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Comparative Evaluation of the Vitek 2 Yeast Susceptibility Test and CLSI Broth Microdilution Reference Method for Testing Antifungal Susceptibility of Invasive Fungal Isolates in Italy: the GISIA3 Study[▽]

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The newly available AST-YS01 Vitek 2 cards were evaluated, and the results were compared with those obtained by the CLSI M27-A2 microdilution reference method. Clinical fungal isolates, including 614 isolates of *Candida* spp., 10 *Cryptococcus neoformans* isolates, 1 *Geotrichum capitatum* isolate, and 2 quality control strains, were tested for their susceptibilities to amphotericin B, fluconazole, and voriconazole using both methods. The majority of fungal isolates were susceptible to all antifungal agents tested: the MIC_{90} values determined by the Vitek 2 and CLSI methods were 0.5 and 1 μg/ml, respectively, for amphotericin B; 8 and 16 μg/ml, respectively, for fluconazole; and <0.12 and 0.25 μg/ml, respectively, for voriconazole. Overall there was excellent categorical agreement (CA) between the methods (99.5% for amphotericin B, 92% for fluconazole, 98.2% for voriconazole), but discrepancies were observed within species. The CAs for fluconazole were low for *Candida glabrata* and *Candida krusei* when the results of the CLSI method at 48 h were considered. Moreover, the fully automated commercial system did not detect the susceptibility of *Cryptococcus neoformans* to voriconazole. The Vitek 2 system can be considered a valid support for antifungal susceptibility testing of fungi, but testing of susceptibility to agents not included in the system (e.g., echinocandins and posaconazole) should be performed with other methods.

Antifungal susceptibility testing (AFST) has become increasingly common in clinical practice in recent years. This is a result of both the improved performance of antifungal susceptibility testing methods and the introduction of antifungal drugs with various mechanisms of action, such as the echinocandins and triazoles (7, 9, 10). It is generally considered that the outcome of invasive fungal infections, in particular, candidemia, is improved by prompt initiation of appropriate antifungal therapy (13). Treatment of invasive *Candida* infections is currently based on the updated IDSA guidelines (15), but knowledge of the susceptibilities of local clinical isolates to antifungal agents can further guide physicians' choice of appropriate and safe antifungal agents, which is especially important for long-term treatment (9).

AFST reference methods for fungi have been available since 1997 from the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards) and, more recently, from the subcommittee on AFST of the European Committee for Antimicrobial Susceptibility Testing (EUCAST). However, both of these methods are time-consuming and clinical microbiological laboratory personnel may be unfamiliar with the methodologies (3, 6, 11, 12, 14, 20). Commercially available methods demonstrate variable performance compared with the performance of reference methods; two commercial assays have been approved by the U.S. Food and Drug Administration (FDA) for AFST of fungi with several antifungal agents: Etest (bioMérieux SA, Marcy l'Etoile, France) and the Sensititre YeastOne system (Trek Diagnostic Systems Ltd., East Grinstead, England). Recently, bioMérieux expanded its role in this area with a yeast susceptibility test that determines *Candida* growth spectrophotometrically using the Vitek 2 microbiology systems, performing fully automated testing of susceptibility to flucytosine, amphotericin B, fluconazole, and voriconazole (1, 16–18).

To investigate the reliability of the new AST-YS01 Vitek 2 cards, the susceptibilities of clinical fungal isolates to amphotericin B, fluconazole, and voriconazole, as determined by the Vitek 2 system, were compared with those obtained with the reference CLSI (M27-A2) broth microdilution method (14).

MATERIALS AND METHODS

Clinical isolates. Six hundred thirty-eight fungal isolates were included in the study. The isolates were collected from January 2007 to December 2008 by 13 Italian microbiological laboratories from sterile specimens from critically ill patients hospitalized in general and surgical intensive care units (ICUs) and surgical wards. The isolates were identified by standard procedures (8), including morphology on cornmeal agar plates, germ tube production in serum, and biochemical analysis with the Vitek system or API 20CAUX or ID 32C panels (bioMérieux, Rome, Italy). Prior to susceptibility testing, each isolate was subcultured on Sabouraud dextrose agar to ensure its viability and purity and that it had optimal growth characteristics. After each isolate was tested, it was stored at −80°C. Quality control strains *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019), from the American Type Culture Collection (ATCC), were used in each working session.

