 10.1111/j.1469-0691.2006.01419.x

Comparison of the EUCAST-AFST broth dilution method with the CLSI reference broth dilution method (M38-A) for susceptibility testing of posaconazole and voriconazole against *Aspergillus* spp.

E. Chryssanthou[1] *and M. Cuenca-Estrella*[2]

[1]Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden and [2]Servicio de Micologia, Centro Nacional de Microbiologia, Instituto de Salud Carlos III, Majadahonda, Spain

ABSTRACT

The susceptibilities of 40 clinical isolates of *Aspergillus* spp. (*Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*) were determined for posaconazole and voriconazole by the CLSI M38-A and EUCAST-AFST broth dilution methods. Where a discrepancy was observed between the methods, the EUCAST method tended to give higher MIC values. Overall, the level of agreement was 92.5% and the intra-class correlation coefficient was >0.90.

Keywords *Aspergillus* spp., broth dilutions, posaconazole, susceptibility testing, voriconazole

Original Submission: 14 October 2005; **Revised Submission:** 14 December 2005; **Accepted:** 5 January 2006

Clin Microbiol Infect 2006; 12: 901–904

INTRODUCTION

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is a standing committee organised by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the national breakpoint committees of Europe. EUCAST was set up to harmonise breakpoints of antimicrobial agents and to standardise susceptibility testing in Europe so that comparable results and interpretations, both in clinical practice and in antimicrobial resistance surveillance, can be achieved. EUCAST has tasked the Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) with preparing guidelines for testing antifungal agents against various fungal species.

EUCAST-AFST has already developed a standard broth microdilution procedure for the determination of antifungal MICs for fermentative species of yeasts (document E.Dis.7.1) [1]. This standard is based on the reference procedure described in document M27-A2 by the CLSI, formerly the NCCLS [2]. In addition, EUCAST-AFST has developed a method for determining broth dilution MICs for *Aspergillus* spp. This is a microdilution method that is intended to produce concordant results with the CLSI reference method for antifungal susceptibility testing of filamentous fungi (document M38-A) [3]. The EUCAST method includes some modifications based on previous studies: (1) RPMI-1640 medium supplemented with glucose 2% w/v (RPMI 2% G) is used as the assay medium; (2) the inoculum is prepared by counting conidia in a haemacytometer; and (3) an inoculum size of 1×10^5 to 5×10^5 conidia/mL is used [4–6].

The agreement and correlation between the various methods has not been evaluated previously for any antifungal agent. The aim of the present study was to investigate the concordance obtained between the CLSI and EUCAST standard methods when determining the MICs of voriconazole and posaconazole for clinical isolates of *Aspergillus* spp.

Corresponding author and reprint requests: E. Chryssanthou, Department of Clinical Microbiology, Karolinska University Hospital, SE-141 86 Stockholm, Sweden
E-mail: Erja.Chryssanthou@karolinska.se

MATERIALS AND METHODS

Test isolates

A panel of 40 clinical isolates belonging to four different *Aspergillus* spp. (ten each of *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus terreus*) was used. The isolates were stored at −70°C and were subcultured twice on

potato dextrose agar slants at 35°C for 2–3 days before testing. Two reference strains, *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019, were included as quality controls, with MICs being within the accepted limits by both methods on all occasions [7].

Antifungal agents

Posaconazole (Schering-Plough, Kenilworth, NJ, USA) and voriconazole (Pfizer, New York, NY, USA) were provided in the form of powders. Both drugs were dissolved in 100% dimethylsulphoxide to make stock solutions of 3.2 g/L, which were stored at −70°C until required. The range of posaconazole and voriconazole concentrations tested was from 0.015 to 8 mg/L.

