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Interlaboratory evaluation of VITEK2 system and Sensititre YeastOne© for antifungal susceptibility testing of yeasts isolated from blood cultures against four antifungal agents

Claudio Farina[1,2], Esther Manso[1,3], Stefano Andreoni[1,4], Marco Conte[1,5], Paolo Fazii[1,6], Gianluigi Lombardi[1,7], Silvana Sanna[1,8], Giuseppe Russello[2]

[1]Comitato Studio Micologia (CoSM), Associazione Microbiologi Clinici Italiani, Milano;
[2]Microbiology Institute, AO 'Ospedale S. Carlo Borromeo', Milano;
[3]Biochemical and Microbiology Laboratory, AO 'Ospedali Riuniti', Ancona;
[4]Microbiology Laboratory, AOU 'Maggiore della Carità', Novara;
[5]Microbiology Laboratory, AO 'Domenico Cotugno', Napoli;
[6]Biochemical and Microbiology Laboratory, PO 'Santo Spirito', Pescara;
[7]Microbiology and Virology Laboratory, AO 'Niguarda-Ca' Granda', Milano;
[8]Microbiology Institute, Università degli Studi, Sassari

SUMMARY

An interlaboratory evaluation (seven centers) of VITEK2 System and Sensititre YeastOne® was conducted to test the antifungal susceptibilities of yeasts. The MICs of amphotericin B, fluconazole, flucytosine, and voriconazole were determined for 70 isolates of *Candida* spp.

Our results demonstrated a higher interlaboratory agreement of VITEK 2 System than Sensititre YeastOne®. A good concordance between the two methods was observed for amphotericin B, fluconazole, voriconazole and 5-fluorocytosine (from 81.4% to 88.6%). The study suggests the potential value of the VITEK2 System as a convenient alternative method for testing the susceptibility of yeasts. It also indicates the need for further optimization of MIC endpoint criteria to improve interlaboratory agreement.

KEY WORDS: Antifungal susceptibility testing, Yeast, Agreement

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INTRODUCTION

Invasive candidiasis is associated with high morbidity and mortality. Differences in the virulence and susceptibility of the various *Candida* spp. to antifungal drugs make the identification and rapid MIC determination very important for clinical management (Avolio *et al.,* 2009). Ready-to-use tests should be reliable, reproducible and easy to perform for routine application in clinical laboratories. The standardization of reference procedures for antifungal susceptibility testing (AST) of yeasts and moulds by the Clinical Laboratory Standards Institute (CLSI) have served to develop several automated or semi-automated commercial systems to provide simple, flexible and affordable alternative susceptibility testing methods for use in the clinical laboratory (CLSI, 2008a; CLSI, 2008b; Cuenca-Estrella *et al.,* 2010). The VITEK2 Antifungal Susceptibility System (bioMérieux S.A., Marcy l'Etoile, France) is a fully automated commercial method that spectrophotometrically determines the yeast growth and allows both fungal identification and MIC determination simultaneously. The system incorporates the AST-YS01 card which is designed for susceptibility testing of amphotericin B (AMB),

Corresponding author
Claudio Farina
U.O.C. Microbiologia e Virologia
AO 'Ospedale San Carlo Borromeo'
Via Pio II, 3 - 20153 Milano, Italia
E-mail: farina.claudio@sancarlo.mi.it

fluconazole (FLC), flucytosine (5FC), and voriconazole (VRC) which are essentially miniaturized versions of the double dilution technique used for determining the MIC by microdilution methodology in mg/L (Al Sweih *et al.*, 2005; Morrell *et al.,* 2005; Pfaller *et al.,* 2010).
The aim of this study was the intra- and inter-laboratory evaluation of the commercial technique VITEK 2 System for susceptibility testing of antifungal agents and its comparison with results obtained by a commercial system based on the micro-broth dilution reference method proposed by CLSI (Sensititre YeastOne®, SYO; Trek Diagnostic Systems, UK) by testing 70 *Candida* spp. isolated from blood. MIC interpretative criteria were referred to those described in the CLSI document M27-S3.

