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Correlation between the procedure for antifungal susceptibility testing for *Candida* spp. of the European Committee on Antibiotic Susceptibility Testing (EUCAST) and four commercial techniques

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ABSTRACT

The correlation between results obtained with the European Committee on Antibiotic Susceptibility Testing (EUCAST) antifungal susceptibility testing procedure (document 7.1) and four commercial systems was evaluated for a collection of 93 clinical isolates of *Candida* spp. Overall, agreement between the EUCAST procedure and the Sensititre YeastOne and Etest methods was 75% and 90.4%, respectively. The correlation indices ($p < 0.01$) between the EUCAST and commercial methods were 0.92 for Sensititre YeastOne, 0.89 for Etest, − 0.90 for Neo-Sensitabs, and 0.95 for Fungitest. Amphotericin B MICs obtained by Sensititre YeastOne were consistently higher than with the EUCAST method and, although very major errors were not observed, 91% of MICs were misclassified. Amphotericin B- and fluconazole-resistant isolates were identified correctly with Sensititre YeastOne, Etest and Fungitest. Neo-Sensitabs identified amphotericin B-resistant isolates, but misclassified > 5% of fluconazole-resistant isolates as susceptible. The commercial methods, particularly Etest and Fungitest, appeared to be suitable alternatives to the EUCAST procedure for antifungal susceptibility testing of clinical isolates of *Candida*.

Keywords Amphotericin B, antifungal testing, *Candida* spp., EUCAST, fluconazole, susceptibility testing

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INTRODUCTION

The Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing (AFST-EUCAST) has developed a standard broth microdilution procedure for the determination of antifungal MICs for fermentative species of yeasts [1]. This standard is based on the NCCLS reference procedure described in document M27-A2 [2], but includes some modifications to allow for automation of the method and to permit the incubation period to be shortened from 48 to 24 h. A multicentre evaluation has demonstrated that the EUCAST procedure for antifungal susceptibility testing is a reproducible method, with 94% agreement between laboratories [3]. In addition, a two-laboratory study evaluated the correlation between the NCCLS M27-A and EUCAST microdilution procedures with a panel of 109 bloodstream isolates of *Candida* spp., tested against amphotericin B, flucytosine, fluconazole and itraconazole, and demonstrated an overall agreement of 92% and a correlation coefficient of 0.90 ($p < 0.01$) [4]. However, standard reference procedures are generally not practical for use in routine clinical laboratories, since they involve rather complex methods for susceptibility testing. Many microbiologists prefer to use other systems with advantages such as ease of performance, economy or more rapid results. Several techniques based on agar diffusion or use of a colorimetric oxidation–reduction indicator have been developed. Some of these techniques are available commercially, and are rapid and simple alternatives to the procedures

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developed by either the EUCAST or NCCLS [5–8].

A significant use of reference procedures is to provide a standard from which other methods can be developed and compared. Many studies have analysed the correlation between the NCCLS procedure and various commercially available systems [5–24], including some suitable for susceptibility testing of *Candida* spp. However, only one study [25] has compared the EUCAST procedure with commercial systems. Therefore, the aim of the present study was to analyse results obtained with the EUCAST procedure and four commercially available systems for a collection of clinical isolates of *Candida* spp.

MATERIALS AND METHODS

Fungi

A collection of 93 non-duplicate clinical isolates of *Candida* spp. was tested. Most ($n = 49$) were obtained from blood cultures, while the remainder were from deep-site specimens ($n = 18$) or oropharyngeal exudates ($n = 26$). The isolates were selected to represent broad in-vitro susceptibility ranges. Each isolate was sent to the Centro Nacional de Microbiología, Madrid, Spain for identification or antifungal susceptibility testing. Isolates were identified by routine microbiological techniques (Table 1) and were maintained at −70°C. *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as quality control strains in each set of experiments.

