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Limitations of the Current Microbial Identification System for Identification of Clinical Yeast Isolates

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The ability of the rapid, computerized Microbial Identification System (MIS; Microbial ID, Inc.) to identify a variety of clinical isolates of yeast species was compared to the abilities of a combination of tests including the Yeast Biochemical Card (bioMerieux Vitek), determination of microscopic morphology on cornmeal agar with Tween 80, and when necessary, conventional biochemical tests and/or the API 20C Aux system (bioMerieux Vitek) to identify the same yeast isolates. The MIS chromatographically analyzes cellular fatty acids and compares the results with the fatty acid profiles in its database. Yeast isolates were subcultured onto Sabouraud dextrose agar and were incubated at 28°C for 24 h. The resulting colonies were saponified, methylated, extracted, and chromatographically analyzed (by version 3.8 of the MIS YSTCLN database) according to the manufacturer's instructions. Of 477 isolates of 23 species tested, 448 (94%) were given species names by the MIS and 29 (6%) were unidentified (specified as "no match" by the MIS). Of the 448 isolates given names by the MIS, only 335 (75%) of the identifications were correct to the species level. While the MIS correctly identified only 102 (82%) of 124 isolates of *Candida glabrata*, the predictive value of an MIS identification of unknown isolates as *C. glabrata* was 100% (102 of 102) because no isolates of other species were misidentified as *C. glabrata*. In contrast, while the MIS correctly identified 100% (15 of 15) of the isolates of *Saccharomyces cerevisiae*, the predictive value of an MIS identification of unknown isolates as *S. cerevisiae* was only 47% (15 of 32), because 17 isolates of *C. glabrata* were misidentified as *S. cerevisiae*. The low predictive values for accuracy associated with MIS identifications for most of the remaining yeast species indicate that the procedure and/or database for the system need to be improved.

Patients are increasingly being colonized and infected with a variety of yeast species due to debilitating diseases such as AIDS, diabetes mellitus, and malignancies, as well as to the increasing use of indwelling central venous catheters, organ transplants, anticancer drugs, broad-spectrum antibiotics, and corticosteroid therapy (2, 4, 9, 11, 14, 18, 20, 21). The specific medical procedure or type of disease implicated in a yeast infection may be more likely to be associated with certain yeast species than with others (11, 21). For example, patients with leukemia are more likely to be infected with *Candida albicans* or *Candida tropicalis* than with *Candida glabrata*, while patients with solid tumors are at a greater risk for infection with *C. glabrata* (21). In addition, yeast species can vary greatly in their relative virulence (21) as well as their susceptibilities to antifungal agents (3, 11, 15). Because of the either natural or acquired resistance of some of the yeast pathogens to antifungal drugs, the severity of systemic yeast infections, and the increasing desire to limit the duration of patients' hospital stays in order to control costs, the rapid, accurate identification of a wide variety of yeast species that can be recovered from patients with well-documented infections is clinically important (7, 11, 13, 18, 20).

By conventional identification methods including carbohydrate assimilation and fermentation, the correct species identification of many clinical yeast isolates is often complex and time-consuming (6, 10, 18, 20). The automated Microbial Identification System (MIS; Microbial ID, Inc., Newark, Del.) has provided a reasonably accurate, rapid, and cost-effective alternative for the identification of many aerobic gram-positive and gram-negative bacterial species (1, 12, 17). The system also has a database for the identification of yeast species. The MIS includes a gas chromatograph with a flame ionization detector, along with an autosampler, an integrator, and a computer. The system identifies and quantifies the fatty acid methyl esters of the microorganisms. The computer then searches a software library of fatty acid compositions, compares the fatty acid profile of the isolate with those of known species, and generates a report giving the most likely species name of the isolate along with the extent of correlation of the isolate's profile with a species in the database, listed as the similarity index (19).

