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Comparative evaluation of Fungitest®, Neo-Sensitabs® and M27T-NCCLS broth microdilution methods for antifungal drug susceptibility testing of *Candida* species and *Cryptococcus neoformans*

Vergleichende Bewertung von Fungitest®, Neo-Sensitabs® und M27T-NCCLS Bouillon-Mikroverdünnungsmethoden zur Empfindlichkeitsprüfung von *Candida*-Arten und *Cryptococcus neoformans*

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Key words. *Candida*, *Cryptococcus neoformans*, susceptibility testing, Fungitest®, Neo-Sensitabs®, microdilution test.

Schlüsselwörter. *Candida*, *Cryptococcus neoformans*, Empfindlichkeitsprüfung, Fungitest®, Neo-Sensitabs®, Mikrodilutionstest.

Summary. Two commercial antifungal susceptibility testing systems (Fungitest® and Neo-Sensitabs®) were compared with the M27T-NCCLS reference broth microdilution method using one hundred isolates of *Candida* sp. and *Cryptococcus neoformans*. Six different antifungal drugs were tested: amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, ketoconazole and miconazole. The overall agreement between the Fungitest and the reference methods was much better than between the Neo-Sensitabs and the reference methods: the agreement for the Fungitest ranged from 100% for amphotericin B to 76.7% for itraconazole whereas for the Neo-Sensitabs, it ranged from 90.4% for amphotericin B to 36% for ketoconazole. For the total number of tests performed with Neo-Sensitabs, there were 37.8% of discrepancies with the reference method whereas for the tests performed with Fungitest, there was only 16.5% of discrepancies. Major discrepancies, defined as results that classified an isolate as susceptible by one method and resistant by another, occurred in 21 cases for the Neo-Sensitabs test and only in four cases with the Fungitest, namely 0.6% of the cases. We conclude that the Fungitest method constitutes a simple and reliable procedure for antifungal drug susceptibility testing.

Zusammenfassung. Zwei kommerzielle antifungale Empfindlichkeitstestsysteme (Fungitest® und Neo-Sensitabs®) wurden mit der M27T-NCCLS Referenz-Bouillon-Mikroverdünnungsmethode verglichen, wobei hundert Isolate von *Candida* sp. und *Cryptococcus neoformans* verwendet wurden. Sechs verschiedene Antimykotika wurden getestet: Amphotericin B, 5-Fluorocytosin, Fluconazol, Itraconazol, Ketoconazol und Miconazol. Die Übereinstimmung zwischen Fungitest und der Referenzmethode war deutlich besser als zwischen Neo-Sensitabs und der Referenzmethode: Die Übereinstimmung für Fungitest lag zwischen 100% für Amphotericin B und 76.7% für Itraconazol, während für Neo-Sensitabs die Übereinstimmung zwischen 90.4% für Amphotericin B und 36% für Ketoconazol lag. Für die Gesamtheit der Tests mit Neo-Sensitabs ergab sich eine Diskrepanz von 37.8% gegenüber der Referenzmethode, während sich bei den mit Fungitest durchgeführten Tests eine Diskrepanz von nur 16.5% ergab. Größere Diskrepanzen, d.h. Resultate, die ein Isolat als empfindsam bei einer Methode klassifizierten und als resistent bei der anderen, ergaben

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sich in 21 Fällen bei Neo-Sensitabs und in nur 4 Fällen bei Fungitest, d.h. in 0.6% der Fälle. Wir schließen daraus, dass die Fungitest-Methode eine einfache und verläßliche Methode für den Antimykotika-Empfindlichkeitstest darstellt.

Introduction

Serious fungal infections in immunocompromised patients are increasing in frequency and so there is a need to have reliable methods of *in vitro* testing of antifungal drugs. The National Committee for Clinical Laboratory Standards (NCCLS) has developed a standardized broth macrodilution method which was later modified into a broth microdilution method for the testing of *Candida* spp. and *Cryptococcus neoformans* [1, 2]. However, this reference broth microdilution method remains time-consuming and has not eliminated the need for easier methods.

Neo-Sensitabs® is an agar diffusion method using tablets for determining the sensitivity to antifungal drugs which is commonly used in our routine laboratory [3]. Fungitest® is a new kit which allows the determination of the sensitivity of yeasts to antifungal agents according to a standardized method adapted from the NCCLS reference method. It includes six antifungal agents at two different concentrations in modified RPMI 1640 buffered medium in the presence of a redox indicator.

