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Evaluation of the Microbial Identification System for Identification of Clinically Isolated Yeasts

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The Microbial Identification System (MIS; Microbial ID, Inc., Newark, Del.) was evaluated for the identification of 550 clinically isolated yeasts. The organisms evaluated were fresh clinical isolates identified by methods routinely used in our laboratory (API 20C and conventional methods) and included *Candida albicans* ($n = 294$), *C. glabrata* ($n = 145$), *C. tropicalis* ($n = 58$), *C. parapsilosis* ($n = 33$), and other yeasts ($n = 20$). In preparation for fatty acid analysis, yeasts were inoculated onto Sabouraud dextrose agar and incubated at 28°C for 24 h. Yeasts were harvested, saponified, derivatized, and extracted, and fatty acid analysis was performed according to the manufacturer's instructions. Fatty acid profiles were analyzed, and computer identifications were made with the Yeast Clinical Library (database version 3.8). Of the 550 isolates tested, 374 (68.0%) were correctly identified to the species level, with 87 (15.8%) being incorrectly identified and 89 (16.2%) giving no identification. Repeat testing of isolates giving no identification resulted in an additional 18 isolates being correctly identified. This gave the MIS an overall identification rate of 71.3%. The most frequently misidentified yeast was *C. glabrata*, which was identified as *Saccharomyces cerevisiae* 32.4% of the time. On the basis of these results, the MIS, with its current database, does not appear suitable for the routine identification of clinically important yeasts.

Over the past several decades there has been a significant increase in the number of fungal infections caused by yeasts and yeast-like fungi. Although infections can occur in normal hosts, most of these infections have been seen primarily in patients who are immunosuppressed or immunocompromised in some way, such as AIDS patients, transplant patients receiving concomitant immunosuppressive therapy, and patients receiving corticosteroids, anticancer drugs, and anti-AIDS drugs. Other predisposing factors responsible for yeast infections include overuse of broad-spectrum antibiotics, the presence of indwelling catheters, and intravenous drug abuse. Yeasts can be acquired from exogenous sources (rare), e.g., soil and plant material, and endogenous sources. They are normal microfloras of the oropharynx, gastrointestinal tract, and skin, and their isolation in culture may or may not be of clinical significance. Although the clinical significance of a yeast isolated from a clinical specimen ultimately rests with a physician, the accurate and timely identification of the organism by a laboratory can aid in making the correct diagnosis. The yeasts most commonly isolated from clinical specimens, in decreasing order of occurrence, include *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Saccharomyces* spp., *Candida krusei*, *Candida guilliermondii*, *Rhodotorula* spp., *Trichosporon* spp., and *Cryptococcus neoformans* (7, 10).

There are a variety of methods available for identifying yeasts from clinical specimens. These include (i) rapid methods, e.g., enzymatic and fluorogenic tests; (ii) conventional methods, e.g., the germ tube test, morphology studies, and carbohydrate utilization; and (iii) commercially available methods, e.g., the API 20C (bioMerieux Vitek, Hazelwood, Mo.), Uni-Yeast Tek (Remel Laboratories, Lenexa, Kans.), Baxter Yeast ID Panel (Baxter Healthcare Corporation, West Sacramento, Calif.), and Vitek Biochemical Card (bioMerieux Vitek) tests. Each method has its advantages and limitations, and frequently more than one method may be required to identify an organism to the species level (7, 10).

Cellular fatty acid analysis by gas-liquid chromatography has been used to identify bacteria, mycobacteria, and fungi from environmental and clinical sources (2, 4, 11). In particular, a commercially available, computer-assisted microbial identification system (MIS; Microbial ID, Inc., Newark, Del.) has been evaluated by several investigators for its ability to identify gram-negative anaerobic bacilli (9), gram-negative nonfermentative bacteria (5), and mycobacteria (8) from clinical specimens. Recently, the manufacturer released a computer database (version 3.8) specifically for identifying yeasts from clinical specimens (Clinical Yeast Library). The purpose of this study was to evaluate the MIS for its ability to identify commonly isolated yeasts.

MATERIALS AND METHODS

Yeasts. A total of 550 yeasts were analyzed in this study. These organisms were fresh clinical isolates, 412 of which were cultured from specimens received in the Microbiology Laboratory, Polyclinic Medical Center, and 138 of which were provided through the courtesy of Tasna Kitch, Milton S. Hershey Medical Center, Hershey, Pa. (87 isolates), and Robert L. Sautter, Harrisburg Hospital, Harrisburg, Pa. (51 isolates). The isolates tested were from a variety of sources, which included 214 urine samples, 176 respiratory tract specimens, 53 wounds, 44 stools, 23 blood samples, 2 spinal fluid samples, 12 specimens from other body fluids, and 26 specimens from other sources not stated. Isolates were maintained on Sabouraud dextrose agar at room temperature until tested.