Antifungal agents and reference method panels. Fluconazole and voriconazole were obtained as standard powders from Pfizer Pharmaceuticals (Groton, CT),

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and amphotericin B was purchased from Sigma-Aldrich (Milan, Italy). Fluconazole was dissolved in sterile water, and the other antifungal agents were solubilized in dimethyl sulfoxide. Broth microdilution panels were prepared in each laboratory according to the CLSI M27-A2 method (14) and were stored at −80°C for testing within 6 months. The concentration ranges tested were 0.125 to 256 μg/ml for fluconazole and 0.016 to 32 μg/ml for amphotericin B and voriconazole.

Inoculum suspension. After overnight growth on Sabouraud dextrose agar at 35°C, each isolate was suspended in 5 ml of sterile distilled water and vortexed. The turbidity at a wavelength of 530 nm was adjusted to a McFarland standard of 0.5 with sterile distilled water. This suspension (approximately 1×10^6 to 5×10^6 CFU/ml) was used for the broth microdilution method, after appropriate dilution according to the standardized protocol (14). Inoculum suspensions for use with the AST-YS01 Vitek 2 cards were obtained from the same overnight cultures, with the turbidity being adjusted to a 1.8 to 2.2 McFarland standard using the Bio-Mérieux Densicheck instrument, according to the manufacturer's recommendations.

Susceptibility testing. For the CLSI method, the inoculum suspension was serially diluted to 0.5×10^3 to 0.25×10^3 CFU/ml with RPMI 1640 medium. Reference panel plates were inoculated with 0.1-ml aliquots and incubated at 35°C. An initial visual reading was made after 24 h, with the lowest concentration inhibiting visible growth being recorded as the MIC value for the tested agent. Following 48 h of incubation, the panels were analyzed spectrophotometrically, after they were shaken. The spectrophotometric reading has been preferred to the recommended visual one to avoid the bias related to the reader's expertise (5). MICs for fluconazole and voriconazole were determined to be the lowest concentration with a significant decrease in turbidity (≥50%) compared with the turbidity of the control growth. Amphotericin B MICs were determined to be the lowest concentration in which no visible growth was detected.

Susceptibility testing with the Vitek 2 system was performed according to the manufacturer's instructions. The standardized suspension was placed in a Vitek 2 cassette along with a sterile polystyrene test tube and an AST-YS01 Vitek 2 card containing 2-fold serial dilutions of amphotericin B (range, 0.03 to 16 μg/ml), flucytosine (range, 0.125 to 64 μg/ml), fluconazole (range, 1 to 64 μg/ml), and voriconazole (range, 0.125 to 16 μg/ml). Following loading of the cassette, dilution of the fungal suspensions and card filling were performed automatically by the Vitek 2 system. The incubation time varied according to the growth rate measured in the drug-free control well. Quality control strains were included in each working session.

Data recording and analysis of results. In accordance with the M27-A2 document, the results from the 48-h reading were used. Complete data (from the CLSI and Vitek 2 methods) for each fungal isolate tested were recorded on an electronic data report form (E-DRF). The E-DRF was automatically checked by an electronic validation program to verify the consistency of the data. If the E-DRF passed this check, the data were automatically saved, printed, and sent to the data management unit; otherwise, the user made the appropriate corrections. The printed copy of the E-DRF was signed and filed on site.