Growth assessment is based on reduction of the coloured indicator which turns the medium from blue to pink. When growth is inhibited by the antifungal agent, the medium remains blue.

The aim of the study was to compare the two methods with the NCCLS broth microdilution method.

Materials and methods

Isolates

A total of 100 isolates were tested. They included 36 *Candida albicans*, 15 *Candida krusei*, 10 *Candida glabrata*, eight *Candida parapsilosis*, seven *Candida tropicalis*, eight *Candida lusitaniae* and 16 *Cryptococcus neoformans* of which nine were of the variety *neoformans* and seven of the variety *gattii*.

Eight reference strains (*C. albicans* ATCC 90028-ATCC 76615-ATCC 24433; *C. parapsilosis* ATCC 22019; *C. krusei* ATCC 6258; *C. tropicalis* ATCC 750; *C. glabrata* ATCC 90030; *Cr. neoformans* ATCC 90112) were included.

To ensure quality control, at least one reference strain was run in each batch of the broth microdilution test. The majority of the strains came from the collection of the Institute of Tropical Medicine of Antwerp. Eight *C. albicans* strains were provided by the University of Antwerp and four ATCC reference strains received from the Scientific Institute Louis Pasteur, Brussels.

Isolates were retrieved from lyophilisates and subcultured on Sabouraud glucose agar (Difco, Detroit, USA) supplemented with 0.5% (w/v) chloramphenicol. Prior to testing, subcultures on Sabouraud glucose agar were incubated at 35 °C for 24 h for *Candida* spp. and for 48 h for *Cryptococcus neoformans*.

Reference broth microdilution method

The reference broth microdilution method was performed according to the guidelines of NCCLS document M27-T [4]. Analytical-grade powders of the antifungal drugs except for amphotericin B, were obtained from the respective manufacturers: 5-fluorocytosine from Roche (Basle, Switzerland), fluconazole from Pfizer (Sandwich, UK), miconazole, ketoconazole and itraconazole from Janssen-Cilag (Beerse, Belgium).

Ketoconazole, miconazole and itraconazole were dissolved in dimethyl sulfoxide, 5-fluorocytosine and fluconazole in sterile distilled water.

Amphotericin B was purchased as the Fungizone® intravenous preparation in sodium desoxycholate (Bristol-Myers Squibb Belgium S.A., Brussels, Belgium).

According to Odds *et al.* 1995, there seems to be no difference in the results obtained with this commercial preparation and those obtained with pure amphotericin B [5].Stock solutions were diluted with RPMI-1640—with L-glutamine but without bicarbonate—(Sigma Chemical Co, St Louis, MO, USA) supplemented with 0.2% glucose and buffered to pH 7.0 with 0.165 M morpholinopropanesulfonic acid (MOPS, Sigma).

The final concentration ranges were 0.03 to 16 $\mu g\,ml^{-1}$ for amphotericin B, ketoconazole, itraconazole and miconazole, 0.125 to 128 $\mu g\,ml^{-1}$ for fluconazole and 0.125 to 64 $\mu g\,ml^{-1}$ for 5-fluorocytosine.

Testing was performed in 96-well round-bottom microtitre plates. Cell suspensions were prepared in physiological water (8.5 $g\,l^{-1}$ NaCl) and were adjusted to give a final inoculum concentration of about 1×10^3 cells ml^{-1}. The plates were incubated at 35 °C and read after 46–50 h for *Candida* sp. and 70–74 h for *Cr. neoformans*.The minimal inhibition concentration (MIC) of amphotericin B was defined as the lowest concentration at which there was 100% inhibition of growth (MIC_{100}) and the MIC of 5-fluorocytosine and azoles were

defined as the lowest concentrations at which there was 50% inhibition of growth compared to the drug-free control (MIC_{50}).

Agar diffusion method

Neo-Sensitabs tablets (Rosco Diagnostica, Toastrup, Denmark) preloaded with a constant amount of diffusible antifungal (amphotericin B: 10 μg; fluconazole: 15 μg; itraconazole: 10 μg; ketoconazole: 15 μg; miconazole: 10 μg and 5-fluorocytosine: 10 μg) drugs were used for the agar diffusion method.