API 20C and conventional methods. Yeast-like colonies growing on primary isolation media, e.g., sheep blood agar, chocolate agar, and Sabouraud dextrose agar, were routinely Gram stained, and if found to be yeasts microscopically, were identified by the germ tube test (1), rapid colorimetric tests for the enzymes L-proline aminopeptidase and beta-galactosaminidase (6), and API 20C (bioMerieux Vitek) (3). Isolates tested with API 20C whose identifications were questionable or of low selectivity were subjected to supplemental testing as recommended by the manufacturer or, if needed, additional conventional methods (8).

Gas-liquid chromatography (MIS). Gas-liquid chromatography with the MIS was performed as previously described (5, 8, 9). Briefly, yeasts were inoculated onto Sabouraud dextrose agar and incubated at 28°C for 24 h for analysis. One 4-mm loopful (approximately 40 mg) of yeast-like cells was placed in the bottom

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TABLE 1. Strains tested and accuracy of MIS identifications

Organism	No. of strains tested	No. of strains (mean SI value/SI range)		
		Correctly identified	Incorrectly identified	Not identified
Candida albicans	294	233 (0.640/0.402–0.915)	32 (0.630/0.425–0.855)	29
Candida glabrata	145	93 (0.700/0.430–0.889)	45 (0.580/0.431–0.703)	7
Candida tropicalis	58	11 (0.510/0.410–0.697)	7 (0.490/0.419–0.546)	40
Candida parapsilosis	33	25 (0.670/0.402–0.918)	3 (0.430/0.417–0.448)	5
Candida lusitaniae	7	0	0	7
Candida krusei	3	3 (0.780/0.700–0.865)	0	0
Candida kefyr	1	1 (0.479)	0	0
Candida guilliermondii	1	1 (0.881)	0	0
Cryptococcus neoformans	5	5 (0.770/0.725–0.808)	0	0
Saccharomyces cerevisiae	3	2 (0.770/0.596–0.947)	0	1
Total	550	374 (0.650/0.402–0.918)	87 (0.580/0.417–0.855)	89

of a screw-cap tube (13 by 100 mm). The cells were lysed, and fatty acids were saponified by addition of a solution of NaOH in aqueous methanol and heated at 100°C in a water bath. The fatty acids were converted to fatty acid methyl esters by addition of a solution of HCl in aqueous methanol and heated at 80°C. Fatty acid methyl esters were extracted from the aqueous phase with a hexane–methyl-*tert*-butyl ether reagent. A dilute solution of NaOH in distilled water was used to remove free fatty acids and residual reagents from the organic extracts. The upper solvent phase was placed in gas-liquid chromatography vials. The MIS uses a Hewlett-Packard (Avondale, Pa.) model 5890A gas chromatograph with a flame ionization detector. It is equipped with a 25-m methyl phenyl silicone-fused capillary column and uses hydrogen as the carrier. The output from the chromatograph is fed to an integrator for calculation of peak retention times and areas. These data are then transferred to a computer for peak identification and comparison of the profile with reference profile libraries which generate a similarity index (SI). An SI of 1.000 indicates a perfect match. The lower the SI, the less likely the identification is correct. MIS output includes a chromatogram, profile report, library search report, and similarity comparison chart. The isolates studied were compared with those in the Yeast Clinical Library (database version 3.8).

Data analysis. For the purpose of this study, an identification with the MIS was considered in agreement with the result of a routine identification method if it was the first choice given, regardless of the SI value. When the MIS gave no identification on initial testing, the analysis was repeated with a new sample.

