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Antifungal Susceptibility of 205 *Candida* spp. Isolated Primarily during Invasive Candidiasis and Comparison of the Vitek 2 System with the CLSI Broth Microdilution and Etest Methods[▽]

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Infections due to *Candida* spp. are frequent, particularly in immunocompromised and intensive care unit patients. Antifungal susceptibility tests are now required to optimize antifungal treatment given the emergence of acquired antifungal resistance in some *Candida* species. An antifungal susceptibility automated method, the Vitek 2 system (VK2), was evaluated. VK2 was compared to the CLSI broth microdilution reference method and the Etest procedure. For this purpose, 205 clinical isolates of *Candida* spp., including 11 different species, were tested for fluconazole, voriconazole, and amphotericin B susceptibility. For azoles, essential agreement ranged from 25% to 100%, depending on the method used and the *Candida* species tested. Categorical agreements for all of the species averaged 92.2% and ranged from 14.3 to 100%, depending on the 24-h or 48-h MIC reading by the Etest and CLSI methods and on the *Candida* species. Results obtained for *Candida albicans* showed excellent categorical and essential agreements with the two comparative methods. For *Candida glabrata*, the essential agreement was high with the CLSI method but low with the Etest method, and several very major errors in interpretation were observed between VK2 and the Etest method for both azoles. Low MICs of fluconazole were obtained for all of the *Candida krusei* isolates, but the VK2 expert software corrected all of the results obtained to resistant. Amphotericin B results showed MICs of ≤1 mg/liter for 201 (VK2), 190 (CLSI), and 202 (Etest) isolates. The AST-YS01 Vitek 2 card system (bioMérieux) is a reliable and practical standardized automated antifungal susceptibility test. Nevertheless, more assays are needed to better evaluate *C. glabrata* fluconazole sensitivity.

Invasive candidiasis infections are of increasing concern, particularly in immunodeficient or intensive care unit patients. The emergence of antifungal resistance and the development of new echinocandin class and broad-spectrum azole fungal agents have complicated the choice of antifungal treatment for candidiasis.

Antifungal choice is first based on *Candida* species identification, but antifungal susceptibility testing will play an increasingly important role when selecting which antifungal drug to use (3). Standardized methods for antifungal susceptibility testing have been available for many years. The Clinical and Laboratory Standards Institute (CLSI) standardized broth microdilution method remains a reference for antifungal susceptibility testing (4). Indeed, clinically relevant interpretative breakpoints are available and quality control strains are validated (11). Nevertheless, this method is complex and laborious to use as a routine method. Alternative, standardized and reliable methods adapted to hospital laboratories, such as the Etest, are now commonly used (2).

The objective of this study was to evaluate and compare a new automated antifungal susceptibility test system (AST-YS01 Vitek 2 cards; bioMérieux) to both the CLSI reference broth microdilution method and Etest procedures. For this purpose, 208 clinical isolates of *Candida* spp. isolated primarily from patients with invasive candidiasis were tested with fluconazole, voriconazole, and amphotericin B.

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MATERIALS AND METHODS

***Candida* isolates.** All of the *Candida* sp. strains used in this study were isolated during candidiasis. Of these isolates, 81 were derived from blood cultures, 62 were from sterile sites (abdominal, cerebral, cardiac, esophageal, urine, and bone tissue samples), 51 were from deep pulmonary samples, and the other 14 were from different sites in multicolonized patients. In each case, an antifungal treatment was prescribed.

Candida species identification was carried out using chromogenic medium, YST Vitek 2 system cards, or ID32C strips (bioMérieux, Craponne France). The

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isolates were frozen on CryoBeads (AES Laboratory, Bruz, France) at −80°C after routine identification. Before the antifungal tests were performed, all of the isolates were passaged twice on Sabouraud agar for viability and purity and subcultured 24 h prior to testing. In total, 208 clinical yeast isolates were tested, including 85 of *C. albicans*, 57 of *Candida glabrata*, 22 of *Candida parapsilosis*, 17 of *Candida tropicalis*, 14 of *Candida krusei*, 5 of *Candida inconspicua*, 2 of *Candida lusitaniae*, 2 of *Candida norvegensis*, 2 of *Candida kefyr*, 1 of *Candida dubliniensis*, and 1 of *Candida guilliermondii*.