The tablets of amphotericin B, miconazole and itraconazole were placed on casitone agar (bacto-casitone: 9 g; yeast extract: 5 g; sodium citrate: 10 g; glucose: 20 g; bactoagar: 18 g; phosphate buffer: 1000 ml; KH_2PO_4: 0.54 g; Na_2HPO_4: 3.34 g) and those of 5-fluorocytosine, fluconazole and ketoconazole on Shadomy medium (yeast nitrogen base: 6.7 g; glucose: 10 g; L-asparagine: 1.5 g; agar: 15 g; phosphate buffer: 1000 ml; K_2HPO_4: 0.92 g; Na_2HPO_4: 0.33 g).

The inoculum of yeasts was prepared by adjusting the density of yeast cells in sterile distilled water to a 1.0 MacFarland standard, namely, 3×10^6 cells ml^{-1}.

A sterile cotton swab was dipped into the suspension, the agar plate was inoculated by streaking the swab three times over the entire surface and then placing the tablets onto the agar medium. After 24 h at 37 °C for *Candida* sp. and 48 h at 35 °C for *Cr. neoformans*, the diameter of the zone of inhibition of yeast growth around the discs was measured to the nearest millimetre (mm) and recorded.

Fungitest

The Fungitest microplates (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) were set up according to the manufacturer's instructions. Cell suspensions were prepared in sterile distilled water and were adjusted to a turbidity corresponding to a 1.0 MacFarland standard, namely, 3×10^6 cells ml^{-1}; 100 μl of this suspension was added to 1.9 ml of sterile distilled water and this was further diluted by adding 20 μl to 3 ml of the suspension medium furnished with the kit. This gave a final inoculum concentration of 1000 cells ml^{-1}. The microplates were inoculated by placing 100 μl of the appropriate cell suspension into each well.

The plates were incubated at 37 °C and read after 48 h for *Candida* sp. and incubated at 30 °C and read after 72 h for *Cr. neoformans*.

Each antifungal drug was tested at two concentrations, selected to distinguish resistant isolates from susceptible ones. The drug concentrations were as follows: amphotericin B, 2 and 8 μg ml^{-1}; 5-fluorocytosine, 2 and 32 μg ml^{-1}; fluconazole, 8 and 64 μg ml^{-1}; itraconazole and ketoconazole, 0.5 and 4 μg ml^{-1} and miconazole, 0.5 and 8 μg ml^{-1}.

Analysis of the results

As for the agar dilution method, the sizes of the zones of inhibition are interpreted as indicated in Table 1. Those interpretations are recommended for antifungals used in systemic infections.

For the Fungitest, isolates that were inhibited from growth at the two drug concentrations were classified as susceptible, those inhibited from growth only at the higher of the two concentrations as susceptible-dose-dependent and those not inhibited by any of the two concentrations as resistant.

To permit comparison with the NCCLS method, the breakpoints of Fungitest were applied to the reference broth microdilution method. Major discrepancies were defined as results that classified an isolate as susceptible by one method and resistant by another and minor discrepancies were defined as variations from resistant or susceptible to susceptible-dose-dependent or vice-versa.

Results

In each batch of tests, the MIC for the quality control strains were within the accepted limits for each of the antifungal drugs tested (data not shown).

Table 2 summarizes the *in vitro* susceptibility of the 100 isolates to the six antifungal drugs as measured by the reference broth microdilution method and gives the repartition of the 100 isolates

Table 1. Interpretation of the diameters of inhibition zones (tablets included)

Antifungal agents[1]	Diameter (mm)		
	Susceptible	SDD[2]	Resistant
AB	>15	10–14	no zone
5FC	>30	23–29	<22
FLU	>30	23–29	<22
ITR	>20	12–19	<11
KE	>30	23–29	<22
MI	>20	12–19	<11

[1] AB, amphotericin B; 5FC, 5-fluorocytosine; FLU, fluconazole; ITR, itraconazole; KE, ketoconazole; MI, miconazole.
[2] SDD, susceptible-dose-dependent.