METHODS

Study design

The study was designed to compare the MIC results for amphotericin B, flucytosine, voriconazole and fluconazole obtained by the VITEK 2 yeast susceptibility test to those obtained by YeastOne® method in seven laboratories: four laboratories tested 70 clinical isolates of *Candida* spp. by the VITEK 2 system (Ancona, Milan "San Carlo", Milan "Niguarda", and Novara) and three (Napoli, Pescara, Sassari) by YeastOne® system. The MIC results obtained with the VITEK2 system after 18 hours of incubation were compared to those obtained with the reference YeastOne® system after 24 hours of incubation.

Fungal strains

Seventy yeasts isolated from blood cultures (one per patient) were studied. Yeasts were selected between positive blood cultures diagnosed in seven laboratories, located in different parts of Italy. Only the first isolate from each patient was included in the study. Strains were sent to all the participating laboratories and stored at -80°C until testing. Yeasts were chosen according to the Italian etiological distribution of fungemias, including 20 *C. albicans*, 14 *C. parapsilosis*, 10 *C. glabrata*, 6 *C. guilliermondii*, 6 *C. tropicalis*, 5 *C. krusei*, 2 *C. kefyr*, 2 *C. lusitaniae*, 2 *C. lypolitica*, 2 *C. norvegiensis*, 1 *C. pelliculosa*.
Candida krusei ATCC 6258 and *C. parapsilosis* ATCC 22019 were used as control strains in susceptibility testing assays.

Identification

Identification of *Candida* spp. was performed with the API systems (bioMérieux S.A.) and/or by VITEK2 system (YST-2143 cards) according to the manufacturer's instructions.

Susceptibility tests

VITEK2. Susceptibility tests with the VITEK2 system were performed with AST-YS01 cards, according to the manufacturer's instructions. The 64-well AT-N011 card contains the following antimicrobial agents (as dehydrated substances) at the indicated concentrations: amphotericin B, 1, 4, 16, and 32 mg/L; fluconazole, 1, 4, 8, and 16 mg/L; flucytosine, 4, 8, 16, and 64 mg/L; voriconazole, 0.5, 1, 4 and 8 mg/L. The inoculum suspensions for the VITEK2 were arranged in sterile saline to a turbidity equal to a 2. McFarland standard by using the bioMérieux DensiChek instrument. MICs and clinical categories defined by the expert system (to simulate an actual situation in the clinical laboratory) were considered and compared with those obtained by the reference method. YeastOne®. Susceptibility to amphotericin B, fluconazole, voriconazole, and flucytosine was determined utilizing the system Y08 Sensititre Yeast One, a microtiter broth dilution assay with an indicator dye (Alamar Blue).
The YeastOne® Y08 colorimetric antifungal panel (Trek Diagnostic Systems Ltd., East Grinstead, England) plate is formed in a 96-well microtiter plate, prepared with 11 dilutions of each drug. It contained from rows 2 to 12 of each strip dried amphotericin B (concentrations ranging from 0.008 to 16 mg/L), fluconazole (0.03-64 mg/L), itraconazole (0.008-16 mg/L), ketoconazole (0.008-16 mg/L), voriconazole (0.008-16 mg/L), posaconazole (0.008-8 mg/L), 5-fluorocytosine (0.125-256 mg/L) and caspofungin (0.008-16 mg/L). Each well incorporated Alamar Blue, which changed from blue to pink in the presence of microbial growth. Assays have been performed according with CLSI M27-A3 document (CLSI, 2008b).

Data analysis

Reproducibility was the percentage agreement within +1 $\log_2$ dilution. For each one of the four

drugs and each reading mode, the essential agreements between VITEK2 and YeastOne® system were assessed. Essential agreement was considered as the percentage agreement within +1 $\log_2$ dilutions of the reference MIC.

RESULTS

Tables 1-4 summarize the in vitro amphotericin B, fluconazole, flucytosine and voriconazole susceptibilities of 70 isolates of *Candida* spp. as determined by the VITEK 2 and YeastOne® system. MIC readings for the two quality control strains were within the limits reported in the CLSI M27-A3 document and those described in each one of the guides for the commercial methods.
MIC_{90} values generated by the YeastOne® were higher than those obtained by VITEK2 system in *C.albicans* for voriconazole and fluconazole and lower in *C. albicans* for flucytosine.
Tables 1-4 present also a summary of the interlaboratory agreement and the concordance between the two methods.