Antifungals. Fluconazole, voriconazole, and amphotericin B were tested in this assay. The Vitek 2 AST-YS01 card contains serial twofold dilutions ranging from 1 to 64 mg/liter for fluconazole, from 0.125 to 8 mg/liter for voriconazole, and from 0.25 to 16 mg/liter for amphotericin B.

For the CLSI method, serial twofold dilutions ranging from 0.125 to 64 mg/liter for fluconazole (Pfizer Pharmaceuticals Group), from 0.03 to 16 mg/liter for voriconazole (Pfizer Pharmaceuticals Group), and from 0.03 to 16 mg/liter for amphotericin B (catalog no. A4888; Sigma Aldrich, St. Louis, MO) were prepared.

The Etest method was performed using Etest strips (AB Biodisk, Solna, Sweden) with tested MICs ranging from 0.016 to 256 mg/liter, from 0.002 to 32 mg/liter, and from 0.002 to 32 mg/liter for fluconazole, voriconazole, and amphotericin B, respectively.

Vitek 2 system cards. AST-YS01 cards are FDA approved and CE marked. All of the tests were performed according to the manufacturer's recommendations. A yeast suspension was adjusted to a McFarland standard of 1.8 to 2.2 with a Vitek 2 DensiChek instrument (bioMérieux). MICs were spectrophotometrically measured after various incubation times, depending on the growth control of each isolate tested.

CLSI broth microdilution. CLSI broth microdilution tests were performed according to the CLSI M27-A3 document′s recommendations approved in April 2008. Standardized inoculum suspensions equivalent to a 0.5 McFarland standard were prepared for each isolate. The MICs were determined after 24 and 48 h of incubation at 35°C. Azole MICs were determined as the lowest concentrations that inhibited growth ≥50% compared to that of the drug-free control, and the amphotericin B MIC was determined as the lowest concentration inhibiting any growth.

Etest system. A standardized inoculum suspension of each isolate equivalent to a 0.5 McFarland standard was prepared. Etest strips (AB Biodisk) were applied to RPMI medium plates (AES Laboratory), and MICs were determined after 24 and 48 h of incubation at 37°C. Azole MICs were read as the lowest concentrations producing an 80% reduction of growth. The amphotericin B MIC was determined as the lowest concentration inhibiting any growth.

Quality control. All of the methods were validated using quality control strains ATCC 90028 (*Candida albicans*), ATCC 22019 (*C. parapsilosis*), and ATCC 6258 (*C. krusei*).

Breakpoints. CLSI interpretative breakpoints for fluconazole (4) (susceptible, ≤8 mg/liter; susceptible dose dependent [SDD], 16 to 32 mg/liter; resistant, ≥64 mg/liter) and voriconazole (susceptible, ≤1 mg/liter; SDD, 2 mg/liter; resistant, ≥4 mg/liter) were used to determine categorical agreement percentages (CAs). CAs were calculated at the 24-h and 48-h end points of the comparative methods. These breakpoints are also those recommended by AB Biodisk to interpret Etest results. The Etest MICs of 12 mg/liter for fluconazole and 1.5 mg/liter for voriconazole were considered SDD.

The CLSI method was taken as the reference method for comparisons with the Vitek 2 system (VK2). Similarly, the Etest method was also considered as a basis for comparison with VK2. Discrepancies were categorized into three groups: (i) very major errors (VME; i.e., VK2 interpreted the isolate as susceptible, whereas the other comparison method found the isolate resistant), (ii) major errors (ME; i.e., the isolate was resistant by VK2 but susceptible by the other comparison method), and (iii) minor errors (mE; i.e., either susceptible or resistant by one method and SDD by the other).