RESULTS AND DISCUSSION

The identification of yeasts, other than *C. albicans*, can take 24 to 72 h or longer by commercial or conventional identification systems. The MIS can provide a same-day identification once the yeast is harvested from a Sabouraud dextrose agar plate. This would provide an identification more rapidly than can be provided by many of the currently available identification systems. The MIS correctly identified 374 of 550 (68.0%) of the yeasts isolated. Eighty-seven isolates (15.8%) were incorrectly identified, and 89 isolates (16.2%) were not identified (Table 1). Of the four most commonly isolated yeasts, 79.2% of the *C. albicans*, 64.1% of the *C. glabrata*, 19.0% of the *C. tropicalis*, and 75.7% of the *C. parapsilosis* organisms were correctly identified. The MIS identified 100% of the *C. krusei*, *C. kefyr*, *C. guilliermondii*, and *Cryptococcus neoformans* organisms tested, although there were too few organisms in these groups to draw any conclusions. Repeat testing of the yeasts not identified on initial testing resulted in an additional 3.3% (18 isolates) being correctly identified. However, the number of organisms misidentified also increased by 1.8% (10 isolates) (Table 2). An analysis of discrepant results is presented in Table 3. The most frequently misidentified organism was *C. glabrata*, which was identified as *S. cerevisiae* 32.4% of the time. Of the 47 misidentifications, *C. glabrata* was given as a second choice 64% of the time and the SI units between the first and second choices rarely exceeded 0.200 units. Similarly, when *C. glabrata* was identified correctly, *S. cerevisiae* was frequently given as a second choice (data not shown). This indicates that these two organisms may have very similar fatty acid profiles which are not easily separated by gas-liquid chromatography. Of the strains of *C. tropicalis* and *C. lusitaniae* tested, 68.9 and 100%, respectively, were not identified on initial testing, indicating that there may be an insufficient number of strains included in the current database (Table 1).

In studies using the MIS to identify bacteria, several investigators have used SI values to help determine the reliability of results (5, 8, 9). Frequently a cutoff value of ≥0.500 (which can range from 0.400 to 0.600, depending on the species) was used, with identifications having SI values above the cutoff having

TABLE 2. MIS results of repeat testing of strains giving no identification on initial testing

Organism	No. of strains tested	No. of strains (mean SI value/SI range)		
		Correctly identified	Incorrectly identified	Not identified
Candida albicans	29	11 (0.500/0.407–0.619)	3 (0.540/0.401–0.657)	15
Candida glabrata	7	4 (0.590/0.486–0.716)	2 (0.490/0.461–0.511)	1
Candida tropicalis	40	3 (0.500/0.450–0.587)	2 (0.510/0.484–0.539)	35
Candida parapsilosis	5	0	0	5
Candida lusitaniae	7	0	2 (0.450/0.447–0.461)	5
Saccharomyces cerevisiae	1	0	1 (0.501)	0
Total	89	18 (0.520/0.407–0.716)	10 (0.500/0.401–0.657)	61

TABLE 3. Discrepancies between routine identification methods and MIS

Identification of organism by API 20C and conventional methods	No. of strains	MIS test result					
		First choice			Second choice		
		Identification of organism	No. of strains	Mean SI value	Identification of organism	No. of strains	Mean SI value
C. albicans	35	*C. guilliermondii*	17	0.770	*C. albicans*	14	0.680
		C. tropicalis	9	0.540	*C. albicans*	6	0.520
		C. parapsilosis	6	0.560	*C. albicans*	2	0.530
		C. krusei	1	0.474			
		C. kefyr	1	0.505			
		C. glabrata	1	0.657			
C. glabrata	47	*S. cerevisiae*	47	0.610	*C. glabrata*	30	0.540
C. tropicalis	9	*C. albicans*	9	0.500			
C. parapsilosis	3	*C. albicans*	3	0.430	*C. parapsilosis*	1	0.430
C. lusitaniae	2	*C. kefyr*	2	0.450			
S. cerevisiae	1	*C. parapsilosis*	1	0.501			

been accepted as correct and those falling below the cutoff having been considered incorrect, requiring repeat MIS analysis or additional testing. No attempt was made in the present study to utilize SI values to establish a cutoff or to determine the reliability of the result. It has been our experience and that of others (5, 8, 9) that when the MIS is used to identify bacteria, identifications are frequently obtained with SI values in the range of 0.001 to 0.400. This is in contrast to the results obtained in the present study with yeasts, in which over 95% of the identifications obtained had SI values greater than 0.500 and all had SI values greater than 0.400 (Tables 1, 2, and 3). Although establishing a cutoff value would most likely reduce the number of incorrect identifications, it might or might not increase the number of correct identifications and would increase the number of isolates requiring repeat or additional testing. For example, applying a cutoff value of $\geq$0.500 to the data presented in Table 1 for *C. albicans* would result in a decrease of incorrect results from 32 to 25 (-2.4%), a decrease in correct results from 233 to 202 (-10.5%), and an increase in isolates requiring repeat or supplemental testing from 29 to 67 ($+12.9\%$). In either case, the number of isolates requiring repeat or additional testing is unacceptably high. Given the poor results obtained with the MIS in this study, the use of SI values to evaluate the data is unlikely to improve the performance of the method.

We have found fatty acid analysis by the MIS to be a useful supplemental and reference method for difficult-to-identify gram-negative nonfermentative bacteria, anaerobic bacteria, mycobacteria, and fastidious organisms. However, the MIS, with its current database, cannot be recommended at this time for the routine identification of clinically isolated yeasts.

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