TABLE 1 - *Amphotericin B susceptibilities of 70 isolates of Candida spp. as determined by the VITEK2 and YeastOne® test. Amphotericin B MIC interlaboratory agreement and concordance between the methods.*

Strains	*No. Tested*	*Test method*	*MIC (mg/L)*			*% interlaboratory agreement*	*%VITEK2 vs YeastOne agreement*
			Range	*MIC_{50}*	*MIC_{90}*		
C. albicans	20	VITEK2	0.25-0.5	0.5	0.5	100	85.0±8.0
		YeastOne					
			0.25-2.5	0.75	1,5	90.0±4.7	
C. parapsilosis	14	VITEK2	0.25-0.75	0.25	0.5	100	100
		YeastOne	0.25-1.5	0.5	0.75	100	
C. glabrata	10	VITEK2	0.5-0.75	0.5	-	100	90.0±9.5
		YeastOne	0.5-1.5	0.75	-	90.0±9.5	
C. guilliermondii	6	VITEK2	0.25-0.5	0.25	-	100	83.3
		YeastOne	0.25-0.75	0.25	-	100	
C. tropicalis	6	VITEK2	0.5-0.75	0.5	-	100	100
		YeastOne	0.75-1	1	-	100	
C. krusei	5	VITEK2	0.2-0.5	0.5	-	100	60
		YeastOne	0.5-1	0.5	-	100	
C. lipolytica	2	VITEK2	0.25-0.25	0.25	-	100	100
		YeastOne	0.25-0. 5	0.5	-	100	
C. norvegensis	2	VITEK2	0.25-0.5	0.25	-	100	100
		YeastOne	0.25-0.5	0.25	-	100	
C. kefyr	2	VITEK2	0.25-0.5	0.25	-	100	50
		YeastOne	0.25-0.75	0.25	-	100	
C. lusitaniae	2	VITEK2	0.25-0.5	0.25	-	100	50
		YeastOne	0.25-0.75	0.25	-	100	
C. pelliculosa	1	VITEK2	0.25	0.25	-	100	100
		YeastOne	0.25	0.25	-	100	
All Candida spp.	70	VITEK2	0.25-0.75	0.5	0.5	100	84.3±4.3
		YeastOne	0.25-2.5	0.5	1	95.8±2.3	

The amphotericin B MICs determined by both VITEK2 and YeastOne® showed the highest level of interlaboratory reproducibility among the centers: the essential agreement (EA) was 100% and 95.8% respectively. The rates of EA value was 100% for all species using VITEK2. On the other hand, when YeastOne® was used, the EA for amphotericin B MICs ranged from 90.0% to 100% with the lowest value for *C. albicans* and *C. glabrata* (90.0%). Furthermore, a good global correlation between the two methods was also observed (84.3%). A high level of interlaboratory reproducibility was also found for voriconazole and fluconazole with both VITEK2 and YeastOne®. The EA was lower with YeastOne® (from 66.7% to 100% for voriconazole and from 60.0 to 100% for fluconazole) than with VITEK2 system (from 83.3 to 100% for voriconazole and from 50.0% to 100% for fluconazole) in all the tested species except for fluconazole and voriconazole in *C. norvegensis* and for fluconazole in *C. pelliculosa*. The technical agreement for fluconazole on *C. krusei* strains is complete. However, this chemosensitivity testing does not have to be considered, because all the standardization documents declare the lack of validity of this assay.
The global agreement was 88.6% for voriconazole and 81.4% for fluconazole.

TABLE 2 - *Fluconazole susceptibilities of 70 isolates of Candida spp. as determined by the VITEK2 and YeastOne® Test. Fluconazole MIC interlaboratory agreement and concordance between the methods.*

