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 56 clinical isolates (26 C. albicans, 12 C. glabrata, 1 C. tropicalis, 2 C. krusei, 1 S. cerevisiae), with 2 drugs: Amphotericin B (FZ), fluconazole (FC). The reference method (NCCLS, M27-A) was used as reference method. 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 initial concentrations in broth medium. Reading was performed after 24 or 48 h. Neo-sensitabs® was an agar diffusion method on Shadomy agar using antifungal tablets. Reading was made after 24 h. For all strains Neo-sensitabs® was in accordance with NCCLS for FC (p=0.05, 0.9999, 0.9999, 0.9999, 0.9999), Fungitest® correlated with NCCLS method for one antifungal after 24 and 48 h (p<0.05) with sensibility indices for FC, fluconazole (0.9999, 0.9999), itraconazole (0.9999, 0.9999). Concordance with M27-A criteria for Fungitest® was not achieved for C. krusei, C. tropicalis, C. parapsilosis. For Neo-sensitabs®, it was 55% for after 48h. Our results suggest that Fungitest® is appropriate for routine yeasts susceptibility testing because Fungitest® reading time 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 susceptibility testing since 1996, 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. kefuri, 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.

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.

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

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

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Conclusion

1. Fungitest® and Neo-sensitabs® methods correlate with the reference NCCLS M27-A procedure. Both techniques could easily 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 better 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 Fungitest® testing could be also reviewed to enhance the correlation degree with the reference method.

3. Neo-sensitabs®:

Flucytosine 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 less susceptible species like C. glabrata.

References

1. Davey K., Holmes A. Johnson E., Szekety A., Wamock D. Comparative evaluation of Fungitest and broth microdilution method, Fungitest®, and the agar diffusion method Neo-sensitabs® to The aim of our study was to compare a new commercially available i


3. 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.


7. Rijs A., Verduyn F., Meis J. The in vitro Susceptibility of yeasts blood culture isolates and CBS isolates: |


10. Fluconazole and itraconazole testing by Neo-sensitabs® gave unreliable results and this method

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

12. There is no significative difference between results obtained after 24 or 48h (Rank test, p>.05).

13. Results are in agreement with NCCLS method (Cohen's kappa coefficient, p<.05) and are reported in table I.

14. The least good results were obtained with Itraconazole after 48h(55%).

15. 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 (59%).