Statistics. The reproducibility of MICs was assessed by coefficient of variation, and MICs were considered reproducible if the coefficient of variation was <0.5. The MICs obtained with the VK2 AST-YS01 cards were compared with those obtained with the CLSI and Etest methods. A >2-dilution MIC difference was required to calculate the essential agreement (EA) between two methods. EAs were calculated at the 48-h end point of the method. Interpretative breakpoints were used to examine CAs. When the CA was not 100%, a symmetry test was performed in order to determine if misclassifications were randomly assigned or preferentially due to one of the methods. Analysis was conducted with SAS software (SAS Institute, Cary, NC). The significance level was 5% (0.05).

RESULTS

Two hundred eight isolates of *Candida* spp. were tested for in vitro susceptibility to fluconazole, voriconazole, and amphotericin B with the VK2, CLSI, and Etest methods.

For the CLSI and Etest methods, all of the isolates grew enough to be read at 24 h and 48 h, but 3 isolates out of 208 (1 each of *C. glabrata*, *C. kefyr*, and *C. albicans*) failed to grow sufficiently for VK2, despite a new defrosting and a second test of the isolates. For the 205 remaining isolates tested, VK2 results were, on average, available within 15 h 13 min, with a range of 12 h 15 min (*C. albicans*) to 26 h 30 min (*C. glabrata*).

Validation and reproducibility of VK2 results were checked by testing three control American Type Culture Collection (ATCC) reference strains. Each strain was tested at least seven times, for a total of 22 tests. The reproducibility of the MICs obtained with the reference strains was excellent, with coefficients of variation ranging from 0 to 0.46. MICs for ATCC 90028 (*C. albicans*) and ATCC 22019 (*C. parapsilosis*) were always in the range of expected values for the three antifungals. On the contrary, the MICs obtained with *C. krusei* ATCC 6258 were lower than expected for both fluconazole and voriconazole in all of the test series, thus invalidating the results for this species. However, the Vitek 2 expert software corrects and systematically interprets *C. krusei* as resistant to fluconazole. We therefore decided to analyze all of the results obtained with this species by taking into consideration the results of the VK2 expert software.

All of the MICs recorded with the above-mentioned reference strains consistently validated both the CLSI and Etest methods.

The in vitro susceptibilities of the 205 isolates of *Candida* sp. to azoles that were determined with the three different methods are shown in Tables 1 and 2.

MICs for *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and the other *Candida* (*C. lusitaniae*, *C. dubliniensis*, *C. kefyr*, and *C. guilliermondii*) isolates (n = 128) were very low by all three methods. For both azoles, the EAs were excellent, ranging from 95.4% to 100% between VK2 and the CLSI method and from 64.7% to 100% between VK2 and the Etest method (Table 1). The isolates were mostly susceptible to azoles, regardless of the method used. Only one *C. albicans* isolate was found to be resistant to fluconazole by all three methods. Clinical resistance to fluconazole was observed in this human immunodeficiency virus type 1-infected patient with esophageal candidiasis. This isolate was also found to be SDD to voriconazole by both the 48-h Etest and 48-h CLSI methods but susceptible according to the VK2 method (MIC, 0.5 mg/liter), accounting for the only mE observed among those 128 isolates.

For these seven species, VK2 was fully concordant for fluconazole and voriconazole compared with the CLSI and Etest methods at 24 h and 48 h, with a CA of 100%, except for *C. albicans* with voriconazole at 48 h (CA = 98.8%; test for symmetry not performed, only one misclassification) (Table 2).

The results obtained for *C. glabrata* (n = 56) with the CLSI method at 24 h were very similar to the 48-h results. For fluconazole with the VK2 method, only five isolates were resistant and nine were SDD, compared to three and eight with the 48-h CLSI method, respectively. The CA between the VK2

TABLE 1. Fluconazole and voriconazole MIC_{50}s and MIC_{90}s obtained with VK2, CLSI (24 h and 48 h), and Etest (24 h and 48 h)