Table 2. Antifungal susceptibilities of 100 isolates as determined by the reference broth microdilution method (BMM)

Organism (No. of isolates)	Antifungal agent [5]	MIC ranges[1] (μg ml^{-1})	No. S[2]	No. SDD [3]	No. R [4]
C. albicans (36)	AB	0.25–2	36	–	–
	5FC	<0.12–>64	34	1	1
	FLU	<0.25–32	23	13	–
	ITR	<0.03–4	25	11	–
	KET	<0.03–8	35	–	1
	MIC	<0.03–4	22	14	–
C. glabrata (10)	AB	0.5–2	10	–	–
	5FC	<0.125	10	–	–
	FLU	2–128	6	3	1
	ITR	0.125–2	5	5	–
	KET	0.03–1	9	1	–
	MIC	<0.03–0.125	10	–	–
C. krusei (15)	AB	1–2	15	–	–
	5FC	4–32	–	13	2
	FLU	16–128	–	10	5
	ITR	0.25–2	2	13	–
	KET	0.125–2	4	11	–
	MIC	0.25–2	1	14	–
C. lusitaniae (8)	AB	0.25–2	8	–	–
	5FC	<0.125–64	7	–	1
	FLU	0.25–64	6	1	1
	ITR	0.06–1	6	2	–
	KET	<0.03–0.5	7	1	–
	MIC	<0.03–4	6	2	–
C. parapsilosis (8)	AB	0.5–1	8	–	–
	5FC	<0.125	8	–	–
	FLU	0.5–4	8	–	–
	ITR	0.06–0.5	8	–	–
	KET	<0.03–0.25	8	–	–
	MIC	0.06–1	5	3	–
C. tropicalis (7)	AB	0.5–2	7	–	–
	5FC	<0.125	7	–	–
	FLU	0.25–2	7	–	–
	ITR	0.06–1	6	1	–
	KET	<0.03–0.125	7	–	–
	MIC	0.03–0.5	4	3	–
Cr.neoformans	AB	0.25–2	7	2	–
var.*neoformans* (9)	5FC	<0.125–>64	5	2	2
	FLU	1–32	8	1	–
	ITR	0.06–0.5	9	–	–
	KET	0.03–0.25	9	–	–
	MIC	0.03–0.5	8	1	–
Cr.neoformans	AB	0.25–2	7	–	–
var.*gattii* (7)	5FC	<0.125–2	5	2	–
	FLU	4–32	4	3	–
	ITR	<0.03–>16	6	–	1
	KET	0.03–8	6	–	1
	MIC	0.06–1	6	1	–

[1], Minimal inhibition concentration; [2], susceptible; [3], susceptible-dose-dependent; [4], resistant.
[5]AB, amphotericin B; 5FC, 5-fluorocytosine; FLU, fluconazole; ITR, itraconazole; KE, ketoconazole; MI, miconazole.

in susceptible (S), susceptible-dose-dependent (SDD) and resistant (R).

Ninety eight isolates were susceptible to amphotericin B with MIC_{100} ranging from 0.25 to 2.0 μg ml^{-1}.

For 5-fluorocytosine, 18 isolates were susceptible-dose-dependent with MIC_{50} ranging from 2 to 32 μg ml^{-1} and six were classified as resistant with $MIC_{50} > 32$ μg ml^{-1}.

For fluconazole, 31 isolates were susceptible-dose-dependent with MIC_{50} ranging from 8 to 64 μg ml^{-1} and seven were classified as resistant with $MIC_{50} > 64$ μg ml^{-1}. Five out of the seven resistant strains were *C. krusei* isolates.

For itraconazole, 32 isolates were susceptible-dose-dependent with MIC_{50} from 0.5 to 4 μg ml^{-1} and only one was classified as resistant with a $MIC_{50} > 4$ μg ml^{-1}.

For ketoconazole, 13 isolates were susceptible-dose-dependent with MIC_{50} from 0.5 to 4 μg ml^{-1} and two isolates were classified as resistant with $MIC_{50} > 4$ μg ml^{-1}.

For miconazole, 38 isolates were susceptible-dose-dependent with MIC_{50} from 0.5 to 8 μg ml^{-1} and none was classified as resistant.

In conclusion, we noticed that a majority of isolates were susceptible to the antifungal drugs for all the species, except for *C. krusei*.

The results obtained for the two varieties of *Cr. neoformans* demonstrated that there was no important difference between them and that there was no reason to evaluate them separately.

Table 3 gives the percentage agreement between the two commercial systems and the reference method. Overall agreement between the agar diffusion method and the reference broth microdilution method ranged from 90.4% for amphotericin B to 85% for 5-fluorocytosine, 80.4% for miconazole, 66% for itraconazole, 41.7% for fluconazole and 36% for ketoconazole. It ranged from 89.6% for *C. parapsilosis* to 80.1% for *Cr. neoformans*, 74.8% for *C. lusitaniae*, 62% for *C. albicans*, 61.8% for *C. tropicalis*, 53.3% for *C. glabrata* and 44.3% for *C. krusei*.

