



Letter to Editor



Factors to Consider While Ordering Antifungal Susceptibility Testing in Clinical Practice

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KEYWORDS: Antifungal; Clinical breakpoint; Candida, Aspergillus

Dear Editor,

In recent decades, there has been a significant rise in the population susceptible to invasive fungal infections (IFIs). This surge correlates with the expanding population with immunocompromised status or the administration of immunosuppressive drugs, as well as those undergoing treatment in intensive care units. It is approximated that there are 1.5 to 5 million distinct fungal species, among which over 300 are identified as agents of infections in humans.¹ These numbers are anticipated to climb further as molecular techniques become more widespread in detecting and diagnosing novel fungal diseases, alongside the continuous growth in the susceptible population. Regrettably, access to antifungal susceptibility testing (AFST) remains restricted in Asia/Pacific, with only 38.3% of acute care hospitals providing AFST internally or through a reference laboratory.² Because of this, clinicians must recognize various restricted situations where AFST will provide the most significant value. Commonly employed methods for assessing in vitro phenotypic antifungal activity include agar diffusion and broth dilution techniques, which measure the ability of a particular drug to inhibit the growth of fungi over a range of concentrations. The readout is the minimum inhibitory concentration (MIC), which corresponds to the lowest

antifungal concentration that inhibits the organism's growth. The Clinical and Laboratory Standards Institute (CLSI) is a major international organization that guides clinical laboratories globally on performing standardized antifungal drug susceptibility testing. While similar, these guidelines exhibit methodological and interpretative differences in breakpoints tailored to the drug dosages used in specific regions.³ While ordering AFST, the clinician must ensure that the testing laboratory is enrolled in an external quality assurance program and validates the accuracy and reliability of susceptibility data reported by microbiology laboratories.

AFST should be conducted on samples obtained from normally sterile sites, such as blood, cerebral spinal fluid, joint fluid, pleural fluid, pericardial fluid, and ocular tissue. The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) has recommended AFST for all *Candida* isolates obtained from blood and other deep-seated sites. Additionally, they highlighted the importance of AFST for isolates obtained from patients experiencing treatment failure, isolates from uncommon species (including *C. auris*), and isolates from species prone to developing

Citation: Garg R, Agarwala P. Factors to Consider While Ordering Antifungal Susceptibility Testing in Clinical Practice. *JASPI*. 2024;2(1):41-43. DOI: [10.62541/jaspi021](https://doi.org/10.62541/jaspi021)

resistance.³ The Infectious Diseases Society of America (IDSA) has also advocated for antifungal susceptibility testing, particularly for azoles, for all bloodstream and clinically significant *Candida* isolates. They further suggested considering echinocandin testing for patients infected with either *Nakaseomyces glabrata* (formerly *C. glabrata*) or *C. parapsilosis*.⁴

Both IDSA and ESCMID do not advise routine AFST for all *Aspergillus spp.* isolates due to the variability in susceptibility to antifungal therapies among many species.^{3,4} However, a growing prevalence of cross-resistance to azole antifungals is observed in *A. fumigatus* isolates, leading to unfavorable patient outcomes. Consequently, AFST is recommended when *A. fumigatus* is isolated, particularly in regions where azole resistance exceeds 10%.⁵

In some situations, susceptibility testing is unnecessary, mainly when dealing with intrinsic resistance (IR). IR refers to inherent or innate antimicrobial resistance that is not acquired, and it is indicated by elevated MIC in a wild-type population for most or all representatives of a species against a specific antimicrobial agent. As per the CLSI document (M100), IR is identified using a 3% threshold.⁶ So, IR is established when at least 97% of isolates from a specific species are not inhibited at the highest concentration of the particular antifungal drug in vitro testing. As a result, susceptibility testing becomes redundant because resistance is highly prevalent. *Pichia kudriavzevii* (formerly *C. krusei*) and

fluconazole are notable examples of intrinsic antifungal resistance.⁷

However, in specific interactions between a few pathogenic organisms and antifungals, even though conditions for IR are not fulfilled, recommendations still advise against conducting AFST and using particular antifungals to treat infections (Table 1). For example, with amphotericin B, this caution applies to *Clavispora lusitaniae* (formerly *Candida lusitaniae*) and *Aspergillus terreus*.

In the case of infection by *C. lusitaniae*, both IR and acquired resistance can happen after exposure to amphotericin B in clinical settings. Regarding *A. terreus* against amphotericin B drug, MICs are typically high, usually exceeding one µg/mL. Although *A. terreus* does not strictly meet the criteria for IR to amphotericin B, clinical studies have reported unfavorable outcomes, leading to the recommendation against using amphotericin B for treating invasive aspergillosis caused by *A. terreus*.⁸

In conclusion, understanding the local susceptibility of antifungals to pathogenic organisms at hospitals is crucial for selecting empirical antifungal therapy when specific culture results indicate positivity in correlation with clinical manifestations, or there is suspicion of IFIs. The selection of antifungal treatment, knowledge on IRs, and the surveillance of resistance rates are particularly significant where ASFT can offer substantial value, especially in resource-constrained countries.

Table 1: In vitro antifungal activity and spectrum of antifungal drugs against Pathogenic Yeast and Molds

Species	AmB	Flu	Itra	Vori	Posa	Isavu	Echino
<i>C. albicans</i>	√	√	√	√	√	√	√
<i>Nakaseomyces glabrata</i> (formerly <i>C. glabrata</i>)	√	√/R	√/R	√/R	√/R	√/R	√
<i>Pichia kudriavzevii</i> (Formerly <i>C. krusei</i>)	√	X	X	√	√	√	√
<i>C. parapsilosis</i>	√	√	√	√	√	√	↑ MIC
<i>C. tropicalis</i>	√	√	√	√	√	√	√
<i>C. auris</i>	√/R	√/R	√/R	√/R	√/R	√/R	√
<i>Aspergillus spp.</i>	√ (not <i>A. terreus</i>)	X	√	√	√	√	√ (in combination)
<i>Mucorales</i>	√	X	X	X	√	√	X
<i>Cryptococcus and Dimorphic fungi</i>	√	√	√	√	√	√	X
<i>Fusarium spp.</i>	√/R	X	X	√/R	√/R	√/R	X
<i>Scedosporium spp.</i>	X	X	X	√/R	√/R	√/R	X
<i>Lomentospora spp.</i>	X	X	X	√/R	X	X	X

Footnotes: X = No In-vitro activity; √ = In-vitro activity; √/R = resistance is common.

Abbreviations: AmB = Amphotericin B formulations; Flu = fluconazole; Itr = itraconazole; Vori = voriconazole;

Posa = posaconazole; Isavu = isavuconazole; Echino = echinocandins (caspofungin, micafungin, anidulafungin)

ACKNOWLEDGEMENT

None

CONFLICT OF INTERESTS STATEMENT

The authors declare no conflict of interest

SOURCE OF FUNDING

None

ETHICS STATEMENT INCLUDING PATIENT CONSENT

Not applicable

AUTHORS' CONTRIBUTIONS

RG: Writing the draft; Resources

PA: Conceptualization; Supervision; Review & editing

AI DECLARATION

No AI tool was used to generate research data, perform data analysis, interpret findings, formulate scientific conclusions, or make editorial decisions. All AI-assisted content was critically reviewed, verified, and revised by the authors. The authors take full responsibility for the accuracy, originality, integrity, and final content of the manuscript.

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