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EUCAST Technical Note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia–forming moulds

*Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST)**

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INTRODUCTION

Antifungal susceptibility tests are performed on those fungi causing disease, especially if they belong to a species exhibiting resistance to commonly used antifungal agents. Antifungal susceptibility testing is also important for resistance surveillance, epidemiological studies and for comparing the *in vitro* activity of new and established agents.

Reference methods for antimicrobial susceptibility testing rely on incremental dilution of the antimicrobial agents to determine the minimum inhibitory concentrations (MICs) and are mainly used to establish the activity of a new agent, to confirm the susceptibility of microorganisms that yield equivocal results in routine tests, or to determine their susceptibility when routine tests are either unreliable or not readily available. There is also a need for standardized methods for determining the *in vitro* susceptibilities of both new and established antifungal agents against clinical isolates of filamentous fungi as there is an increasing number of agents to choose from for treating invasive mould disease, and resistance to antifungal agents in some species has been documented [1–9].

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The Subcommittee on Antifungal Susceptibility Testing (AFST) of the European Committee for Antimicrobial Susceptibility Testing (EUCAST) has developed a broth dilution methodology for determining the antifungal susceptibility of conidia-forming moulds that cause clinically significant invasive fungal disease. This technical note is based on the EUCAST method and the definitive document (E.DEF 9.1) is available in full on the EUCAST website at http://www.eucast.org.

SCOPE

The standard method described in the definitive document provides a valid method for testing the susceptibility by determining the MICs of antifungal agents for moulds able to produce conidia. These MICs show the activity of a given antifungal drug under defined test conditions, and can be used for patient management when other factors, such as pharmacokinetics, pharmacodynamics and resistance mechanisms, are taken into account. The MIC permits moulds to be categorized as ''susceptible'' (S), ''intermediate'' (I), or ''resistant'' (R) to an antifungal drug. In addition, MIC distributions can be used to define wild-type or non-wild-type fungal populations.

The method described in the definitive document is intended to provide a valid, easy, rapid and economic method for testing the susceptibility of moulds to antifungal agents and to facilitate an acceptable degree of conformity, e.g. agreement within specified ranges, among laboratories. Since technical factors are of utmost importance, the standard focuses on testing conditions including inoculum preparation and size, the composition of the growth medium and incubation temperature and duration.

TEST PROCEDURES

Test procedures are similar to those published in the document entitled ''Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts'' [10]. The medium recommended is RPMI 1640 supplemented with glucose to a final concentration of 2%. The preparation of stock and working solutions of antifungal agents and the preparation and storage of microdilution plates is identical to that described in the method for fermentative yeasts [10]. However, inoculum preparation is performed by counting spores in a haemocytometer chamber instead of adjusting the optical density of the culture using a spectrophotometer as this would require separate standardization for each species to compensate for differences in the size and colour of the spores [11–13]. In addition, the endpoints are read visually by recording the degree of growth for each well using a viewing mirror. Two different endpoints are obtained, the MIC and the minimum effective concentration (MEC). The MIC is recorded for polyenes, azoles and terbinafine whereas the MEC is reserved for the echinocandins – caspofungin, micafungin and anidulafungin. The MIC is defined as the lowest concentration of drug that yields no growth whereas the MEC is the lowest concentration of drug that results in macroscopic changes in filamentous growth to microcolonies or granular growth when compared with growth control wells. Reading the MEC requires a degree of expertise which can be acquired by examining under the microscope a small volume removed from each of the wells of the microdilution plate.

INTERPRETATION OF RESULTS

Interpretation of mould MICs is challenging and interpretative breakpoints have yet to be established. The clinical utility and relevance of testing moulds also remains uncertain. Most of the information available is derived from invasive aspergillosis, which is predominantly caused by *Aspergillus fumigatus*.

Amphotericin B

There is no evidence of a clear correlation between the MIC of amphotericin B and outcome of treatment [14–16]. The most useful information is often derived from complete identification of the fungus. Experience indicates that for most *Aspergillus* spp., MICs of amphotericin B are clustered between 0.5 and 2 mg/L. However, isolates of *A. terreus* may exhibit higher MICs [3,17] and, in general, infections due to this species are associated with a poorer response to amphotericin B compared with that found for infections caused by more common species of *Aspergillus* [3]. Therefore, high MICs of amphotericin B should be taken into consideration and alternatives to amphotericin B should be considered when an invasive fungal disease is due to *A. terreus*.

Itraconazole

More is known about the detection of azole resistance than about a relationship between MIC and outcome [1,7]. Two isolates were collected from patients who did not respond to therapy with itraconazole. These isolates were resistant to itraconazole in a murine model of invasive aspergillosis and had elevated itraconazole MICs (MIC ≥ 8 mg/L) [1]. In addition, several studies have demonstrated that mutations in the *cyp*51A gene are associated with high MICs of itraconazole [2,4–6]. Recently, the itraconazole wild-type population of *A. fumigatus* and the corresponding epidemiological cut-off has been described [18].

Voriconazole

There is no evident correlation between the MIC of voriconazole and the outcome of treatment. However, as some isolates with high MICs of itraconazole and mutations in the *cyp*51A gene also exhibited elevated MICs of voriconazole, cross resistance cannot be discounted and should be taken into consideration when choosing therapy [2,4–6]. Recently, the voriconazole wild-type population of *A. fumigatus* and the corresponding epidemiological cut-off has been described [18].

Posaconazole

It is not known whether there is any correlation between the MIC of posaconazole and the outcome of treatment. However, as with voriconazole, isolates with high MICs of itraconazole and mutations in the *cyp*51A gene may also exhibit

elevated MICs of posaconazole, so cross resistance should be considered [2,4–6]. Recently, the posaconazole wild-type population of *A. fumigatus* and the corresponding epidemiological cut-off has been described [18].

Caspofungin

There is no indication of any correlation between either the MIC or the MEC and outcome of treatment with caspofungin.

Micafungin

There are no data available to suggest any correlation between the MIC and outcome of treatment with micafungin.

QUALITY CONTROL

The definitive document provides guidelines to assure the quality of the results by employing control strains as described in detail by the CLSI [19].

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