Candida sp. (no. of isolates), azole, and method	24 h			48 h			EA[a] (%)
	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	
C. albicans (84)							
Fluconazole							
VK2	≤1–≥64[d]	≤1	≤1				
CLSI	<0.125–64	0.125	0.5	<0.125–>64	0.5	2	98.8
Etest	0.094–128	0.25	0.38	0.094–>256	0.625	2	89.3
Voriconazole							
VK2	≤0.12–0.5	≤0.12	≤0.12				
CLSI	<0.03–1	0.03	0.125	<0.03–2	0.03	0.125	100
Etest	0.003–1	0.008	0.023	0.002–2	0.02	0.064	98.8
C. parapsilosis (22)							
Fluconazole							
VK2	≤1	≤1	≤1				
CLSI	<0.125–8	0.125	0.5	<0.125–8	0.25	0.5	95.4
Etest	0.064–0.5	0.315	0.38	0.064–8	1	1.5	90.9
Voriconazole							
VK2	≤0.12	≤0.12	≤0.12				
CLSI	<0.03–0.25	<0.03	<0.03	<0.03–0.25	<0.03	0.03	100
Etest	0.004–0.047	0.012	0.023	0.006–0.19	0.032	0.064	100
C. tropicalis (17)							
Fluconazole							
VK2	≤1–2	≤1	2				
CLSI	<0.125–2	0.25	0.5	0.125–2	0.5	2	100
Etest	0.5–2	1.5	1.5	0.38–6	1.5	3	64.7
Voriconazole							
VK2	≤0.12	≤0.12	≤0.12				
CLSI	<0.03–0.06	0.03	0.06	0.03–0.25	0.06	0.125	100
Etest	0.016–0.094	0.047	0.094	0.012–0.25	0.094	0.19	100
Other *Candida* spp. (5)[c]							
Fluconazole							
VK2	≤1–2	≤1	≤1				
CLSI	<0.125–0.5	0.125	0.125	<0.125–4	0.125	0.125	100
Etest	0.064–3	0.75	0.75	0.064–3	0.75	0.75	100
Voriconazole							
VK2	≤0.12–≤0.12	≤0.12	≤0.12				
CLSI	<0.03–<0.03	<0.03	<0.03	<0.03–<0.03	<0.003	<0.003	100
Etest	0.004–0.094	0.023	0.023	0.004–0.094	0.023	0.023	100
C. glabrata (56)							
Fluconazole							
VK2	≤1–≥64	8	32				
CLSI	<0.125–64	2	8	0.125–>64	6	16	94.6
Etest	0.094–>256	7	16	0.094–>256	>256	>256	25
Voriconazole							
VK2	≤0.12–≥8	≤0.12	2				
CLSI	<0.03–8	0.125	0.5	0.03–8	0.125	2	89.3
Etest	0.016–8	0.125	1.5	0.032–>32	1.5	32	32.1
C. inconspicua (5), *C. norvegensis* (2)							
Fluconazole							
VK2	4–≥64	16	16				
CLSI	<0.125–16	<0.125	<0.125	2–32	16	16	100
Etest	8–24	10	10	24–>256	>256	>256	85.7

Continued on following page

TABLE 1—*Continued*

Candida sp. (no. of isolates), azole, and method	24 h			48 h			EA[a] (%)
	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	
Voriconazole							
VK2	≤0.12–0.25	≤0.12	≤0.12				
CLSI	<0.03–0.5	<0.03	<0.03	0.03–0.5	0.25	0.25	100
Etest	0.19–2	0.19	0.19	0.19–2	0.19	0.19	85.7
C. krusei (14)							
Fluconazole							
VK2	4–32	16	32				100/92.8[b]
CLSI	0.25–64	16	32	8–64	32	64	100[b]
Etest				32–>256	>256	>256	
Voriconazole							
VK2	≤0.12–0.25	≤0.12	0.25				
CLSI	0.03–0.5	0.25	0.5	0.25–1	0.5	1	92.8
Etest	0.19–0.5	0.25	0.25	0.064–1.5	0.25	1.5	64.3

[a] EA between VK2 and the two other methods at 48 h.
[b] EA calculated with Vitek 2 expert software interpretive values.
[c] *C. lusitaniae* ($n = 2$), *C. guilliermondii* ($n = 1$), *C. kefyr* ($n = 1$), and *C. dubliniensis* ($n = 1$).
[d] MICs are in micrograms per milliliter.

