Comparison of MTS, Etest® and EUCAST methods for *in vitro* antifungal susceptibility testing of *Candida* spp.

Antigoni Elefanti[1], Joseph Meletiadis[1], Maria Siopi[1], Loukia Zerva[1], Aristea Velegraki[2]

[1]Clinical Microbiology Laboratory, "Attikon" University General Hospital, [2]Mycology Research Laboratory, Dept of Microbiology National and Kapodistrian University of Athens, Greece

National and Kapodistrian University of Athens

University General Hospital "Attikon"

Correspondence: Joseph Meletiadis, 1 Rimini str, Haidari 124 62, Athens Greece, Tel: +30-210-583-1909, Email: jmeletiadis@med.uoa.gr

Abstract

Objectives: Development of new methods for the *in vitro* antifungal susceptibility testing of *Candida* spp. has become increasingly important, due to the emergence of strains with intrinsic or acquired resistance to antifungal drugs. The new commercially available method, the Liofilchem® MIC Test strips (MTS), which are strips impregnated with gradient concentrations of antifungal drugs as the widely used strips Etest®, is used for the determination of the on-scale Minimun Inhibitory Concentrations (MIC) of antifungals. Gradient concentration strips could be applied in the clinical laboratory due to technical simplicity and speed of outcome obtained and the low cost. The purpose of present study was to compare two commercial methods, MTS and Etest, with the EUCAST reference microdilution method.

Methods: In the present study, 50 isolates from a collection of Greek Candida species (10 strains of *C. albicans*, 10 *C. tropicalis*, 10 *C. krusei*, 10 *C. parapsilosis*, and 10 *C. glabrata*) isolated from blood cultures of immunocompromised patients (period 2008-2011) was chosen and the MICs of 9 antifungals (amphotericin B, flucytocine, fluconazole, itraconazole, voriconazole, posaconazole, caspofungin, micafungin and anidulafungin) were determined for each isolates. The determination performed with 3 different methods, MTS (Liofilchem, Italy), Etest (Biomerieux, France) and the reference microdilution method EUCAST EDF 7.2, according to the corresponding instructions. The percentage of agreement between methods was calculated by converting the values of MICs from MTS and Etest to the nearest value of EUCAST ± 1 fold dilution.

Results: The overall percentage of agreement between MTS and EUCAST ranged from 65% (amphotericin B) to 98% (anidulafungin). Specifically, for azoles the percentages of agreement were ranging from 79% (posaconazole) to 94% (fluconazole) and for echinocandins from 88% (caspofungin) to 98% (anidulafungin). On the other hand the agreement between MTS and Etest was ranging from 83% (posaconazole) to 100% (fluconazole and amphotericin B). Finally, the MIC values obtained from MTS and Etest methods for amphotericin B were higher than those obtained from the EUCAST by 69% and 80%, respectively.

Conclusions: The MTS and Etest strips provided comparable results and these methods appear to be suitable for MIC determination. High amphotericin B MIC (≥ 2 mg/L) with MTS and Etest methods, need to be verified using the reference method.

Introduction & Purpose

Systemic *Candida* infections are associated with high mortality rates and prolonged hospital stay. During last years a noticeable shift towards *Candida* species, other than *C. albicans*, has been observed, whereas intrinsic or acquired resistance to antifungal drugs in several of these species has been reported. Consequently, the antifungal resistance in combination with the extended use of antifungals as well as the increasing number of invasive fungal infections, lead to the need for development of new, reproducible and clinically relevant methods for the *in vitro* antifungal susceptibility testing.

The new commercially available method, the Liofilchem® MIC Test strips (MTS), which are strips impregnated with gradient concentrations of antifungal drugs as the widely used strips Etest®, is used for the determination of the Minimun Inhibitory Concentrations (MIC) of antifungals. Gradient concentration strips could be applied in the clinical laboratory due to major advantages, such as technical simplicity, speed of outcome obtained and the low cost.

Therefore the purpose of the present study was to compare two commercial methods, MTS and Etest®, with the EUCAST reference microdilution method.

Methods

Isolates: In the present study, a collection comprised of 50 strains of Greek *Candida* species, all isolated from blood cultures of immunocompromised patients (period of time 2008-2011), was studied. More specifically, 10 strains of each *C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and *C. glabrata* were selected. All isolates were stored in normal saline with 10% glycerol at -70°C until the study was performed. Prior to testing each isolate was revived by subculturing it twice onto Sabouraud dextrose agar (SDA) with chloramphenicol plates at 37°C for 24 hours.