The MIC values were considered to be in essential agreement (EA) between the two methods when they were within 2 dilutions. Categorical agreement (CA) was assigned to *Candida* sp. susceptibility testing results that fell within the same interpretive categories. Results were analyzed on the basis of the interpretive breakpoints for fluconazole (sensitive [S], ≤8 μg/ml; susceptible dose dependent [SDD], 16 to 32 μg/ml; resistant [R], ≥64 μg/ml) and voriconazole (S, ≤ 1 μg/ml; SDD, 2 μg/ml; R, ≥4 μg/ml) (2). Interpretive criteria for amphotericin B have not been established, but for comparison, isolates inhibited by amphotericin B at ≤1 μg/ml were considered susceptible (4). These breakpoints were also used to determine the CA between the results from the CLSI M27-A2 and AST-YS01 Vitek 2 card methods. CA was assigned where both methods classified the susceptibilities of the isolates within the same interpretive categories (S, SDD, or R). Discrepancies were considered "major" if an isolate classified S by the reference method was classified R by the commercial method and "very major" if an isolate classified R by the reference method was classified S by the commercial method. Errors were considered "minor" when there were discrepancies between the two methods in classifying SDD isolates S or R or classifying S and R isolates SDD.

RESULTS

Six hundred thirty-eight fungal isolates were collected during the study period: 327 isolates of *C. albicans* (51.3%), 299 isolates of non-*C. albicans Candida* spp. (46.8%), and 12 isolates (1.8%) belonging to other genera. Six hundred twenty-five of the collected isolates were evaluable: 324 *Candida albicans* isolates, 74 *C. glabrata*, 15 *C. krusei*, 142 *C. parapsilosis*, 37 *C. tropicalis*, 22 other *Candida* spp., and 10 *Cryptococcus neoformans* isolates and 1 *Geotrichum capitatum* isolate. Thirteen isolates (3 *Candida albicans*, 3 *C. glabrata*, 2 *C. parapsilosis*, and 2 *C. lipolytica* isolates and 1 isolate each of *C. famata*, *C. tropicalis*, and *Saccharomyces cerevisiae*) failed to grow in the AST-YS01 Vitek 2 card control well after the maximum incubation time (35 h), despite several repeated assays, and were excluded.

The Vitek 2 results (70.2%) were available for the majority of isolates within 12 to 15 h; the results for the remaining *Candida* isolates were obtained within 20 h, and the results for the *Cryptococcus neoformans* isolates were obtained at between 19 and 29 h. The results for one *C. glabrata* isolate were available after 35 h. The MICs of the two quality control strains were within the range of expected values (2) and showed reproducibility by both methods. The mode MICs for *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were as follows: for amphotericin B, 0.5 μg/ml for both isolates; for fluconazole, 32 and 2 μg/ml, respectively; and for voriconazole, ≤0.12 μg/ml with the Vitek 2 cards for both isolates and 0.125 and 0.03 μg/ml, respectively, by the CLSI method.

The majority of isolates were susceptible to all antifungal drugs tested by AST-YS01 Vitek 2 cards and the CLSI M27-A2 method, with both methods demonstrating similar MIC_{90} values (the rates of susceptibility to amphotericin B and voriconazole were 97.5% and 99.5%, respectively, for both methods; the rates of susceptibility to fluconazole were 87.3% with the CLSI method and 93.2% with the Vitek 2 cards). Although some differences were observed among species, overall excellent CAs were observed between the two methods: 99.5% for amphotericin B, 92% for fluconazole, and 98.2% for voriconazole (Table 1).

Overall, lower MIC values were obtained with Vitek 2 cards than by the CLSI method at 48 h. The MIC_{90} values from the Vitek 2 cards and the CLSI method at 48 h were 0.5 and 1 μg/ml, respectively, for amphotericin B; 8 and 16 μg/ml, respectively, for fluconazole; and ≤0.12 and 0.25 μg/ml, respectively, for voriconazole. CAs were equal to or nearly 100% for most of the clinically relevant species studied (Table 1). However, there were exceptions, particularly with isolates of *C. glabrata* and *C. krusei*; there was poor agreement between the MIC values for fluconazole with *C. glabrata* isolates (CA, 51.4%) and *C. krusei* isolates (CA, 53.3%). Although most of the discrepancies were minor errors (36.5% for *C. glabrata* and 40% for *C. krusei*), there was also a high rate of occurrence of very major errors (12.2% and 6.6% for *C. glabrata* and *C. krusei*, respectively).