method and the 24-h and 48-h CLSI methods was 78.6% in both cases, with 12 errors. Eleven of these 12 errors were mE, the 12th was a ME, and the EA was very good (94.6%). When the VK2 method was compared to the Etest method, greater differences appeared in MICs determined at the 24- and 48-h end points. The CA with the 24-h Etest was 77.3% with only one VME. However, when reading at 48 h, the CA obtained was 23.2% with 23 VME and a low EA of 25%. The errors observed between the VK2 and CLSI methods (at both 24 and 48 h), as well as between the VK2 and Etest methods (24 h only), were randomly assigned (VK2 versus CLSI, $P = 0.61$ in both cases; VK2 versus Etest, $P = 0.68$). In contrast, the errors between the VK2 and 48-h Etest methods were not randomly assigned ($P < 0.0001$). In total, only 17.86% of the *C. glabrata* isolates were similarly interpreted for fluconazole susceptibility by all three methods at 48 h (seven susceptible, two resistant, and one SDD).

A cross-resistance of the *C. glabrata* isolates to voriconazole was measured as 12.5%, 10.6%, 14.3%, and 51.8% with the VK2, 48-h CLSI, and 24-h and 48-h Etest methods, respectively. The CAs between VK2 and CLSI (both 24 and 48 h) were excellent (89.3 and 87.5%; $P = 0.61$ and $P = 0.54$, respectively), with only mE (Table 2). When the VK2 method was compared with the 48-h Etest method, discrepancies were also observed with this antifungal. There was a low CA (51.8%, $P < 0.001$), a low EA (32.1%), and 20 VME. Similar to fluconazole tests, when looking at MICs read at 24 h with the Etest method, the CA with VK2 was improved (89.2%) without any VME.

In spite of these differences in susceptibility interpretation, both the VK2 MIC_{50} (MIC for 50% of the isolates tested) and MIC_{90} (8 and 32 mg/liter, respectively) of fluconazole and the MIC_{90} (2 mg/liter) of voriconazole were very high (Table 1). MICs obtained with the 48-h Etest method were the highest, resulting in a low EA with VK2 (25% for fluconazole and 32.1% for voriconazole). The EA between VK2 and the 48-h CLSI method was better (94.6% for fluconazole and 89.3% for voriconazole).

Among other species known to have emerging resistance to fluconazole, we also tested five *C. inconspicua* and two *C. norvegensis* isolates. The VK2 method determined higher MICs of fluconazole than those seen with susceptible species (Table 1), but only one out of the seven isolates was found to be resistant, compared with five for the 48-h Etest method and none with the CLSI method (Table 2). Even if the number of isolates was low, EAs and CAs were calculated. The symmetry test was not performed (insufficient number of isolates). For both species, CAs and EAs with the 48-h Etest method were mediocre, whereas CAs were much better with the 24-h Etest method and even a little higher than the CA for the CLSI method (Table 2). EA with the CLSI method was nevertheless perfect. For voriconazole, all of the isolates were susceptible by all three methods at the 24-h end point; only one isolate was SDD by the 48-h Etest method.

It is noteworthy that none of the isolates of *C. krusei*, which is intrinsically resistant to fluconazole, were found to be resistant by the VK2 method and that the MICs corresponded to a susceptible value for 6 of the 14 isolates. The MIC_{50} was only 16 mg/liter by the VK2 method, similar to the MIC determined by the 24-h CLSI method. Nevertheless, it is important to note that the ATCC 6258 MICs were not in the expected range. However, as already mentioned, the VK2 method's expert software always corrected the interpretation to resistant when analyzing a *C. krusei* isolate. There was no cross-resistance to voriconazole among the 14 *C. krusei* isolates tested, by either the VK2 or the CLSI method. Nevertheless, two isolates had a MIC of 1.5 mg/liter by the 48-h Etest method.