A previous study reported that the MIS correctly identified only 71% of the clinical isolates of 10 yeast species to the species level (5). In contemplating the use of the MIS (or any other system) for yeast identification, it is important not only to determine the ability of the system to identify multiple isolates of each species accurately but also to document the predictive values for the accuracies of the MIS identifications for each of the species. The predictive value of an MIS identification for any one species is calculated by dividing the number of correct MIS identifications for that species by the total number of times that the MIS both correctly and incorrectly called yeast isolates by that one name. It is possible that although the MIS may correctly identify well under 90% of the isolates of any one species, for example, *C. glabrata*, the predictive value for MIS identifications of unknown isolates as that species may be acceptably high because few isolates of other yeast species are misidentified by the system as *C. glabrata*. A high predictive value (perhaps ≥95%) associated with an MIS identification of a given species might therefore permit the laboratory to confidently report isolates with that species identification without further expensive and time-consuming tests. In contrast, a low

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predictive value associated with an MIS identification of another species would necessitate further testing of any isolates given that species name by the system, even if greater than 95% of the isolates of that species were correctly identified by the system. The current study was undertaken to determine both the percentage of isolates of each yeast species that were correctly identified by the MIS as well as the predictive values of accuracy associated with MIS identifications of unknown isolates as each of the species.

MATERIALS AND METHODS

The majority (n = 406; 85%) of yeasts used in the current study were freshly isolated from clinical specimens. In addition, 71 (15%) of the isolates studied were stock cultures of infrequently isolated yeasts (from the Laboratories for Mycology, New York State Department of Health) which had previously been recovered from clinical specimens. More than 75% of all of the isolates tested were recovered from urine, genital, and wound specimens submitted for culture. Multiple isolates of the same species from the same patients were excluded from the study. Isolates were initially subcultured onto Sabouraud dextrose agar (SDA; Becton Dickinson Microbiology Systems, Cockeysville, Md.) and onto cornmeal agar with 0.5% Tween 80 (CMT), which were incubated at 25°C.

Conventional identification methods. Each of the fresh clinical yeast isolates was preliminarily identified by determination of its microscopic morphology on CMT and by its colony morphology and pigment production on SDA. Isolates were identified as *C. albicans* by their typical microscopic appearances on CMT, including the production of chlamydospores (9, 11, 20). The identification of isolates of most of the other species was finalized with the Yeast Biochemical Card (YBC; bioMerieux Vitek, Hazelwood, Mo.) (6–8, 10, 13, 16, 18), which was inoculated and incubated according to the manufacturer's specifications. The inoculum for the YBC was adjusted with a colorimeter (bioMerieux Vitek) to 46 to 56% transmission (equivalent to a no. 2 McFarland standard), a standardization of the inoculum that was recommended previously (18). Fresh clinical isolates which could not be conclusively identified by using the combination of their microscopic morphology and the YBC were identified with the API 20C Aux system (bioMerieux Vitek) (8, 13) and/or by additional tests, as appropriate. These additional tests included assimilation of from 7 to 12 carbohydrates (dextrose, maltose, sucrose, lactose, galactose, raffinose, trehalose, inositol, xylose, dulcitol, melibiose, and rhamnose) in disk form on yeast nitrogen base agar, nitrate assimilation on yeast carbon base agar, urease production, ascospore production, and relative growth on SDA incubated at 25 and 37°C (9, 11, 20). Stock cultures of the clinical isolates were identified with the API 20C system (bioMerieux Vitek). The identification obtained by the procedures described above was considered the correct identification.

Chromatographic identification method. Isolated colonies of the yeasts were streaked onto quadrants of SDA plates, and the plates were incubated for 24 ± 2 h at 28 ± 1°C in an aerobic atmosphere, as specified by the manufacturer of the MIS (19). Fatty acid methyl ester extracts were prepared and then analyzed on a 5890 series II gas-liquid chromatograph (Hewlett-Packard, Avondale, Pa.) (19). An external calibration mixture (Microbial ID, Inc.) and an extract of a control strain (*Candida krusei* ATCC 44507) were chromatographically analyzed on each day of testing. Version 3.8 of the YSTCLN database in the MIS computer was used to identify the isolates. For each isolate, the computer printout either listed one or more possible species choices with a similarity index (SI) for each choice ranging from 0 to 1.000 or it reported "no match," which indicated that the MIS was unable to identify the isolate. For the current study, the MIS result was considered correct if the correct species name of an isolate was listed on the MIS printout as the first choice, regardless of the SI, as suggested for gram-negative bacterial species in a previous study of the MIS (17).

