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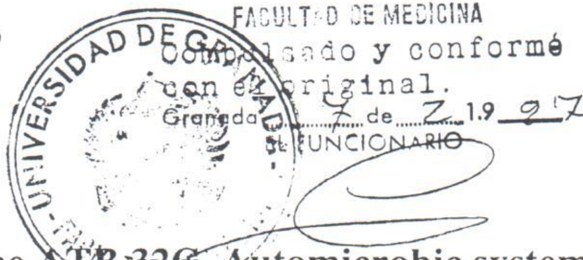

Original article

Evaluation of the ATB 32C, Automicrobic system and API 20C using clinical yeast isolates

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Summary — The ATB 32C (bioMerieux, Spain), AMS-YBC (Vitek System, bioMerieux, Spain) and API 20C (bioMerieux, Spain) systems were evaluated for their reliability in identifying 100 clinical yeast isolates. The ATB 32C, AMS-YBC and API 20C systems correctly identified 97%, 98% and 100% of the isolates respectively. There were no significant differences in incubation periods between ATB 32C and AMS-YBC systems. One isolate of *Candida tropicalis* was wrongly identified by the ATB 32C and the AMS-YBC systems. The *Saccharomyces cerevisiae* isolate was wrongly identified by the ATB 32C system while the AMS-YBC failed to identify it and a third isolate of *Candida krusei* was wrongly identified by the ATB 32C system. The overall accuracy and rapidity of the ATB 32C and AMS-YBC systems were sufficient to permit recommendation of either of these systems for routine use in the clinical microbiology laboratory, although the first system enjoys the advantages of having a wider data-base and the possibility of manual reading.

identification / yeasts / automation

Résumé — Évaluation de l'ATB 32C, du système Automicrobic et de l'API 20C en utilisant des levures isolées en clinique. *L'ATB 32C (Biomérieux, Espagne), l'AMS-YBC (Système Vitek, Biomérieux, Espagne), et l'API 20C (Biomérieux, Espagne) ont été évalués pour leur fiabilité dans l'identification de 100 levures isolées en clinique. Les systèmes ATB 32C, AMS-YBC et API 20C identifient correctement 97%, 98% et 100% de souches isolées. Il n'y a pas de différence significative pour les périodes d'incubation entre les systèmes ATB 32C et AMS-YBC. Une souche de* Candida tropicalis *a été mal identifiée par les systèmes ATB 32C et AMS-YC. Une souche de* Saccharomyces cerevisiae *a été mal identifiée par le système ATB 32C alors que le système AMS-YBC n'a pu l'identifier et une troisième souche de* Candida krusei *a été mal identifiée par le système ATB 32C. La précision et la rapidité des systèmes ATB 32C et AMS-YBC sont suffisantes pour qu'on puisse les recommander pour une utilisation en routine dans les laboratoires de microbiologie clinique bien que le premier système apporte comme avantage d'avoir une plus grande base de données et la possibilité d'une lecture manuelle.*

identification / levure / automate

Introduction

The incidence of fungal infections caused by yeast like agents has increased in recent years [1] especially in immunocompromised and severely debilitated patients [2–4]. Until recently, many of these fungi have been uncommon in clinical specimens [5–8], others are routine isolates which initiate opportunistic infections in immunocompromised individuals [9]. Moreover, *Candida glabrata, Candida lusitaniae* and *Candida krusei* are more resistant to the action of the antifungal agents [10]. The above factors, coupled with the increasing presence diversity of the implicated microorganisms [7, 9, 11], have made it imperative for clinical laboratories to have the capacity to rapidly and accurately identify yeast isolates.

In response to this need, several commercial systems have been developed and evaluated, some are manual [12–15] and provide results as conventional methods [16]. Others are automated, like the Quantum II Microbiology System [17], the AutoMicrobic System Yeast Biochemical Card AMS-YBC (Vitek System, bioMerieux, Spain) [18–20] and the ATB 32C (bioMerieux, Spain) [21]. These automated systems have many advantages over the manual ones including a minimal need for supplementary tests, and a shorter incubation period (24–48 h) to obtain final identification).

The aim os this study was to evaluate the performance of three commercially available systems, API 20C, ATB 32C and AMS-YBC, in the identification of yeast using conventional biochemical tests as a reference.

Materials and methods

A total of 100 clinical yeast isolates, including 96 isolates of commonly encountered taxa (*Candida albicans, Candida tropicalis, C krusei, Candida parapsilosis, C lusitaniae* and *C glabrata*) and four isolates of uncommon taxa, were tested by the API 20C, ATB 32C and AMS-YBC

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Table I. Yeast identification results with the AMS-YBC and ATB 32C systems at 24 and 48 h of incubation. After a 24-h incubation API 20C system provided correct identification of 100% clinical strains.