Antifungal agents: Nine antifungal agents were tested namely amphotericin B, flucytocine, fluconazole, itraconazole, voriconazole, posaconazole, caspofungin, micafungin and anidulafungin.

Inoculum preparation. For the gradient concentration strips methods inoculum suspensions equivalent to a 0.5 McFarland standard were prepared in normal saline according to the manufacturer's instructions for Liofilchem® MTS. CFU counts were affirmed each time by quantitative cultures on SDA plates. For the broth microdilution method a yeast suspensions of 1-5x10^5 CFU/ml were prepared following the EUCAST EDef 7.2 guidelines.

Methods

MTS (Liofilchem®, Italy) and Etest® (Biomerieux, France) strips: Both tests were performed, according to the corresponding instructions, using solidified RPMI 1640 (2% glucose, buffered with MOPS) agar plates as the test medium. Conidial suspensions of indicated final concentration were prepared for all the isolates tested. The inoculums were subsequently evenly distributed on the entire agar surface, containing RPMI 1640 medium and the excess moisture was allowed to be fully absorbed for approximately 15 min, so that the agar surface was completely dry before applying the strips with the antifungal agents. The plates were incubated at 35°C for 48h. and the endpoint readings were performed after 24 and 48h of incubation. The MICs of AMB were determined as the drug concentration that inhibits completely the yeast growth while the MICs for flucytosine, and azoles were recorded as the lowest concentrations at which the border of the elliptical zone of 90% and 80%, respectively, inhibition intersected the strip scale, ignoring trailing growth or microcolonies throughout a discernible ellipse. Isolates were tested in parallel by both methodologies. All experiments were carried out in duplicate and were independently performed on two different days with individually prepared inocula.

EUCAST EDF 7.2: Represents the standard method, which was performed in accordance with the guidelines.

Analysis of the results: The median (range) MICs were determined fro each method, species and drug. Low off-scale MICs left unchanged whereas high off-scale MICs were converted to the next twofold higher concentration. The percentage of agreement between all methods was estimated by converting the MIC values of MTS and Etest® to the nearest one obtained from EUCAST method ± 1 two-fold dilution.

Results

- The median (range) values of MICs, as they were determined from all three methods, for all drugs and species tested are shown in Table 1.
- The overall percentage of agreement between MTS and EUCAST was ranging from 65% (for amphotericin B) to 98% (for anidulafungin) (Table 2). The percentages of agreement for azoles were ranging from 79% (for posaconazole) to 94% (for fluconazole). The percentages of agreement for echinocandins were ranging from 88% (for caspofungin) to 98% (for anidulafungin).
- The overall percentage of agreement between MTS and Etest® was ranging from 83 (for posaconazole) to 100% (for amphotericin B).
- The MICs of amphotericin B that were determined by the MTS and Etest® methodologies were generally higher than those obtained from the standard method EUCAST 7.2 by 80 % and 69%, respectively compared to echinocandins (10-13% and 2-8%, respectively; 46% and 23% for caspofungin) and azole (52-71% and 21-52%, respectively).

Conclusions

- ✓ The MTS and Etest® strip methods provide comparable results in the collection of only susceptible isolates examined.
- ✓ The agreement between MTS and EUCAST methods was very good (79%-98%) for all drugs except amphotericin B (65%).
- ✓ Isolates of *Candida* spp., for which a high MIC value for amphotericin B (≥2 mg/L) is determined according to MTS and Etest® strip methods should be further tested with the standard method.
- ✓ Before routine clinical implementation, further studies are required in order to evaluate the performance of the new test with resistant isolates.

Results

Table 2. Minimum Inhibitory Concentrations (MICs) of all drugs, as they were determined using the MTS, Etest® and EUCAST methods for each *Candida* spp.