When the MIS result was either a misidentification to the species level or "no match," the microscopic morphology of the isolate was determined again and the API 20C Aux system or appropriate conventional test systems were inoculated to confirm the species' identification. In addition, a fresh extract from a new subculture of the isolate on SDA, incubated at 28°C, was analyzed in the chromatograph a second time. If an isolate was misidentified the first time that it was analyzed in the MIS, it was counted as a misidentification, regardless of whether it was correctly or incorrectly identified by the system when it was reanalyzed in the MIS. If it was unidentified by the MIS when it was first tested and then correctly or incorrectly identified when the chromatography was repeated, it was counted as a correct or an incorrect identification, respectively. In addition to calculating the percentage of isolates of each yeast species that were correctly identified by the MIS, the predictive values for the accuracy of MIS assignments of unknown yeast isolates to each of the yeast species were determined as described above.

RESULTS

Of 477 yeast isolates from 23 species studied, the MIS was unable to identify 29 (6%) (Table 1), calling them by the term "no match." Of the 448 isolates identified by the MIS, 403 (90%) were correctly identified to the genus level but only 335 (75%) were correctly identified to the species level. In all, 142 (30%) of the 477 isolates studied were either misidentified or unidentified by the MIS and only 335 (70%) were correctly identified to the species level. When six or more isolates of a species were tested, correct identification by the MIS to the species level ranged from 0% (for isolates of *Cryptococcus albidus*) to 100% (for *Rhodotorula rubra* and *Saccharomyces cerevisiae*). We encountered 32 isolates (4 *Candida famata*, 7 *C. albidus*, 4 *Cryptococcus humicolus*, 2 *Cryptococcus laurentii*, 4 *Cryptococcus terreus*, 3 *Cryptococcus uniguttulatus*, 4 *Hansenula anomala*, 3 *Rhodotorula glutinis*, and 1 *Trichosporon penicillatum*) of yeast species for which the MIS software library had no data. Of these 32 isolates, 6 (1 *C. albidus*, 1 *C. terreus*, all 3 *C. uniguttulatus*, and 1 *R. glutinis*) were correctly called "no match" by the system, and the remaining 26 isolates were incorrectly identified.

The predictive value of the MIS species identifications (the probability that the MIS identifications of unknown isolates as each species were correct) was 75% overall but ranged from 0% (for *Geotrichum candidum* and *Sporobolomyces salmonicolor*) to 100% (for *C. glabrata* and *Kluyveromyces marxianus*) (Table 1). It is of interest that while the MIS correctly identified only 102 (82%) of the isolates of *C. glabrata*, the predictive value of an MIS identification of an unknown isolate as that species was 100% (102 of 102 isolates), since the system did not misidentify any isolates of other species as *C. glabrata*. In contrast, while the MIS correctly identified 100% (all 15) of the isolates of *S. cerevisiae*, the predictive value of an MIS identification of an unknown isolate as that species was only 47% because the system misidentified 17 isolates of *C. glabrata* as *S. cerevisiae* (Table 2). The predictive value of an MIS identification as *C. albicans* was only 84% because 18 isolates of other species, including 15 isolates of *Candida tropicalis*, were called *C. albicans* by the MIS.

Of 477 yeast isolates that were chromatographically analyzed, almost a third (n = 153 [32%]) had to be analyzed a second time because the initial MIS result was either "no match" (57 isolates) or incorrect (96 isolates). For these 153 reanalyzed isolates, the results for only 48 (31%) changed from incorrect or "no match" to correct, the results for another 48 (31%) remained incorrect, the results for 29 (19%) remained "no match," the results for 11 (7%) changed from incorrect to "no match," and the results for 17 (11%) changed from "no match" to incorrect.

