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Comparative evaluation of Fungitest®, Neo-sensitabs® and Broth Microdilution Method for yeasts Susceptibility testing.

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Revised Abstract

The need of a simple and reliable method for routine yeasts susceptibility testing led us to evaluate two commercially available methods. We investigated the in vitro susceptibility of 96 clinical isolates (26 *C. albicans*, 32 *C. glabrata*, 4 *C. krusei*, 2 *C. tropicalis*, 2 *C. kefir*, 1 *S. cerevisiae*) to 6 drugs: flucytosine (FC), amphotericine (A), fluconazole (FZ), itraconazole (I), ketoconazole (K), miconazole (M), comparing two systems, Fungitest® (Sanofi (Pasteur) and Neo-sensitabs® (Rosco). A broth microdilution adaptation from the NCCLS M27-A procedure was used as reference method. Fungitest® consists of individually packed 16-wells microplates containing 6 drugs at 2 critical concentrations in buffered medium. Reading was performed after 24 and 48 h. Neo-sensitabs® is an agar diffusion method on Shadomy agar using antifungals tablets. Reading was made after 24h. For all strains Neo-sensitabs® was in concordance with NCCLS for FC (94%), A (98%), FZ(55%), I (53%) with p<.05. Fungitest® correlated with NCCLS method for all antifungals after 24 and 48 h (p>.05) with respectively 95/95% for FC, 100/100% for A, 80/76% for FZ, 81/55% for I, 93/75% for K and 88/81 for M. *C. glabrata* gave the poorest result with Neo-sensitabs® with 28% concordance for FZ, 39% for I; therefore this method can't be recommended for this species. Fungitest® concordance observed for was 55% for I after 48h. Our results suggest that Fungitest® is appropriate for routine yeasts susceptibility testing however itraconazole testing has to be improved.

Introduction

Infections caused by yeasts has followed the increasing number of immunocompromised patients (1). The emergence of antifungal drug resistance and the development of new available antifungal molecules has raised the need of simple and reliable methods of *in vitro* testing of antifungal drugs.

But several questions remain unsolved for the clinical laboratory. **Which method? Which strain? .Leading to which interpretation?**

Recently, the subcommittee of the National committee for clinical laboratory Standards (NCCLS), working on standardization of antifungal sucseptibility testing since 1986, published the M27-A document (5). Unfortunately, this methodology is time-consuming, labor-intensive, and unsuitable for most laboratories: an easiest reliable method would be much useful.

The aim of our study was to compare a new commercially available method, Fungitest®, and the agar diffusion method Neo-sensitabs® to the reference broth microdilution method (NCCLS, M27-A).

Methods

Clinical isolates: the strains were isolated from patients hospitalized in the university hospital of Liège, belgium. Sixty-five *Candida* isolates were distributed as follows: 25 *C. albicans*, 31 *C. glabrata*, 4 *C. krusei*, 2 *C. tropicalis*, 2 *C. kefir*, 1 *S. cerevisiae*.

ATCC strains: *C. albicans* ATCC 10231 and *C. glabrata ATCC 90030 were used as reference strains.*

Neo-sensitabs®: This agar diffusion method using antifungal tablets on Shadomy agar (2) was performed according the manufacturer's instructions. Plates were incubated at 35°C and read after 18-22 hours.

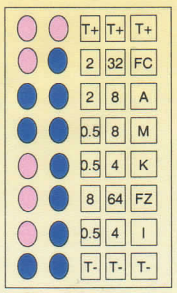

Figure 1.Fungitest® microplate (MICs, µg/ml).

Fungitest®:

This test consists of individually packed 16-wells microplates containing 6 drugs at two concentrations chosen as breakpoints (Figure 1).

The plates were incubated at 35°C and read after 48h as recommended by the manufacturer but also after 24h.

Statistical analysis: the degree of agreement between two methods was determined by the Cohen's kappa coeffficient.