Strains	*No. Tested*	*Test method*	*MIC (mg/L)*			*% interlaboratory agreement*	*%VITEK2 vs YeastOne agreement*
			Range	*MIC_{50}*	*MIC_{90}*		
C. albicans	20	VITEK2	1-4	1	1	96.7±4.0	70.0±10.2
		YeastOne	0.25-16	0.5	4	95.0±4.9	
C. parapsilosis	14	VITEK2	1-32	1	4	95.2±5.7	71.4±12.1
		YeastOne	0.75-16	1.5	8	92.8±6.9	
C. glabrata	10	VITEK2	4->64	16	-	93.3±7.9	90.0±9.5
		YeastOne	8->64	16	-	60.0±15.5	
C. guilliermondii	6	VITEK2	1-8	2	-	100	83.3
		YeastOne	1-8	4	-	100	
C. tropicalis	6	VITEK2	1-2.5	1	-	88.9	100
		YeastOne	0.5-8	2	-	83.3	
C. krusei	5	VITEK2	8-32	16	-	80.0	80
		YeastOne	8-32	16	-	100	
C. lipolytica	2	VITEK2	1-2	1	-	100	50
		YeastOne	1-4	2	-	100	
C. norvegensis	2	VITEK2	2-2	2	-	58.3	100
		YeastOne	2-2	2	-	100	
C. kefyr	2	VITEK2	1-1	1	-	100	50
		YeastOne	1-4	1	-	100	
C. lusitaniae	2	VITEK2	1-1	1	-	100	50
		YeastOne	0.25-1	1	-	100	
C. pelliculosa	1	VITEK2	4-4	4	-	50.0	100
		YeastOne	2-2	2-2	-	100	
All Candida spp.	70	VITEK2	0.25->64	1.5	16	93.2±2.9	81.4±4.6
		YeastOne	0.25->64	2	16	87.5±3.9	

The EA for flucytosine ranged from 58.3% to 100% with VITEK2 system and from 50.0% to 100% with YeastOne®: the lower agreement was obtained for *C. norvegensis* and *C. kefyr* with VITEK2 system and for *C. tropicalis*, *C. kefyr* and *C. lusitaniae* with YeastOne®. The overall agreement between VITEK2 and YeastOne® was the same observed for fluconazole (81.4%).

DISCUSSION

The findings of the present study document the excellent degree of standardization and reproducibility that can be achieved with the VITEK2 yeast susceptibility testing. This system is the first automated approach to antifungal susceptibility testing and provides an improvement in test standardization. In addition to providing highly reproducible results, the VITEK2 system was rapid, with a mean time to result of 13 hours, lower than that required by the microtiter broth dilution assay. The availability of rapid quantitative antifungal susceptibility data will be a major step in optimizing the therapy of invasive *Candida* infections (Alexander *et al.*, 2006; Magill *et al.,* 2006, Mokaddas *et al.,* 2007; Pfaller *et al.,* 2007).

The results of the present study show the excel-

TABLE 3 - *5-Fluorocytosine susceptibilities of 70 isolates of Candida spp. as determined by the VITEK2 and YeastOne® Test. 5-Fluorocytosine MIC interlaboratory agreement and concordance between the methods.*

Strains	*No. Tested*	*Test method*	*MIC (mg/L)*			*% interlaboratory agreement*	*%VITEK2 vs YeastOne agreement*
			Range	*MIC_{50}*	*MIC_{90}*		
C. albicans	20	VITEK2	1-1.5	1	1	100	85.0±8.0
		YeastOne	0.04-4	0.08	0.25	80.0±4.5	
C. parapsilosis	14	VITEK2	1-1	1	1	100	92.8±6.9
		YeastOne	0.06-2.5	0.5	1	85.7±9.3	
C. glabrata	10	VITEK2	1-1	1	-	100	70.0±14.5
		YeastOne	0.02-0.04	0.04	-	80.0±12.6	
C. guilliermondii	6	VITEK2	1-1	1	-	100	66.3
		YeastOne	0.04-1	1	-	83.3	
C. tropicalis	6	VITEK2	1-32	1	-	91.6	66.7
		YeastOne	0.08-4	0.125	-	66.7	
C. krusei	5	VITEK2	8-16	8	-	86.7	100
		YeastOne	8-16	8	-	100	
C. lipolytica	2	VITEK2	2-2	2	-	100	100
		YeastOne	2-2	2	-	100	
C. norvegensis	2	VITEK2	4-8	4	-	58.3	100
		YeastOne	8-8	8	-	100	
C. kefyr	2	VITEK2	1-2.5	1	-	75.0	100
		YeastOne	1-2.5	1	-	66.7	
C. lusitaniae	2	VITEK2	1-1	1-1	-	100	50
		YeastOne	0.25-1	0.25	-	50	
C. pelliculosa	1	VITEK2	1	1	-	100	100
		YeastOne	1	1	-	100	
All Candida spp.	70	VITEK2	1->32	1	4	95.8±2.1	81.4±4.6
		YeastOne	0.008-16	0.125	4	76.4±5.0	

lent degree of standardization and reproducibility of VITEK2 yeast susceptibility test. However, a low EA was found in *C. norvegensis* for voriconazole, fluconazole and flucytosine as previously described by Pfaller *et al.* (2007), in *C. pelliculosa* for fluconazole and in *C. kefyr* for flucytosine.