The processing of one yeast isolate for chromatography by the MIS cost approximately $1.50 for material. The total processing time, from start to finish, for a gas-liquid chromatographic (GLC) analysis was 2 to 2.5 h, but the total labor required was 7 to 15 min per isolate, depending on the number of isolates extracted and chromatographically analyzed each day.

DISCUSSION

Only 70 to 71% of the yeast isolates investigated were correctly identified by the MIS during this and a previous study (5). It is of interest that of the isolates of *C. glabrata* that were misidentified by the GLC system in the current study, all 17 were called *S. cerevisiae*. When the MIS identified an isolate as *S. cerevisiae*, there was a 53% probability (17 of 32) that the isolate was, in fact, an isolate of *C. glabrata* and only a 47% probability that the isolate was *S. cerevisiae*. Nevertheless, because of the high predictive value (100%) associated with MIS identifications of isolates as *C. glabrata*, an MIS identification

TABLE 1. Comparison of the MIS with conventional tests for identification of yeasts grown on SDA

Species identified by conventional tests	No. of isolates tested	No. (%) of isolates tested by GLC				Predictive value of a GLC species identification (% [no. of isolates correctly identified/ total no. tested])[a]
		Correctly identified to species level	Incorrectly identified to species level		Unidentified ("no match")	
			Correct genus	Incorrect genus		
Blastoschizomyces capitatus	1			1 (100)		
Candida spp.						
C. albicans	115	98 (85)	16 (14)		1 (1)	84 (98/116)
C. famata	4		4 (100)			
C. glabrata	124	102 (82)		17 (14)	5 (4)	100 (102/102)
C. guilliermondii	11	7 (64)	4 (36)			23 (7/30)
C. krusei	18	14 (78)			4 (22)	88 (14/16)
C. lusitaniae	8	5 (63)	3 (38)			50 (5/10)
C. parapsilosis	56	53 (95)	2 (4)		1 (2)	78 (53/68)
C. tropicalis	53	14 (26)	28 (53)	1 (2)	10 (19)	78 (14/18)
Cryptococcus spp.						
C. albidus	7		4 (57)	2 (29)	1 (14)	
C. humicolus	4		3 (75)	1 (25)		
C. laurentii	2		1 (50)	1 (50)		
C. neoformans	11	8 (73)		3 (27)		40 (8/20)
C. terreus	4		2 (50)	1 (25)	1 (25)	
C. uniguttulatus	3				3 (100)	
Geotrichum candidum	0					0 (0/2)
Hansenula anomala	4			4 (100)		
Kluyveromyces marxianus	10	6 (60)		3 (30)	1 (10)	100 (6/6)
Rhodotorula spp.						
R. glutinis	3		1 (33)	1 (33)	1 (33)	
R. rubra	11	11 (100)				73 (11/15)
Saccharomyces cerevisiae	15	15 (100)				47 (15/32)
Sporobolomyces salmonicolor	4			3 (75)	1 (25)	0 (0/6)
Trichosporon spp.						
T. beigelii	8	2 (25)		6 (75)		29 (2/7)
T. penicillatum	1			1 (100)		
Total	477	335 (70.2)	68 (14.3)	45 (9.4)	29 (6.1)	74.8 (335/448)

[a] Predictive values are lacking for some species because either the MIS called no isolates by that species name (*B. capitatus*) or the species was not included in the MIS database (the remaining species for which no predictive values are listed).

of an unknown isolate as that species could be reported without additional confirmatory testing. The predictive value of an MIS identification of an unknown isolate as *K. marxianus* was also 100% (although only 10 isolates of that species were tested during the current study) because no other isolates of other yeast species were misidentified by the MIS as *K. marxianus.* The MIS identifications an unknown isolates as most of the remaining species should be routinely confirmed by at least documenting a microscopic and colonial morphology which is compatible with the MIS identification and by performing supplemental biochemical tests when appropriate. This recommendation is similar to suggestions made previously for other rapid yeast identification systems (6–8, 16). The results of the current study confirm and extend the results of the earlier study of MIS yeast identification (5).

