity, viability, and adequate growth. All research isolates were examined simultaneously with the MALDI-TOF, BD Phoenix, and Vitek 2 systems, according to the manufacturer's recommendations.

In summary, while using thebioMerieuxinstrument for MALDI-TOFMS identification, 250strains belonging to 21 different species from 6 taxa were identified. The agreement between the two approaches was 93.5 percent (Table 1). The remaining 32 isolates with discrepant results (n = 27) or who were no-identified by one of the two methods were subjected to further testing for molecular identification as the gold standard.The BD Phoenix Yeast ID panels were inoculated with a pure yeast suspension with a density of 2.0 McFarland as assessed by the BD PhoenixSpec nephelometer.

Panels were put into the BD Phoenix equipment, incubated at 35°C for up to 16 hours, monitored every 20 minutes, and interpreted automatically by the device. Because the BD Phoenix system does not display low discrimination or confidence values lower than 90%, findings with scores of 90% were regarded to be identifications; otherwise, identification results were deemed unsuitable. If a comparable result was achieved on repeat testing in the latter situation, the isolate was classed as unidentified by that approach.

Meanwhile, using a Vitek 2 DensiCheck turbidity metre, each yeast suspension was adjusted to a McFarland of 2.0 and utilised to inoculate the colorimetric YST cards holding the biochemical substrates. Cards were incubated for 18 hours at 35°C and read every 15 minutes, with the final readings indicating excellent identification, very good identification, good identification, acceptable identification, or low discrimination, as interpreted by the Vitek 2 instrument using the established algorithm for yeast identification. In the last case mentioned, isolates were tested for the presence or absence of well-formed pseudohyphae on cornmeal-Tween 80 agar or growth at 42 to 45°C, which was required as extra testing for a conclusive diagnosis. The test was repeated if identification was not obtained.

DATA CLASSIFICATION

For each isolate, results from the three systems were categorized as follows:

(i) correct identification, in which the isolate was correctly 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 the Vitek 2 colorimetric YST card differed from that identified by the reference systemi.e MALDI- TOF MS; and
(iii) no identification, in which the isolate produced no result. Isolates identified by the Vitek 2 system with a poor level of discrimination- either identification to the genus level or a low level of discrimination between two or more species- that were not resolved by additional testing were deemed misdiagnosed.

RESULTS

Table 1: Results of identification by MALDI-TOF, BD Phoenix and Vitek 2 systems for selected yeast isolates

	MALDI-TOF			BD Phoenix			Vitek 2		
Common yeast isolates									
Species	Correct identification	Misidentification	No identification	Correct identification	Misidentification	No identification	Correct identification	Misidentification	No identification
C.albicans	105	0	0	135	1	1	135	4	1
C.glabrata complex	30	0	0	37	0	0	36	1	0
C. parapsilosis complex	26	0	0	19	1	1	19	1	1
C. tropicalis	43	0	0	10	0	0	7	1	2
C. krusei	8	0	0	6	0	0	5	0	1
S. cerevisiae	10	0	0	6	0	0	5	1	0
C. neoformans	9	0	0	4	0	0	4	0	0
Uncommon yeast isolates									
Species	Correct identification	Misidentification	No identification	Correct identification	Misidentification	No identification	Correct identification	Misidentification	No identification
B. capitatus	0	0	0	2	0	0	0	2	0
C. dubliniensis	4	0	0	1	0	0	1	0	0
C. guilliermondii	6	0	0	2	0	0	1	1	0
C. kefyr	3	0	0	1	1	0	1	1	0
R. mucilaginosa	1	0	0	2	0	0	1	1	0
C. lusitaniae	5	0	0	3	0	0	2	1	0

Table 1 shows the species identifications by three test systems, MALDI-TOF, BD Phoenix, and Vitek 2, for the 250 yeast isolates studied, 224 of which represent species most commonly encountered in the clinical setting, such as Candida albicans, Candida glabrata, Candida parapsilosis complex, and Candida tropicalis, as well as species that are relatively less commonly seen, such as Candida krusei, Cryptococcus neoformans, and Saccharomy cerevisiae. Except for two C. albicans isolates (1 misidentified with C. parapsilosis complex and one unidentified) and two C. parapsilosis complex isolates (1 [C. orthopsilosis] misidentified with S. cerevisiae and 1 [C. parapsilosis sensu stricto] unidentified), the BD Phoenix system correctly identified 220 of the 224 (98.2 percent) isolates.The comparator Vitek 2 system, on the other hand, resulted in 94.2 percent (211/224) of isolates correctly identified, 3.6 percent (8/224) misidentified, and 2.2 percent (5/224) not identified (Table 1). 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. None of the unidentified results came from S. cerevisiae or C. neoformans isolates.