Overall agreement between the Fungitest and the reference broth microdilution method ranged from 100% for amphotericin B to 94.4% for ketoconazole, 91.4% for 5-fluorocytosine, 90.7% for fluconazole, 87.1% for miconazole and 76.7% for itraconazole. It ranged from 95.6% for *C. lusitaniae* to 93.8% for *C. parapsilosis*, 92% for *C. albicans*, 90.3% for *C. tropicalis*, 90% for *C. glabrata*, 86.5% for *Cr. neoformans* and 82.1% for *C. krusei*.

For a total of 600 tests (100 isolates × 6 antifungal drugs), we noticed 206 minor and 21 major discrepancies, namely 37.8% discrepancies between the agar diffusion method and the reference broth microdilution method, and 95 minor and four major discrepancies, namely 16.5% between the Fungitest and the reference broth microdilution method.

All the major discrepancies dealt with azoles and are mentioned in Tables 4 and 5.

Table 4 shows the 20 isolates of *C. albicans* (11), *C. parapsilosis* (1), *C. krusei* (2), *C. lusitaniae* (1) and *C. glabrata* (5) which were classified as susceptible with the reference broth microdilution method but were classified as resistant with the agar diffusion method, and the one isolate of *Cr. neoformans* which was classified as resistant with the reference broth microdilution method but was classified as susceptible with the agar diffusion method.

Table 5 shows the one isolate of *C. krusei* which was classified as susceptible with the reference broth microdilution method but was classified as resistant with the Fungitest, and the three isolates of *C. albicans* (1) and *Cr. neoformans* (2) which were classified as resistant with the reference broth microdilution method but were classified as susceptible with the Fungitest.

Table 3. Percentage agreement between the reference broth microdilution method (BMM), the agar diffusion method (ADM) and Fungitest (FT)

Species (No. of isolates)	Antifungal agents[1]	ADM/BMM	FT/BMM
C. albicans (36)	AB	100	100
	5FC	94	97
	FLU	14	94
	ITR	61	78
	KE	25	94
	MI	78	89
C. parasilosis (8)	AB	100	100
	5FC	100	100
	FLU	75	100
	ITR	100	100
	KE	100	100
	MI	63	63
C. krusei (15)	AB	33	100
	5FC	20	87
	FLU	33	73
	ITR	93	67
	KE	0	73
	MI	87	93
C. tropicalis (7)	AB	100	100
	5FC	100	100
	FLU	29	100
	ITR	57	71
	KE	14	100
	MI	71	71
C. lusitaniae (8)	AB	100	100
	5FC	100	100
	FLU	75	87
	ITR	37	87
	KE	50	100
	MI	87	100
C. glabrata (10)	AB	100	100
	5FC	100	100
	FLU	10	100
	ITR	20	40
	KE	0	100
	MI	90	100
Cr. neoformans (16)	AB	100	100
	5FC	81	56
	FLU	56	81
	ITR	94	94
	KE	63	94
	MI	87	94

[1] AB, amphotericin B; 5FC, 5-fluorocytosine; FLU, fluconazole; ITR, itraconazole; KE, ketoconazole; MI, miconazole.

Table 4. Major discrepancies between the reference broth microdilution method (BMM) and the agar diffusion method (ADM)

Organism	Antifungal agent[1]	Variation BMM→ADM	No. of isolates (total: 21)
C. albicans	FLU	S[2]→R[3]	2
	ITR	S→R	3
	KE	S→R	6
C. parapsilosis	FLU	S→R	1
C. krusei	KE	S→R	2
C. lusitaniae	FLU	S→R	1
C. glabrata	FLU	S→R	1
	ITR	S→R	1
	KE	S→R	3
Cr. neoformans	ITR	R→S	1

[1], FLU, fluconazole; ITR, itraconazole; KE, ketoconazole.
[2], susceptible; [3], resistant.

Table 5. Major discrepancies between the reference broth microdilution method (BMM) and the Fungitest (FT)

Organism	Antifungal agent[1]	Variation BMM→FT	No. of isolates (total: 4)
C. albicans	KE	R[2]→S[3]	1
C. krusei	ITR	S→R	1
Cr. neoformans	ITR	R→S	1
	KE	R→S	1

[1], KE: ketoconazole; ITR: itraconazole.
[2], resistant; [3], susceptible.