EUCAST broth dilution method

The assay medium used was RPMI-1640 (Sigma-Aldrich, Stockholm, Sweden), with glutamine but without bicarbonate, buffered to pH 7.0 with 0.165 M morpholinopropanesulphonic acid (Sigma-Aldrich) and supplemented with glucose 2% w/v. Culture medium was prepared as a double-strength solution and sterilised by filtration. Both drugs were diluted in the assay medium, dispensed into flat-bottomed microdilution trays (Corning Costar Europe, Badhoevedorp, The Netherlands) and frozen at −70°C until required. The plates contained 100 μL of two-fold serial dilutions of the drugs. Two drug-free medium wells for growth and sterility controls were used.

Inoculum suspensions were prepared by covering the surface of *Aspergillus* colonies with 5 mL of sterile water containing Tween-20 0.1% v/v and probing with a sterile loop. The conidia were transferred to a sterile tube, shaken vigorously, and then adjusted by microscopic enumeration with a cell-counting haemacytometer to provide a suspension of 1×10^6 to 5×10^6 conidia/mL [4]. These suspensions were diluted 1:10 in water, and each well was inoculated with 100 μL of the corresponding conidial inoculum suspension.

CLSI reference method

Susceptibility testing was performed by following the CLSI M38-A guidelines [3]. Dilutions of both antifungal agents were prepared with RPMI-1640 medium that was buffered to pH 7.0 with morpholinopropanesulphonic acid. The drug dilutions were dispensed into round-bottomed microdilution plates (Corning Costar) and frozen at −70°C until required. The conidial inoculum suspensions were adjusted to an optical density of 80–82%, and diluted 1:50 in RPMI; each well was then inoculated with 100 μL of the corresponding suspension.

Endpoint determination by EUCAST and CLSI procedures

MIC endpoints were defined for both drugs as the lowest drug concentration that caused 100% growth inhibition. The MIC readings were performed with the aid of a concave mirror after incubation for 48 h at 35°C. All experiments were repeated twice on different days.

Analysis of results

Both on-scale and off-scale results were included in the analysis. The reproducibility between the EUCAST and CLSI results was calculated by determining the percentage of agreement between MICs. Agreement was defined as discrepancies between MICs of no more than ±2 dilutions. In addition, the correlation between the two sets of results was calculated by using the intra-class correlation coefficient (ICC), which was expressed to a maximum value of 1 with a 95% CI. In order to approximate a normal distribution, the MICs were transformed to $\log_2$ values, with $p < 0.01$ considered to be of statistical significance. The ICC is a reverse measurement of the variability in the counting values. All statistical analyses were performed with SPSS software v.13.0 (SPSS Inc., Madrid, Spain).

RESULTS

MIC data

The MIC ranges and geometric means obtained by the CLSI and EUCAST methods for posaconazole and voriconazole against 40 *Aspergillus* spp. isolates are summarised in Table 1. All the isolates tested grew well both in round-bottomed (CLSI) and flat-bottomed (EUCAST) microtitre plates, giving detectable endpoints within 48 h. A broad range of on-scale MICs was observed with both antifungal agents. *A. niger* was the most susceptible species, while *A. flavus* had higher

Table 1. Posaconazole and voriconazole susceptibilities of 40 isolates of *Aspergillus* spp. as determined by the CLSI and EUCAST broth microdilution methods

		MIC (mg/L)			
		Posaconazole		Voriconazole	
Species	Method	Range	Geometric mean	Range	Geometric mean
A. fumigatus	CLSI	0.015–1	0.08	0.06–1	0.52
	EUCAST	0.03–1	0.12	0.25–2	0.66
A. flavus	CLSI	0.015–0.25	0.11	1–2	1.36
	EUCAST	0.125–1	0.24	1–2	1.74
A. niger	CLSI	0.015–0.25	0.04	0.03–2	0.48
	EUCAST	0.015–0.25	0.08	0.125–2	0.57
A. terreus	CLSI	0.015–0.125	0.04	0.5–1	0.90
	EUCAST	0.06–0.125	0.08	1–2	1.36

CLSI, Clinical Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Suseptibility Testing.