Update from the Laboratory

Clinical Identification and Susceptibility Testing of Fungi and Trends in Antifungal Resistance

Mohammad T. Albataineh, MD, PhD, Deanna A. Sutton, PhD, Annette W. Fothergill, MA, MBA, MT, Nathan P. Wiederhold, PharmD*

KEYWORDS

• Fungal identification • Antifungal susceptibility testing • Antifungal resistance

KEY POINTS

- Proper identification of fungi to the species level requires more than morphologic/phenotypic assessment. Molecular and proteomic assays are now needed and are often used in combination with classic techniques.
- Antifungal susceptibility testing is a useful tool to provide information to clinicians to help guide therapy. Several commercially available assays, in addition to the Clinical and Laboratory Standards Institute (CLSI) and the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution methods, are available for testing against yeast. Clinical breakpoints have not been established, however, for each antifungal and each fungal species.
- Echinocandin resistance in *Candida glabrata* species is increasing at some US institutions. Many of these isolates may also be resistant to fluconazole. In addition, azole-resistant *Aspergillus fumigatus* is a growing concern worldwide. Treatment options against these resistant fungi are limited.

INTRODUCTION

Invasive fungal infections are associated with significant morbidity and mortality, because these infections are often difficult to diagnosis and treat. Fungi historically associated with invasive disease in humans include yeast within the genera *Candida,*

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Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229, USA
* Corresponding author.
E-mail address: wiederholdn@uthscsa.edu

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Cryptococcus, and *Trichosporon*; the dimorphic fungi *Blastomyces dermatitidis, Coccidioides immitis/Coccidioides posadasii,* and *Histoplasma capsulatum*; molds, including limited species within the genera *Aspergillus, Fusarium,* and *Scedosporium*; and members of the order Mucorales. Over the past 2 decades there has been a significant increase in the number of fungal species associated with invasive disease in humans. Factors that have contributed to this increase include an increase in the number of immunocompromised patients at high risk for invasive fungal infections, such as HIV-AIDS patients, those receiving immunosuppressive chemotherapy for malignancies, and solid organ transplant recipients. Improvements in diagnostic assays and the clinical recognition of patients with risk factors for such infections, as well as improvements in the tools used to identify fungal species, have shortened the time to acquire a proper diagnosis. The treatment of invasive fungal infections can be challenging due to the limited number of clinically available drugs, in addition to drug interactions and toxicities associated with certain classes of antifungals that may limit their effectiveness. Although antifungal resistance has not reached the level of antibiotic resistance seen with some bacterial species, recent studies and publications indicate that this may be an emerging problem with some invasive fungal pathogens. The objectives of this article are to review the methods used for fungal identification in the clinical setting and to discuss methods for susceptibility testing and recent trends in antifungal resistance, including common pathogens that may develop multidrug resistance.

FUNGAL IDENTIFICATION IN THE CLINICAL SETTING

Identification by Morphologic/Phenotypic Characteristics and DNA Sequence Analysis

The identification of fungi in the clinical laboratory has historically relied on morphologic characteristics and physiologic traits. The description of the colony appearance and the microscopic features of the organism, including the reproductive structures, has been the hallmark for fungal identification for many years. Certain phenotypic/physiologic traits are also combined with the morphologic features to obtain the identities of fungal isolates. These include, but are not limited to, the ability of an organism to grow at certain temperatures, tolerance to cycloheximide and benomyl, nitrate assimilation, tolerance to different concentrations of sodium chloride, growth on trichophyton agar, and growth on urea agar.[1–4] Many of these phenotypic/physiologic assays are still used to identify an organism to the genus and possibly species level in clinical microbiology and reference mycology laboratories. Identification to the species level is clinically important because it provides clinicians with information that may be useful in the management of patients and guidance of antifungal therapy. Early identification and the initiation of appropriate therapy have been shown to influence patient outcomes whereas delaying appropriate therapy can be detrimental.[5–8] Identification to the species level is important in helping to guide appropriate therapy, because some fungi are intrinsically resistant to certain drugs. Furthermore, some species within the same species complex may have different antifungal susceptibility profiles and this can influence treatment.[9–11] Identification by morphologic/physiologic characteristics alone can be time consuming, and results may not be available in a timely fashion for clinical decisions. Morphologic identification can also be fraught with errors if done by those without proper training and experience. In addition, the morphologic features of fungi may be variable.[12,13] Different factors can affect these features, including the media used for subculture or exposure to external stressors, such as antifungal agents prior to recovery from clinical specimens that can often occur in patient groups at high risk for invasive fungal infections where empiric or preemptive antifungal therapy is often used.