Rapid reporting of an accurate yeast species identification can provide physicians with important information for patient management. This information is of particular importance for yeast species including *C. glabrata*, *Candida guilliermondii*, *C. krusei*, *Candida lusitaniae*, *Candida parapsilosis*, *C. tropicalis*, *Cryptococcus neoformans*, and *Trichosporon beigelii*, the species that may be resistant to amphotericin B and/or the newer azole antifungal agents (2–4, 11, 15, 20, 21). The initiation of effective antifungal therapy as quickly as possible can only improve a patient's outcome (20). The rapid, accurate identification of the species of a yeast pathogen may help the physician to select quickly and specifically the most appropriate antifungal agent in the absence of susceptibility tests or before the results of those tests become available. For example, *C. albicans* will most likely respond to azoles including miconazole, ketoconazole, and fluconazole, while *C. tropicalis* may respond only to miconazole and fluconazole and *C. krusei* may respond only to miconazole and ketoconazole (3). In the current study, 15 (28%) of the isolates of *C. tropicalis* (which may not respond to ketoconazole) were misidentified by the MIS as *C. albicans*. In addition, 14 (12%) of the isolates of *C. albicans* (which is likely

TABLE 2. Misidentifications of yeast species by the MIS

Species identified by conventional tests (no. of isolates)	No. of isolates misidentified	No. (%) of isolates misidentified as the following species:											
		C. albicans	*C. guilliermondii*	*C. krusei*	*C. lusitaniae*	*C. parapsilosis*	*C. tropicalis*	*C. neoformans*	*G. candidum*	*R. rubra*	*S. cerevisiae*	*S. salmonicolor*	*T. beigelii*
B. capitatus (1)	1				1 (100)								
C. albicans (115)	16		14 (88)		1 (6)		1 (6)						
C. famata (4)	4			1 (25)		3 (75)							
C. glabrata (124)	17										17 (100)		
C. guilliermondii (11)	4	1 (25)		1 (25)		2 (50)							
C. lusitaniae (8)	3					3 (100)							
C. parapsilosis (56)	2	2 (100)											
C. tropicalis (53)	29	15 (52)	9 (31)		2 (7)	2 (7)						1 (3)	
C. albidus (7)	6							4 (67)					2 (33)
C. humicolus (4)	4							3 (75)				1 (25)	
C. laurentii (2)	2							1 (50)					1 (50)
C. neoformans (11)	3								1 (33)	1 (33)			1 (33)
C. terreus (4)	3							2 (67)					1 (33)
H. anomala (4)	4					3 (75)						1 (25)	
K. marxianus (10)	3						3 (100)						
R. glutinis (3)	2							1 (50)		1 (50)			
S. salmonicolor (4)	3					1 (33)				2 (67)			
T. beigelii (8)	6				1 (17)	1 (17)		1 (17)				3 (50)	
T. penicillatum (1)	1								1 (100)				
Total (430)	113	18 (16)	23 (20)	2 (2)	5 (4)	15 (13)	4 (4)	12 (11)	2 (2)	4 (4)	17 (15)	6 (5)	5 (4)

to be susceptible to amphotericin B) were misidentified by the MIS as *C. guilliermondii*, which may not respond to amphotericin B therapy (11, 15). Such erroneous identifications, if reported to physicians, could result in the selection of inappropriate antifungal therapy.

The current study demonstrated that there may be a substantial difference in the percentage of isolates of any one species of yeasts that are correctly identified by a system and the predictive value of accuracy for that same species' identification by the system. That difference was best illustrated by the finding that although the MIS correctly identified only 82% of the isolates of *C. glabrata*, the predictive value of an MIS identification of an unknown yeast isolate as *C. glabrata* was 100%, because no isolates of any other species were misidentified by the MIS as *C. glabrata*. However, both the low predictive values for accuracy associated with MIS identifications for most of the remaining yeast species and the excessive frequency with which isolates had to be reanalyzed in the chromatograph indicate that the procedure and/or database for this potentially useful system need to be improved.

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