Finally, for all of the species, when both azoles were taken into account, 30 errors (24 with fluconazole, 6 with voriconazole) were detected among the 205 isolates tested, with a CA of 85.4% (175/205) between the VK2 and 48-h CLSI methods.

TABLE 2. Fluconazole and voriconazole susceptibilities of 205 *Candida* sp. isolates, CAs, and errors between VK2 and CLSI/Etest methods at 24 and 48 h

Species (no. of isolates), azole, and method	% of isolates (24 h/48 h)[a] that were:			% CA with VK2 (24 h/48 h)[a]	No. of VK2 errors (24 h/48 h)[a]		
	Susceptible	SDD	Resistant		VME	ME	mE
C. albicans (84)							
Fluconazole							
VK2	98.8	0	1.2				
CLSI	98.8	0	1.2	100	0	0	0
Etest	98.8	0	1.2	100	0	0	0
Voriconazole							
VK2	100	0	0				
CLSI	100/98.8	0/1.2	0	100/98.8	0	0	1
Etest	100/98.8	0/1.2	0	100/98.8	0	0	1
C. parapsilosis (22), *C. tropicalis* (17), other spp. (5)[b]							
Fluconazole + voriconazole							
VK2	100	0	0				
CLSI	100	0	0	100	0	0	0
Etest	100	0	0	100	0	0	0
C. glabrata (56)							
Fluconazole							
VK2	75	16.1	8.9				
CLSI	80.3	14.3	5.4	78.6	0	1	11
Etest	71.5/12.5	16/23.2	12.5/64.3	77.3/23.2	1/23	1/0	12/20
Voriconazole							
VK2	87.5	5.4	7.1				
CLSI	89.3/85.7	7.2/10.7	3.5/3.6	89.3/87.5	0	0	4/5
Etest	89.3/48.2	7.2/10.7	3.5/41.1	89.2/51.8	0/20	1/0	5/7
C. inconspicua (5), *C. norvegensis* (2)							
Fluconazole							
VK2	42.85	42.85	14.3				
CLSI	28.6	71.4	0	71.4	0	0	1/2
Etest	28.6/0	71.4/28.6	0/71.4	75/14.3	0/1	0	1/5
Voriconazole							
VK2	100	0	0				
CLSI	100	0	0	100	0	0	0
Etest	100/85.7	0/14.3	0	100/85.7	0	0	0/1
C. krusei (14)							
Fluconazole							
VK2[c]	0	0	100				
CLSI	35.7/7.1	57.1/64.3	7.1/28.6	7.1/28.6	0	5/1	8/9
Etest	0	7.1	92.9	92.9	0	0	1
Voriconazole							
VK2	100	0	0				
CLSI	100	0	0	100	0	0	0
Etest	100/85.7	0/14.3	0	100/85.7	0	0	2

[a] Interpretative results obtained at 48 h if different from results at 24 h for CLSI and Etest methods only.
[b] Two strains of *C. lusitaniae*, one strain of *C. guilliermondii*, one strain of *C. kefyr*, and one strain of *C. dubliniensis*.
[c] Interpretative values and errors based on Vitek 2 expert software expertise.

Amphotericin B in vitro susceptibility results are presented in Table 3. A MIC of ≤1 mg/liter was found for 201 (VK2), 190 (48-h CLSI), and 202 (48-h Etest) isolates. Only one isolate of *C. albicans* had a MIC of 2 mg/liter by the 48-h CLSI method. One isolate of *C. glabrata* had a MIC of ≥2 mg/liter by all three methods (48-h end point for both comparative methods), two MICs were high only by the VK2 method (4 and 8 mg/liter), and five were high only by the 48-h CLSI method (2 mg/liter for all of the isolates). Two isolates of *C. krusei* had a MIC of >1 mg/liter by both the 48-h Etest and 48-h CLSI methods, one only by the VK2 method, and two only by the 48-h CLSI method. EAs between the VK2 and CLSI methods were good,

TABLE 3. Amphotericin B susceptibilities of the 205 *Candida* sp. isolates used in this study