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Results

Results obtained with the two methods are in agreement with the NCCLS procedure (Cohen's kappa coefficient, p<.05).

•FUNGITEST® : the

- •There is no significative difference between results obtained after 24 or 48h (Rank test, p>.05).
- •Results are in agreement with NCCLS method (Cohen's kappa coefficient, p<.05) and are reported in table I.
- •The least good results were obtained with itraconazole after 48h(55%).

•NEO-SENSITABS®:

- •Results are in agreement with NCCLS method and reported in Table 1.
- •The poorest susceptibility results are obtained with *C. glabrata* for two drugs: fluconazole (28%) and itraconazole (39%).

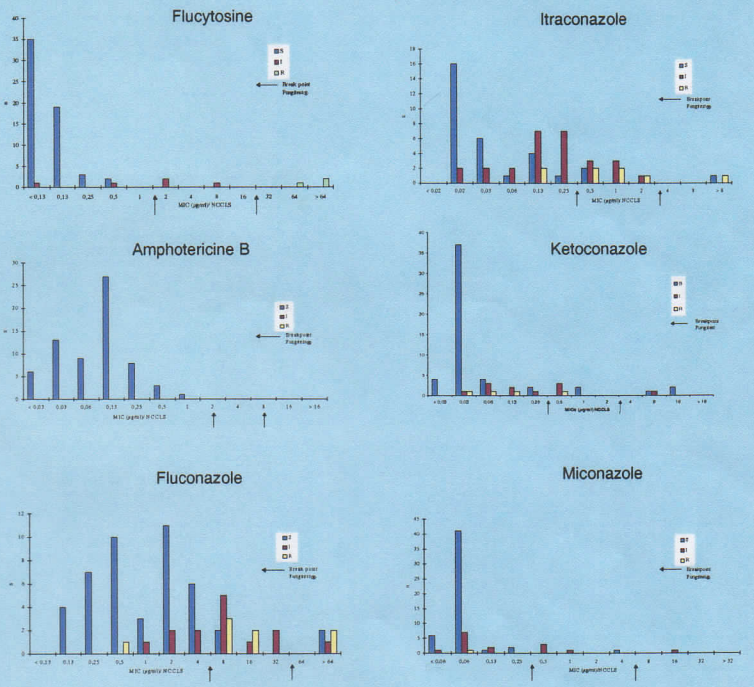

Figure 2. MICs values distribution of the 67 strains determined by following NCCLS in comparison to the Fungitest results. S: susceptible, I: intermediate, R: resistant, n: strains number.

	FC	A	FZ	I	K	M
Neo-sensitabs®	94	98	55	53	/	/
C. glabrat a(n=31)	100	97	28	39	/	/
C. albicans (n=28)	96	100	84	92	/	/
Fungitest® >24h	95	100	80	81	93	88
Fungitest® >48h	95	100	76	55	75	81
Fungitest® >48h *C. glabrata*(n=31)	100	100	77	75	96	93
Fungitest® >48h *C. albicans* (n=28)	100	100	88	92	100	96

Table I. Concordance (%) between Neo-sensitabs® and Fungitest® methods in comparison to NCCLS M27-A procedure.

Conclusions

1. Fungitest® and Neo-sensitabs® methods correlate with the reference NCCLS M27-A procedure. Both techniques could easely be performed by clinical laboratories.

2. Fungitest® :

There is no significative difference between the 24 hours and 48 hours incubation time but the concordance percentages are beter after 24h. Then this shorter incubation time (24h) could be chosen for this method.

We suggest modifications of the breakpoints used for itraconazole and we think that fluconazole testing could be also reviewed to enhance the correlation degree with the reference method.

3. Neo-sensitabs®:

Fluconazole and itraconazole testing by Neo-sensitabs® gave unreliable results and this method can't be recommended on Shadomy agar to test azoles susceptibility of *Candida* species, particularly for less susceptible species like *C. glabrata*.