Drug	Method	Mean values and range of Minimum Inhibitory Concentration of all *Candida* spp.				
		C. krusei	*C. glabrata*	*C. albicans*	*C. tropicalis*	*C. parapsilosis*
Amphotericin B	**Etest**	2(0.5-3)	0.88(0.5-1.5)	0.63(0.5-1)	0.625(0.25-1)	0.38(0.25-0.75)
	MICtest	2(0.75-4)	1.5(0.75-1.5)	0.75(0.75-1)	0.75(0.38-1)	0.38(0.38-0.5)
	EUCAST	1(0.25-2)	0.5(0.5-1)	0.25(0.12-0.5)	0.5(0.25-1)	0.5(0.25-0.5)
Flucytocin	**Etest**	0.38(0.03-0.75)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.01(0.01-0.01)
	MICtest	0.5(0.01-0.75)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.01(0.01-0.01)
	EUCAST	0.5(0.06-0.5)	0.01(0.01-0.01)	0.01(0.01-0.06)	0.01(0.01-0.06)	0.01(0.01-0.03)
Echinocandins						
Caspofungin	**Etest**	0.12(0.03-0.75)	0.03(0.01-0.25)	0.01(0.01-0.03)	0.01(0.001-0.12)	0.75(0.23-3)
	MICtest	0.13(0.094-1)	0.03(0.01-1)	0.01(0.01-0.047)	0.01(0.01-0.125)	0.88(0.25-2)
	EUCAST	0.06(0.06-1)	0.01(0.001-0.5)	0.01(0.01-0.06)	0.01(0.01-0.5)	1(0.5-2)
Micafungin	**Etest**	0.25(0.06-0.5)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.12(0.03-0.25)
	MICtest	0.5(0.125-0.5)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.06(0.06-0.12)
	EUCAST	0.12(0.12-0.5)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.12(0.12-0.25)
Anidulafungin	**Etest**	0.04(0.03-0.06)	0.01(0.01-0.01)	0.01(0.001-0.01)	0.01(0.01-0.01)	0.12(0.06-0.25)
	MICtest	0.06(0.03-0.094)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.19(0.12-0.25)
	EUCAST	0.06(0.03-0.12)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.01(0.01-0.01)	0.25(0.12-0.25)
Azoles						
Fluconazole	**Etest**	6(2-8)	8(0.12-256)	0.47(0.25-0.75)	0.47(0.25-3)	4(1-16)
	MICtest	6(4-8)	12(0.25-256)	0.5(0.25-1)	0.5(0.25-2)	4(2-16)
	EUCAST	4(2-8)	8(0.12-256)	0.25(0.12-0.5)	0.25(0.25-4)	2.5(1-16)
Posaconazole	**Etest**	0.05(0.03-0.47)	0.47(0.06-2)	0.03(0.01-0.06)	0.018(0.01-0.047)	0.01(0.01-0.12)
	MICtest	0.09(0.06-0.47)	0.75(0.012-3)	0.03(0.01-0.047)	0.023(0.01-0.125)	0.02(0.01-0.02)
	EUCAST	0.06(0.03-0.5)	0.5(0.03-2)	0.03(0.01-0.03)	0.12(0.01-0.12)	0.01(0.01-0.12)
Itraconazole	**Etest**	0.06(0.03-0.47)	2(0.02-4)	0.03(0.01-0.06)	0.07(0.01-0.47)	0.12(0.01-0.38)
	MICtest	0.09(0.064-0.47)	1.5(0.032-6)	0.04(0.01-0.064)	0.023(0.012-0.38)	0.09(0.023-0.47)
	EUCAST	0.12(0.06-0.5)	1(0.06-2)	0.01(0.01-0.06)	0.01(0.01-0.12)	0.12(0.01-0.25)
Voriconazole	**Etest**	Not determined	Not determined	0.01(0.01-0.12)	0.015(0.01-0.06)	0.01(0.01-0.12)
	MICtest	0.09(0.047-0.125)	0.19(0.023-0.25)	0.01(0.01-0.06)	0.016(0.012-0.06)	0.01(0.01-0.06)
	EUCAST	Not determined	Not determined	0.01(0.01-0.12)	0.03(0.01-0.03)	0.02(0.01-0.03)

Table 1. The overall percentage of agreement between all methods was calculated by converting the values of MICs from MTS and Etest® to the nearest value of EUCAST ± 1 two-fold dilution.

Drug	Etest vs. MTS	Etest vs. EUCAST	MTS vs. EUCAST
Amphotericin B	100%	86%	65%
Flucytocin	98%	86%	84%
Caspofungin	88%	80%	88%
Micafungin	96%	91%	91%
Anidulafungin	98%	91%	98%
Fluconazole	100%	96%	94%
Posaconazole	83%	75%	79%
Itraconazole	90%	86%	82%
Voriconazole	96%	75%	90%

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