Species (no. of isolates) and method	% of isolates (48 h) with MIC (mg/liter) of:		24 h			48 h			EA[a] (%)
	≤1	>1	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	
C. albicans (84)									
VK2	100		≤0.25–1[c]	0.5	1				
CLSI	98.8	1.2	0.125–1	0.5	0.5	0.25–2	1	1	98.8
Etest	100		0.094–0.38	0.094	0.19	0.016–0.5	0.125	0.25	29.8
C. parapsilosis (22)									
VK2	100		≤0.25–1	0.5	0.5				
CLSI	81.8	18.2	0.125–0.5	0.25	0.5	0.5–2	1	2	86.4
Etest	100		0.094–0.5	0.125	0.25	0.064–1	0.38	0.75	63.6
C. tropicalis (17)									
VK2	100		≤0.25–0.5	0.5	0.5				
CLSI	100		0.5–1	0.25	1	0.5–2	1	2	100
Etest	100		0.047–0.25	0.125	0.19	0.032–0.75	0.25	0.5	70.6
Other *Candida* spp. (5)[b]									
VK2	100		≤0.25–0.5	0.5	0.5				
CLSI	100		0.06–1	0.5	0.5	0.25–1	0.5	0.5	100
Etest	100					0.047–0.38	0.25	0.25	100
C. glabrata (56)									
VK2	94.6	5.4	≤0.25–8	0.5	1				
CLSI	89.3	10.7	0.125–2	0.5	1	0.25–2	1	1	98.2
Etest	98.2	1.8	0.016–1	0.25	0.5	0.023–>32	0.38	0.75	73.2
C. inconspicua (5), *C. norvegensis* (2)									
VK2	100		≤0.25–1	≤0.25	≤0.25				
CLSI	100		0.03–1	0.125	0.125	0.25–1	0.25	0.25	100
Etest	100					0.016–0.75	0.064	0.064	100
C. krusei (14)									
VK2	92.9	7.1	0.5–2	0.5	1				
CLSI	71.4	28.6	1–2	1	2	0.25–2	1	2	100
Etest	85.7	14.3	0.094–1	0.75	0.75	0.19–2	0.875	2	78.6

[a] EA between VK2 and the two other methods at 48 h.
[b] *C. lusitaniae* ($n = 2$), *C. guilliermondii* ($n = 1$), *C. kefyr* ($n = 1$), and *C. dubliniensis* ($n = 1$).
[c] MICs are in micrograms per milliliter.

ranging from 86.4% to 100% according to the *Candida* sp. tested. When the VK2 method was compared to the 48-h Etest, the EAs ranged from 63.6 to 83.3% for "non-*C. albicans*" species, but the EA was very low (29.8%) for *C. albicans*. Nevertheless, the MIC_{90}s were ≤1 mg/liter for all of the species and methods tested.

DISCUSSION

The goal of this study was to evaluate the new automated Vitek 2 antifungal susceptibility test. For this purpose, we analyzed *Candida* sp. isolates that had all previously required susceptibility testing in clinical laboratories. This new method was then compared by the CLSI broth microdilution reference method, and by one of the other available antifungal susceptibility tests, the Etest method, which has been recommended for routine use in French Hospital laboratories (2).

The VK2 method demonstrated excellent reproducibility, which underscores its excellent level of standardization. Spectrophotometric readings remove any subjectivity from the MIC determination. Furthermore, *Candida* species identification and in vitro antifungal susceptibility are obtained in less than 26.5 h (mean, 15.2 h), thus reducing the time necessary for optimizing antifungal treatment decisions. However, three isolates failed to grow sufficiently in the VK2 growth control well. There was no obvious explanation for this, as those three isolates grew well enough in both the CLSI and Etest methods.

Low-level fluconazole MICs were observed for all of the *C. krusei* isolates, but the VK2 expert software systematically gave a resistant MIC result. In current clinical management practices, fluconazole is not recommended as a treatment for *C. krusei* candidiasis (5) or for susceptibility testing. For voriconazole, the VK2 method was fully concordant (CA = 100%) with the 48-h CLSI method, despite the low values observed for the *C. krusei* ATCC strain.