These discrepancies were observed when the Vitek 2 results were compared with the results of the CLSI test at 48 h; when the results of the CLSI test at 24 h for *C. glabrata* isolates were considered, the CA was higher (90.5%), the incidence of minor errors was lower (8.1%), and there was only one very major error (the isolate that produced this very major error grew slowly in the AST-YS01 Vitek 2 cards, requiring 35 h for growth). In addition, the very major error observed between the Vitek 2 cards and the CLSI method at 48 h in a *C. krusei* isolate was not confirmed when the results of the CLSI method at 24 h were considered. However, it should be remembered

TABLE 1. CA of *in vitro* susceptibilities to amphotericin B, fluconazole, and voriconazole, determined by the AST-YS01 Vitek 2 card and CLSI M27-A2 methods, in clinical fungal isolates

Species (no. of isolates)	Drug	Method	MIC (μg/ml)			CA
			Range	50%	90%	
All isolates (625)	Amphotericin B	CLSI	0.12–2	0.5	1	
		Vitek 2	≤0.25–4	0.5	0.5	99.5
	Fluconazole	CLSI	≤0.12–128	0.5	16	
		Vitek 2	≤1–≥64	≤1	8	92.0
	Voriconazole	CLSI	≤0.008–8	0.015	0.25	
		Vitek 2	≤0.12–4	≤0.12	≤0.12	98.2
Candida albicans (324)	Amphotericin B	CLSI	0.12–1	0.5	1	
		Vitek 2	≤0.25–1	0.5	0.5	100
	Fluconazole	CLSI	≤0.12–16	0.25	0.5	
		Vitek 2	<1–16	≤1	≤1	100
	Voriconazole	CLSI	≤0.008–0.25	0.008	0.015	
		Vitek 2	≤0.12–1	≤0.12	≤0.12	100
Candida glabrata (74)	Amphotericin B	CLSI	0.12–2	1	1	
		Vitek 2	≤0.25–2	0.5	1	98.6
	Fluconazole	CLSI	0.5–128	16	128	
		Vitek 2	≤1–≥64	8	16	51.4
	Voriconazole	CLSI	≤0.008–8	0.25	2	
		Vitek 2	≤0.12–4	≤0.12	0.25	90.5
Candida krusei (15)	Amphotericin B	CLSI	0.5–2	1	2	
		Vitek 2	0.5–2	1	2	86.6
	Fluconazole	CLSI	8–128	32	64	
		Vitek 2	8–32	16	32	53.3
	Voriconazole	CLSI	0.12–1	0.25	1	
		Vitek 2	≤0.12–0.25	≤0.12	≤0.12	100
Candida parapsilosis (142)	Amphotericin B	CLSI	0.12–1	0.5	1	
		Vitek 2	≤0.25–1	0.5	0.5	100
	Fluconazole	CLSI	0.25–32	1	4	
		Vitek 2	≤1–32	≤1	2	97.2
	Voriconazole	CLSI	≤0.008–2	0.015	0.06	
		Vitek 2	≤0.12–1	≤0.12	≤0.12	98.8
Candida tropicalis (37)	Amphotericin B	CLSI	0.25–1	0.5	1	
		Vitek 2	≤0.25–1	0.5	0.5	100
	Fluconazole	CLSI	0.25–32	1	4	
		Vitek 2	≤1–16	≤1	8	94.6
	Voriconazole	CLSI	0.008–1	0.03	0.25	
		Vitek 2	≤0.12–0.5	≤0.12	≤0.12	100
Cryptococcus neoformans (10)	Amphotericin B	CLSI	0.12–1	0.5	1	
		Vitek 2	≤0.25–0.5	0.5	0.5	NA[a]
	Fluconazole	CLSI	0.25–4	2	4	
		Vitek 2	≤1–4	1	2	NA
	Voriconazole	CLSI	0.015–0.06	0.03	0.06	
		Vitek 2	ND[b]	ND	ND	ND
Other fungi[c] (23)	Amphotericin B	CLSI	0.12–1	0.5	1	
		Vitek 2	≤0.25–4	0.5	1	100
	Fluconazole	CLSI	0.12–128	1	16	
		Vitek 2	≤1–≥64	2	4	95.7
	Voriconazole	CLSI	≤0.008–4	0.03	0.12	
		Vitek 2	≤0.12–4	≤0.12	0.25	91.3