Organisms	*No of isolates correctly identified by:*				*No of isolates misidentified by:*	
	ATB 32C		*AMS-YBC*		*ATB 32C*	*AMS-YBC*
	24 h	*48 h*	*24 h*	*48 h*		
Candida albicans (34)	34	0	34	0	0	0
Candida tropicalis (17)	13	2	15	1	2	1
Candida krusei (11)	0	10	0	11	1	0
Candida parapsilosis (10)	7	3	7	3	0	0
Candida lusitaniae (7)	7	0	7	0	0	0
Candida glabrata (17)	17	0	17	0	0	0
Candida famata (1)	1	0	1	0	0	0
Geotrichum capitatum (1)	1	0	1	0	0	0
Sporobolomices salmonicolor (1)	1	0	1	0	0	0
Saccharomyces cerevisiae (1)	0	0	0	0	1	1

yeast identification systems (table I). All of the yeasts had been isolated from clinical samples sent to the microbiology laboratory of the Valme University Hospital. The isolates were subcultured at least twice on Sabouraud dextrose agar (Difco) in plates and incubated at 30°C for at least 18 h before being tested by the three identification systems. Strains were identified in the reference laboratory by conventional methods [22, 23]. These included tests for carbohydrate fermentation and assimilation, cycloheximide resistance, nitrate assimilation, growth at 37°C, urease production on Christensen urea agar slant and tetrazolium reduction. When necessary, organisms were checked for ascospore formation and morphology on Dalmau plates. Control strains was utilized, from bioMerieux Laboratoires (France), of *C glabrata* NCYC 350, *Candida guilliermondii* ATCC 6260, *Candida humicola* CBS 2041, *C krusei* CBS 573, *C pseudotropicalis* ATCC 4135 and *Saccharomyces cerevisiae* NCPF 3178.

Api 20C yeast (bioMerieux, Spain)

In the API 20C system yeast identification is based on 19 carbohydrate assimilation tests read by assessing cupules for turbidity. All API 20C tests were conducted as directed by the manufacturer. The strip was incubated at 30°C in a plastic incubation tray and then read for the presence of growth at 24, 48 and 72 h. A final identification was made when the first-choice biocode number listed was excellent very good or acceptable. For isolates having low selectivity, identification was confirmed by consult the API identification services. The cost by strip is 7,3$.

ATB 32C

The ATB 32C system consists of a single-use disposable plastic strip with 32 wells to perform: 29 assimilation tests (carbohydrates, organic acids, aminoacids), 1 negative control for an assimilation test, one susceptibility test (cycloheximide) and 1 colorimetric test (esculin). This system includes a data base with 63 different species or species groups or clinical, industrial and environmental yeasts. It can be used manually in association with ATB equipment for fully automated identification. All ATB 32C tests were conducted as directed by the manufacturer. The strips were incubated for 24 h at 30°C. The reactions were read employing the API automated equipment. It was reincubate 24 h if the identification at 24 h is invalid. The cost by strip is 9$.

AMS-YBC

The YBC system consists of a 30 well disposable plastic card containing 26 conventional biochemical tests and four negative control. All AMS-YBC procedures were conducted as recommended by the manufacturer. The cards inoculated were incubated at 30°C for either 24 or 48 h, depending on the readings provided by the instrument. The cards were read in a single reading in the reader incubator module, and the biochemical patterns were analyzed by the Vitek computer program. Responses were expressed as the one or two most likely possibilities. Only those matches, whose first choise had an 85% or greater probability of being correct, were considered the most likely identification for the yeast. The cost by card is 14$.

The tests were repeated three times and it was considered valid result when its were always the same. Posteriorly, this were compared with the original reference findings. In the statistical analysis we used the 'comparison of Poisson rates' test between the values obtained in each methods and class of the yeast [24].

Results

The control strains were correctly identified and we obtained the same result when the assay was carried out three times by the three systems.

After a 24-h incubation, the API 20C system provided correct identification of 100% clinical strains. The ATB 32C system correctly identified 97 (97%) of the isolates; 82 (84.5%) were identified after 24 h of incubation and 15 (15.4%) (10 *C krusei*, 2 *C tropicalis* and 3 *C parapsilosis*) required 48 h of incubation. There were discrepancies among the yeast taxa: a) one isolate of *C tropicalis* was misidentified as *C humicola*, on the basis of false positive arabinose, inositol, cellobiose, lactose, mellibiose, raffinose and resistance to cyclohemixide; b) one isolate of *C krusei* was misidentified as *Candida inconspicua*, on the basis of false positive glycerol, with unreliable data; and c) one isolate of *S cerevisiae* was misidentified as *Candida utilis*, on the basis of false positive cellobiose xylose.