Reference susceptibility testing

Standard powders of amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole and ketoconazole were supplied by Sigma Aldrich Quimica (Madrid, Spain), Pfizer (Madrid, Spain) and Janssen (Madrid, Spain). MICs were determined with the AFST-EUCAST reference procedure (document 7.1) [1]. In brief, testing was performed with RPMI-1640 medium supplemented with glucose 2% w/v, an inoculum size of 10^5 CFU/mL and flat-bottom microdilution plates [26]. MIC endpoints were determined spectrophotometrically after 24 and 48 h. For amphotericin B, the MIC endpoints were defined as the lowest drug concentration that resulted in a reduction in growth of ≥90% compared with that of a drug-free control well. For flucytosine and azoles, the MIC endpoint was defined as a 50% reduction in optical density.

Commercial techniques

Four commercial methods were investigated: Sensititre YeastOne panel (Trek Diagnostic Systems, East Grinstead, UK); Etest strips (AB Biodisk, Solna, Sweden) on RPMI-1640/glucose 2% w/v agar; Fungitest panel (Bio-Rad, Madrid, Spain); and the agar diffusion method with Neo-Sensitabs (A/S Rosco, Taastrup, Denmark). Susceptibility testing, reading and interpretations of the results were performed in accordance with the manufacturers' instructions. Susceptibility testing was performed in triplicate on three separate days.

Data analysis

Both on-scale and off-scale results obtained by the EUCAST reference procedure were included in the analysis. The low off-scale MICs were left unchanged, and the high off-scale MICs were converted to the next highest concentration. The reproducibility of the results obtained with the EUCAST technique and the commercial methods was evaluated by using distinct statistical tests, depending on the commercial technique investigated, as test results were expressed in different units (i.e., Sensititre YeastOne and Etest results were expressed in mg/L; Fungitest results in susceptible, intermediate and resistant categories; and Neo-Sensitabs results in inhibition (cm) diameters).

The reproducibility between the EUCAST results and MICs obtained by Sensititre YeastOne and Etest was calculated by determining the percentage of agreement between MICs. Agreement was defined as a discrepancy in MICs of no more than two doubling dilutions. Results obtained by Etest were adjusted to the nearest doubling dilution, up or down, as tested by the EUCAST method. In addition, the correlation between results was evaluated by using the intra-class correlation coefficient (ICC), which was expressed to a maximum value of 1 and with a 95% CI. In order to approximate a normal distribution, the MICs were transformed to $\log_2$ values. A p value of < 0.01 was considered to be statistically significant. The ICC is a reverse measurement of the variability of the counting values and was calculated using the formula ICC = (group mean square − error mean square)/(group mean square + error mean square); it thus has a maximum value of 1 if there is a perfect correlation and a minimum value of −1 if there is a complete absence of correlation. The ICC evaluates the correlation between values offering statistical

Table 1. Results obtained with the EUCAST procedure for *Candida* isolates included in the study

	MIC values (mg/L)						
Species	**No. of isolates**	**Amphotericin B**	**Flucytosine**	**Fluconazole**	**Itraconazole**	**Voriconazole**	**Ketoconazole**
C. albicans	21	0.03–2.0	0.12–128.0	0.12–128.0	0.01–16.0	0.01–16.0	0.01–4.0
C. tropicalis	21	0.03–8.0	0.06–1.0	0.12–128.0	0.01–16.0	0.01–16.0	0.01–8.0
C. parapsilosis	12	0.03–1.0	0.12–0.50	0.12–2.0	0.01–0.12	0.01–0.03	0.01–0.06
C. glabrata	10	0.06–0.25	0.12–0.25	2.0–64.0	0.25–0.50	0.03–0.50	0.06–2.0
C. krusei	10	0.03–0.25	2.0–16.0	32.0–128.0	0.06–0.25	0.25–1.0	0.25–1.0
C. lusitaniae	11	0.03–1.0	0.12–0.25	0.12–64.0	0.01–0.12	0.01–0.50	0.01–1.0
C. guilliermondii	8	0.03–1.0	0.12–1.0	2.0–64.0	0.12–2.0	0.06–2.0	0.03–2.0
Total	93	0.03–8.0	0.12–128.0	0.12–128.0	0.01–16.0	0.01–16.0	0.01–8.0