[a] NA, not applicable.
[b] ND, not done because the system did not allow determination of the voriconazole MIC in the absence of an established breakpoint for *C. neoformans*.
[c] Other fungi include seven *C. lusitaniae*, seven *C. guilliermondii*, three *C. famata*, two *C. sake*, and two *C. utilis* isolates and one isolate each of *C. lipolytica* and *G. capitatum*.

that the last CLSI methodology (3) recommends use of the 48-h readings for isolates with borderline MICs, such as *C. glabrata* and *C. krusei.*

Among the other species, minor errors (S isolates classified as SDD or vice versa) were observed in several isolates of *C. parapsilosis* (4/142) and *C. tropicalis* (2/37), but the essential agreements between the MIC values were equal to or nearly 100%.

Discrepancies in the classification of susceptibility to voriconazole were observed in several *C. glabrata* isolates (3/74); the isolates were classified resistant by the results of the CLSI method at 48 h, whereas they were classified susceptible by the Vitek 2 system (two isolates with a voriconazole MIC of ≤ 0.12 μg/ml and one isolate with a voriconazole MIC of 1 μg/ml). Again, this discrepancy was not detected when the results of the CLSI method at 24 h were considered. Among the other species, only 2 of 142 *C. parapsilosis* isolates showed minor errors during classification of the voriconazole MICs. The voriconazole susceptibilities of the *Cryptococcus neoformans* isolates could not be compared between the two methods, as the

Vitek 2 system did not allow determination of voriconazole MICs in the absence of an established breakpoint for *C. neoformans*.

DISCUSSION

The overall agreement between the two methods for determining the MIC values of the antifungal drugs tested was high (CA, 92 to 99.5%). However, the CAs for the *C. krusei* and *C. glabrata* isolates were substantially lower (51.4% and 53.3%, respectively), and a high rate of occurrence of very major errors was observed. Discordant data for *C. krusei* may be avoided with use of the Vitek 2 expert software, which takes into account the innate resistance of this species and corrects the low MIC results for fluconazole accordingly (1).

Many discrepancies between the AST-YS01 Vitek 2 card results and the results of the CLSI method at 48 h were undetected upon comparison with the results of the CLSI method at 24 h. Three *C. glabrata* isolates were classified sensitive to voriconazole according to the Vitek 2 card result but resistant according to the results of the CLSI method at 48 h. However, the voriconazole MICs at 24 h may not be as clinically useful as the voriconazole MICs at 48 h, given the importance of the identification of resistant isolates and the use of reference MICs at 48 h to define categorical susceptibility according to the stated breakpoint (6).

The main drawback encountered with use of the Vitek 2 system in this study was the inability to detect the susceptibility of *Cryptococcus neoformans* isolates to voriconazole. This deficiency should be considered during clinical use, as voriconazole may potentially be used in central nervous system (CNS) cases of cryptococcosis, due to its high level of distribution in this site; an improved outcome has been demonstrated in a patient with CNS aspergillosis receiving voriconazole treatment (19).

In conclusion, the Vitek 2 system represents a practical tool for AFST of clinically relevant fungi, even if the small number of fungi, other than the five more representative *Candida* spp., used cannot allow any conclusion about the other fungi to be drawn. The results obtained with this fully automated system generally correlated well with those of the CLSI reference method. Moreover, the spectrophotometric reading of results eliminates the subjectivity of the visual MIC determination that is required in other methods. However, Vitek 2 cards including other antifungal drugs available for the treatment of invasive fungal infections (such as the echinocandins and posaconazole) are necessary to improve the value of this system for routine clinical use; in the meantime, the Vitek 2 system must be supplemented with alternative methods for testing for susceptibility to these agents.

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