The AMS-YBC system correctly identified 98 (98%) isolates: 83 (84.6%) were identified after a 24-h incubation and 15 (15.4%) required a 48-h incubation (11 *C krusei*, 3 *C parapsilosis* and 1 *C tropicalis*). Among the yeast taxa, there was a discrepancy in one isolate of *C tropicalis* which was misidentified as *C guilliermondii* on the basis of false-positive mellibiose and false resistance to cycloheximide (this same isolate was also misidentified as *C humicola*, by the ATB 32C system) and an isolate of *S cerevisiae* is not identified by the AMS-YBC system after a 48-h incubation on the false positive xylose.

There were no significant differences in results and incubation periods for API 20C, ATB 32C and AMS-YBC systems.

Discussion

Under this conditions, the rate of correct identification of 100% obtained with API 20C was close to rates reported in the literature of 96%, 86% and 94% respectively [13, 14, 25]. However, our rate was somewhat higher; this may be explained by the fact that we did consult the API identification services when profiles obtained for some of the strains were not found in the database. On the other hand, our result was notably better than the rate of 77% reported by Bergan *et al* [26].

The ATB 32C is a system for the automated identification of clinical and industrial yeasts, using 32 standarizedandminiaturized tests. After an incubation period of 24 or 48 h at 30°C, the profile of a strain is interpreted by referring to the standard data base, which includes 63 different species or species groups. The ATB instrumentation allows the automation of different steps of the procedure. Manual use is also possible [21]. The AMS-YBC system is designed to provide the clinical laboratory with the capability for rapid, accurate reference level yeast identification. The major criticisms experienced by previous investigators were that the data-base (which included 36 T different species) was too limited, and that certain key biochemical characteristics were improperly weighted when the taxonomic key was devised. A minor criticism concerned the nessicity for a large inoculum [18–20]. In this investigation, the ATB 32C system correctly identified most of the isolates studied within 24 h with no significant differences between the common and uncommon clinical isolates. A previous study [21] reported an accuracy of 94%. Our results were comparable to those of such investigators. In contrast, our results were slightly better than those obtained with 24 h of incubation (84.2% *vs* 64%). Doucet *et al* [21] found a similar percentage of misidentified isolates (2.6% *vs* 3%) and failed to identify 3.4% of yeast isolates, while we did not report any isolate as 'unidentified' by the ATB 32C system. We found that the misidentifications reported by this system were due to false assimilation results of different carbohydrates, and by variable resistance to cycloheximide.

In previous studies, the YBC of the Vitek system was reported to have an overall agreement, ranging from 83 to 98.8% [17–20]. The present evaluation showed an overall agreement of 98%. In identifying the common clinical isolates, our results were comparable to those reported by other investigators [20] (98.9% *vs* 99.2%). Of the correctly identified yeast isolates, 84.6% were reported after a 24-h incubation while El-Zaatari *et al* [20] obtained inferior results (70.8%). Occasionally, more slowly growing isolates (*C krusei, C tropicalis* and *C parapsilosis*) required more than 24 h to assimilate the YBC biochemicals. These results were similar to those obtained by El-Zaatari *et al* [20]. Our number of misidentified isolates of *C tropicalis* was less than the one obtained by Hasyn *et al* [18] and Pfaller *et al* [17] and similar to the results reported by El-Zaatari *et al* [20]. In respect to the unidentified *S cerevisiae*, our results were similar to those of other investigators [18, 19] and inferior to those of El-Zaatari *et al* [20]. We agree with other authors [18] that the misidentifications were due to wrong carbohydrate assimilation results and to cycloheximide resistance. For correct identification of all *C krusei* isolates, we needed a morphological differentiation as did the other investigators [18].

We can conclude that the bests results were obtained with API 20C. The ATB 32C and AMS-YBC systems evaluated are faster and useful and are comparable in accuracy (97% and 98%) respectively when they are used to identify clinical yeast isolates. It is interesting to note that the ATB 32C and AMS YBC systems both gave the same 'false' reading as compared to the reference system. Both systems are totally automated but the new ATB 32C has the advantages of having a wider data-base and a manual reading possibility; besides, comsumables are cheaper.

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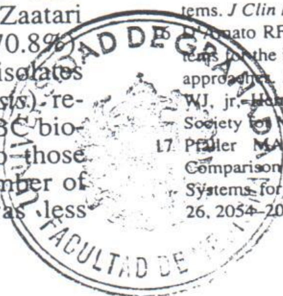

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