The YeastOne® system showed a good EA (from 76.4% to 95.8%) even if some intralaboratory discrepancies were observed probably due to intrinsic difficulties in the correct reading the color changes in wells. Furthermore, ranges of antifungal agents in the VITEK2 System do not exactly match those of YeastOne®. In particular, the tested concentrations were 0.03-64 mg/L (YeastOne®) *versus* 1-16 mg/L (VITEK2) for fluconazole, 0.008-16 mg/L (YeastOne®) *versus* 0.5-8 mg/L (VITEK2) for voriconazole. The present conformation of the VITEK2 cards does not permit to evaluate the azole results according to the EUCAST breakpoints but only with CLSI criteria, because voriconazole breakpoints values are ≤0.125 mg/L - >0.125 mg/L for EUCAST *versus*: ≤1 mg/L - ≥4 mg/L for CLSI, and for fluconazole it is impossible to discriminate between I and R results (CLSI, 2010; EUCAST, 2008). For this reason, only YeastOne® can match with EUCAST interpretative criteria, suggesting the need for a rap-

TABLE 4 - *Voriconazole susceptibilities of 70 isolates of Candida spp. as determined by the VITEK2 and YeastOne®.*

Strains	*No. Tested*	*Test method*	*MIC (mg/L)*			*% interlaboratory agreement*	*%VITEK2 vs YeastOne agreement*
			Range	*MIC_{50}*	*MIC_{90}*		
C. albicans	20	VITEK2 YeastOne	0.12-0.5 0.008->16	0.12 8	0.12 >16	98.3±2.9 95.0±4.9	95.0±4.9
C. parapsilosis	14	VITEK2 YeastOne	0.12-0.5 0.016-32	0.12 0.064	0.25 0.25	95.2±5.7 92.8±6.9	93.0±6.8
C. glabrata	10	VITEK2 YeastOne	0.12-1 0.12-1.5	0.12 0.75	- -	100 80±12.6	88.9±9.9
C. guilliermondii	6	VITEK2 YeastOne	0.12-0.25 0.064-0.25	0.12 0.12	- -	100 83.3	16.6
C. tropicalis	6	VITEK2 YeastOne	0.12-0.12 0.008-0.5	0.12 0.25	- -	100 66.7	50
C. krusei	5	VITEK2 YeastOne	0.12-0.25 0.12-0.25	0.12 0.12	- -	100 100	100
C. lipolytica	2	VITEK2 YeastOne	0.12-0.5 0.12-0.12	0.12 0.12	- -	100 100	50
C. norvegensis	2	VITEK2 YeastOne	0.12-0.25 0.12-0.25	0.12 0.12	- -	83.3 100	100
C. kefyr	2	VITEK2 YeastOne	0.12-0.12 0.12-0.12	0.12 0.12	- -	100 100	100
C. lusitaniae	2	VITEK2 YeastOne	0.12-0.12 0.12-0.12	0.12 0.12	 -	100 100	100
C. pelliculosa	1	VITEK2 YeastOne	0.25-0.25 0.25-0.25	0.25 0.25	- -	100 100	100
All Candida spp.	70	VITEK2 YeastOne	0.12-1 0.008-32	0.125 0.125	0.25 1.5	98.1±1.6 88.8±3.1	88.6±3.4

id revision of the antifungal concentrations in the VITEK2 cards, even if Pfaller *et al.* foresee the next revisions for the CLSI fluconazole breakpoints (R if >4 mg/L for *C. albicans*, *C. tropicalis* and *C. parapsilosis*; >32 mg/L for *C. glabrata*) (Pfaller *et al.,* 2010).

Furthermore, in this study VITEK2 and YeastOne® MICs showed a good quantitative agreement for all species of *Candida* tested (from 81.4% to 88.6%); the best global agreement was observed for voriconazole (88.6%).

In conclusion, the results of the present interlaboratory evaluation of the VITEK2 System and the comparisons of this method with the CLSI procedures demonstrated that the VITEK2 System is potentially a reliable, practical, and easy method for use in the clinical laboratory.

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