As concerns the ability of VK2 to detect resistance in the other species, the accuracy of the other two ATCC strains tested was perfect. Azole testing with the two reference methods demonstrated that the VK2 results were more related to those obtained with the 24-h CLSI and Etest methods than

with the 48-h Etest method. CA between the VK2 and 48-h CLSI method results was very good at 88.3% (181/205) for fluconazole and 97.1% (199/205) for voriconazole, without any VME. These observations are coherent with studies that compared the VK2 method to the CLSI method. CAs ranged from 91 to 96% for fluconazole (depending on the species), and the CA was about 91% for voriconazole (12). Furthermore, according to Posteraro et al. (10), CAs higher than those in our study were observed for *C. glabrata* isolates, with 96.4 and 97.6% for fluconazole and voriconazole, respectively. Nevertheless, such a good correlation with the CLSI method, for every *Candida* sp. isolate tested, is comparable or even superior to other available antifungal susceptibility testing systems (7, 8). In addition, we found CA with both the 24-h and 48-h CLSI methods to be very similar (Table 2), whereas Pfaller et al. (7) found that the VK2 method was more correlated with the 24-h CLSI method for fluconazole.

In the comparison of VK2 with the Etest method for azoles, the results depended on the end point reading time. For fluconazole, the CA was higher for the 24-h end point at 86.3%, with one VME, than at the 48-h end point at 75.6% with 24 VME. For voriconazole, the CAs were 96.1% without any VME and 84.9% with 20 VME at 24- and 48-h end points, respectively.

In both cases, VME were primarily found with *C. glabrata* isolates. For this species, it is noteworthy that 48-h MICs demonstrated the presence of macrocolonies of 27 isolates of *C. glabrata*, which were not clearly visible at 24 h and probably not detected by VK2, with a MIC reading obtained at a mean of 16.5 h. Nevertheless, we suggest that the manufacturer add a strain of *C. glabrata* to the required list of quality control organisms, in order to validate in vitro results of the cards.

Conflicts between the comparative methods have also been observed. In two studies (1, 9), this is a recurring problem for *C. glabrata*. In the study by Pfaller et al. (9), the Etest method also resulted in MICs slightly higher than those of the CLSI method, as in our study.

All of these data are of major interest for the clinical management of candidiasis. Actually, if the use of high doses of fluconazole for the treatment of *C. glabrata* infections is based on antifungal test results, potential discrepancies in antifungal choice could occur, depending on the test used. Nevertheless, the Infectious Diseases Society of America recommendations for *C. glabrata* invasive infection do not include the use of fluconazole as a first-line treatment (5). The VK2 interpretative thresholds should probably be revised for species less sensitive to azoles (*C. krusei* and *C. glabrata*), with species-specific breakpoints. Another alternative might be the use of Eucast breakpoints to interpret VK2 MICs. In any case, complementary studies are necessary to validate this hypothesis, with *in vivo-in vitro* correlation for this new susceptibility testing. Thus, before further investigations, 48-h Etest results should be those taken into account in order to avoid any risk for the patient.

For amphotericin B, an excellent correlation between VK2 and the CLSI method was observed, with low dispersion of MICs. This is in total agreement with the literature (8). In contrast, the MICs obtained with the Etest method displayed a broad range of values. We noticed that the majority (92% to 98.5%) of the MICs were <1 mg/liter and that only two isolates had MICs of ≥ 1 mg/liter by all three methods. Thus, the low rate of amphotericin B-resistant isolates persists no matter what method was used (6, 8).

Finally, the AST-YS01 Vitek 2 card system (bioMérieux) is a reliable and practical standardized, automated antifungal susceptibility test that correlated well with the CLSI method but presented some discrepancies with the Etest method and certain discordances depending on the *Candida* species tested. Moreover, the new class of echinocandins should be added to the VK2 cards to well represent the entire range of antifungals used to treat invasive candidiasis. In addition, further study is required to establish the correlation between in vivo and in vitro susceptibilities in order to determine the accuracy of all of these results, especially for species like *C. glabrata*, whose susceptibility varies according to the method used.

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