Discussion

We performed a total of 600 tests (100 isolates × six antifungal drugs) with the three methods and we noticed that the great majority of the isolates could be classified as susceptible to the antifungal drugs (Table 2). This was observed for all the species except for *C. krusei* which is a species well known for inducing therapeutical problems [6].

Table 3 gives the percentage agreement between the reference method and the two commercial systems and shows a good performance of Fungitest in comparison with Neo-Sensitabs. With the latter, we observed 37.5% discrepancies whereas with the Fungitest, there were only 16.5% discrepancies.

Neo-Sensitabs gives a good agreement for amphotericin B and 5-fluorocytosine except for *C. krusei*. In contrast, with the azoles, the level of agreement was completely different from one species to the other with in the case of *C. glabrata* isolates, zero percentage agreement for ketoconazole susceptibility.

The results were much better with the Fungitest. The percentage of agreement for amphotericin B and 5-fluorocytosine were, respectively, 100 and 91.4%. As for the azoles, there was around 90% agreement except for itraconazole which has agreement of 76.7%. In general, an excellent agreement was obtained for all the species with a lesser accordance for *Cr. neoformans* (86.5% agreement) and for *C. krusei* (82.1% agreement).

For both the Neo-Sensitabs and the Fungitest, there were minor discrepancies, respectively, of 34.3 and 15.8%.

With regard to this, we must remember that for the interpretation of the Neo-Sensitabs, a stricter interpretation was used. Indeed we chose the interpretation to be applied for yeasts from serious systemic infections with the consequence that isolates which would be classified as susceptible-dose-dependent with the interpretation to be applied for yeasts from superficial infections might be classified as resistant and that isolates which would be classified as susceptible became susceptible-dose-dependent isolates.

This could be the case in 153 out of the 206 individual tests giving minor discrepancies with the Neo-Sensitabs system.

As for the major discrepancies given in Tables 4 and 5, they always involved azoles: fluconazole, itraconazole and ketoconazole for the Neo-Sensitabs system and only itraconazole and ketoconazole for the Fungitest.

Candida tropicalis isolates were never involved with either of the two methods.

In Table 4, we can see that 20 out of the 21 major discrepancies (3.5%) concern susceptible isolates classified as resistant with Neo-Sensitabs and a resistant isolate of *Cr. neoformans* classified as susceptible with the commercial test.

For Fungitest, in contrast, we only had four major discrepancies (0.6%). In three out of the four cases, it concerned resistant organisms wrongly classified as susceptible and only in one case a susceptible *C. krusei* isolate classified as resistant with the Fungitest.

Finally, the results obtained for the Fungitest were compared to those obtain by Davey *et al.* 1998 [7].

For *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. tropicalis*, *C. lusitaniae*, *C. glabrata* and *Cr .neoformans*, those authors obtained, respectively, 95; 94.1; 81.6; 81.6; 93.3; 71 and 89.1% whereas our percentages agreement were for the same species 92; 93.8; 82.1; 90.3; 95.6; 90 and 86.5%.

Looking at the antifungal drugs, amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, ketoconazole and miconazole, those authors obtained, respectively, 100; 92.5; 78.1; 85.5; 82.4 and 80.7% whereas we obtained 100; 91.4; 90.7; 76.7; 94.4 and 87.1%.

As can be seen, there was agreement between the results obtained by the two research teams for all the species except for *C. tropicalis* and *C. glabrata*. As for the antifungal drugs, there was agreement for amphotericin B and 5-fluorocytosine. For the azoles, we obtained better results than them except for itraconazole. Our bad results were due to the *C. glabrata* isolates which gave only 40% agreement. Nevertheless, as this species was never involved in major discrepancies (cf. Table 5), we concluded that the low percentage agreement between the Fungitest and the reference method for *C. glabrata* was due to minor discrepancies.

In conclusion, even if we admit that, perhaps, the breakpoint concentrations selected for the antifungal agents included in the Fungitest need to be adjusted as was suggested by Davey *et al.* 1998 [7], we conclude that the percentage agreement between the Fungitest and the NCCLS reference method seems to be quite sufficient to recommend the Fungitest for routine testing.

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