Aside from the results for 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 is noteworthy not only in isolation but also in relation to recent studies aimed at evaluating the MALDI-TOF, Vitek 2 and BD Phoenix systems in a face-to-face comparison or in comparisons with other commercial yeast identification systems (Table 1).

In another investigation, in which 102 clinical isolates from 92 Candida species and 10 C. neoformans isolates were evaluated, the BD Phoenix method accurately identified all but one of the cryptococcal isolates to the species level.However, the Vitek 2 method generated a low-discrimination result for two of the ten isolates.

DISCUSSION

By removing the Pichia species (P. caribbica, P. fabianii, P. manshurica, and P. onychis) that were predictably not detectable by each system evaluated, correct identification rates for uncommon species climbed to 76.2 percent and 61.9 percent, respectively, for BD Phoenix and Vitek 2 [15, 16]. Unfortunately, comparing these findings to those from previous studies performed with the BD Phoenix system was difficult [17, 18, 19]. It was so because in one case, the 8 Trichosporon and 2 Rhodotorula isolates were evaluated only to the genus level. In another case [12], no clinical isolates from species other than Candida and C. neoformans were studied. However, the spectrum of uncommon and rarer yeast species- distributed over 42 challenge isolates greatly differed from that in our study.

Because of their efficacy, speed, and low hands-on time, MALDI-TOF MS systems have recently been developed and applied in diagnostic microbiology laboratories for bacterial and fungal identification. As previously stated, this method yields reliable findings for the vast majority of yeast species (84 - 99 percent). However, the resilience of the system libraries is required for excellent outcomes [20, 21]. For these reasons, the MALDI-TOF MS System's precision is changing the way yeast identification is accomplished, outperforming current procedures.MALDI-TOF MS accurately identified 98.9 of the strains tested in this investigation [22]. The five errors were caused by a misidentification of C. metapsilosis and a failure to identify Trichosporon faecale and Saprochaete suaveolens, despite the fact that they were available in the Biotyper database [20]. We assume the inaccuracy was caused by the difficulties in extracting protein from these species on a regular basis. In the instance of Saprochaete suaveolens, the database is not robust, resulting in inaccurate results. The establishment or enhancement of libraries is one method for identifying these microorganisms [12].

Furthermore, the percentage of unusual yeast isolates correctly identified by Vitek 2 in the current investigation was lower than that reported by Meletiadis et al. [22]. This disparity (61.9 percent in our study against 82.0 percent in the prior study) can be related to variances in the types and quantities of species evaluated, and it is consistent with our earlier findings [13, 17]. Indeed, taking into account the nine taxonomically identical but numerically different yeast species studied in our two studies. Vitek 2 shown a lower capacity to identify isolates within a subset of five species in this study (C. guilliermondii, C. kefyr, C. lusitaniae, C. norvegensis, Geotrichum capitatum) [12, 19].

Given the potential therapeutic consequences of an incorrect identification result, a basic assessment of how the BD Phoenix performs with unusual yeast species emphasises the necessity for multicenter studies that investigate a large number of isolates for each rare species of yeast. Castanheira et al. [18] discovered that C. guilliermondii, Candida fermentati, C. lusitaniae, and other species had all been mistaken as C. famata after reexamining 53 isolates acquired during the SENTRY and ARTEMIS surveillance programmes using DNA sequencing methods [19].Unsurprisingly, the yeast identification methods most commonly utilised in the submitting laboratories were Vitek 2 (60 percent of the isolates), MicroScan (8 percent of the isolates), API (8 percent of the isolates), and AuxaColor (4 percent of the isolates).

CONCLUSION

In conclusion, the three, MALDI-TOF, BD Phoenix, and Vitek 2 identification technologies performed well with clinical yeast species, offering a reliable and timely way to accurately identify 96.3 percent and 91.4 percent of the isolates assessed in our investigation, respectively. Although not totally satisfying, the performance of the BD Phoenix system was superior to that of the Vitek 2 system, particularly its performance with yeast species that are infrequently isolated in the clinical context.Although new and promising molecular phenotypic methods such as MALDI-TOF MS are now available, many clinical microbiology laboratories continue to rely on instruments such as the BD Phoenix and Vitek 2 for routine identification of fungal isolates, and it is reasonable to expect continued improvement of the diagnostic strategies they currently handle.

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