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Commentaries Article

Microbiology Section

Fungal Infections in Intensive Care Unit: Challenges in Diagnosis

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ABSTRACT

Opportunistic fungi have emerged as serious threats particularly among patients of Intensive Care Unit (ICU) over the past few decades. The patients in ICU present special challenges, they are at risk for a variety of complications including the development of new infections. Recent advances in medical technology have not only increased the survival rate of critically ill patients admitted to ICU but at the same time have led to an increase in the number of life-threatening infections due to opportunistic fungi. The most frequently encountered fungal infections are caused by *Candida albicans, Cryptococcus neoformans*, and *Aspergillus fumigatus*. There are reports about increase in the prevalence of infections caused by non-albicans *Candida* spp., non-*fumigatus Aspergillus* spp., Zygomycetes and hyaline molds (e.g., *Fusarium* and *Scedosporium* spp.). This myriad of infection makes diagnosis a challenge for the clinicians. Traditional diagnostic methods, such as histopathology and culture, which are still considered as the gold standards, have low sensitivity and time-consuming. The other alternative to the conventional method, becoming popular is serologic and molecular techniques. Tests like β-glucan test for invasive *Candida* spp. as well as molds and galactomannan antigen test to identify *Aspergillus* with a simple blood sample have already been established as important diagnostic tool and are implemented in routine clinical practice. On the other hand, use of PCR-based assays, DNA sequencing and other molecular approaches, such as Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry, have shown promising results in clinical trials. However they still need to undergo standardization before they are use to accurately detect fungal pathogens.

Keywords: Antifungal agents, Immunocompromised, Mold infection, Opportunistic fungi

INTRODUCTION

Fungal infection is a problem of increasing relevance in health care setting and in particular for Intensive Care Units (ICUs). A rise in the number of individuals with impaired immunity and people more prone to fungal infections generate pressure on the cost of human life and health care. The increasing incidence, reduced susceptibility to antifungal agents and changing epidemiology of fungal infections in these critically ill patients pose a significant diagnostic and therapeutic challenge [1].

Etiology of Fungal Infection in ICU

Over the last several decades the advent of the human immunodeficiency virus (HIV) epidemic, the increasing use of immunosuppressive drugs for serious medical conditions and prolonged stay in ICU, have dramatically increased the number of persons who are severely immunocompromised. This has resulted in an increase in the number of life-threatening infections due to opportunistic fungi, in this cohort of cases. Likewise, the increasing use of invasive monitoring and aggressive therapeutic technologies in ICU has not only resulted in improved survival of individuals with life-threatening illnesses but has also broken-down the defensive anatomical barriers, such as the skin and mucous membrane, which is the major risk factor for invasive fungal infections [2-4]. The other developments in medical practice that resulted in significant changes in the incidence of fungal infections among the different groups of patients at risk include the increased use of antifungal prophylaxis with azoles and the widespread use of antifungal agents for empirical treatment [5].

Spectrum of Fungal Infection in ICU

The most frequently encountered infections are caused by the yeasts *Candida albicans* and *Cryptococcus neoformans*, and by the filamentous fungus, *Aspergillus fumigatus* [6,7]. Recently, *Candida* spp. has become the fourth most frequent causal microorganism responsible for nosocomial sepsis [8]. Furthermore, the prevalence of infections caused by non-*Candida albicans* (essentially *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei*) and other yeast genera are increasing according to recent studies [9,10]. Additionally, significant regional differences have been noticed in the distribution and antifungal susceptibility

pattern among the different species [11]. The infections with rare/emerging pathogens e.g. opportunistic yeast-like fungi (e.g., *Trichosporon* and *Rhodotorula* spp.), non-*fumigatus Aspergillus* spp., Zygomycetes and hyaline molds (e.g. *Fusarium* and *Scedosporium* spp.) is a big concern for the intensivists today[8].

Advances in Diagnosis of Fungal Infection

One of the most challenging aspects of treating invasive fungal infection involves appropriate diagnosis. Traditional methods of diagnosing fungal infection include clinical evaluation, culture, radiographic evidence, and histopathology. Blood culture which is considered the gold standard for diagnosing candidemia (the commonest fungemia in ICU), takes a minimum of 24-48 hours to become positive. Further, species-specific identification in these culture positive isolates is must because of an increase in infections caused by innately azole resistant non-*albicans Candida* species. The conventional methods of identification which rely on phenotypic characteristics is time-consuming and of low accuracy thus, many closely related species (such as *C. albicans* and *C. dubliniensis*) may be misidentified due to indistinguishable physiological characteristics. The automated system like Vitek 2 (Biomerieux) identifies most clinically important *Candida* spp. reliably within 15 hours, appears to be an excellent alternative identification method for clinical laboratories [12]. The Clinical and Laboratory Standards Institute (CLSI) has developed two reference methods for antifungal susceptibility testing of yeasts, broth micro dilution (BMD) (M27-A3 document) and disk diffusion method (DD) (M44-A2) [13,14]. The other Agar-based susceptibility testing method like E-Test (ET) has been widely used by clinical laboratories and have found it to be a reliable alternative to the reference method [15]. Susceptibility testing for filamentous fungi faced many challenges until 2010 when CLSI published a reference method (M51-A) to guide the clinical laboratory in disk diffusion antifungal susceptibility testing of non-dermatophyte filamentous fungi [16]. The method proved to be an alternative simple, rapid, and cost-effective approach to determine the susceptibility of molds to various classes of antifungal agents. The earlier methods were labor intensive and scanty data on local MIC values prevented most of the clinical laboratories from performing it [15].

Within the past 10 years, various advancements have been made in the field of non-culture based fungal diagnostic methods. Most prominent among these is the development and release of two new diagnostic tests: the galactomannan assay and the beta-glucan assay. The galactomannan assay was a much anticipated test for its ability to identify *Aspergillus* with a simple blood sample [17]. The diagnostic capability for the detection of invasive aspergillosis has improved through the use of this enzyme immune assay over the past few years. The test finds approval for prospective screening for invasive aspergillosis in Hematopoietic Stem Cell Transplantation (HSCT) recipients. Unfortunately, the assay is associated with false-positive results in patients receiving β-lactam antibiotics, most notably piperacillin/tazobactam and in patients with intestinal mucositis. Concomitant use of mold-active, antifungal therapy in some patients can cause false-negative results, thus reducing the sensitivity of the assay in patients who are receiving antifungal prophylaxis.

The beta-glucan test is an antigen-based test that detects the presence of 1,3-β-D-glucan, another important component of the cell wall of most fungi. This non-specific diagnostic test approved by Food and Drug Administration (FDA) of the United States of America, detects the presence of many types of fungi by targeting a component of the fungal cell wall. Although, the test can detect common medically important fungi like *Candida* spp. and *Aspergillus* spp. yet it does not detect *Cryptococcus* spp. or members of Zygomycetes. Obvious limitation includes false positive results due to several conditions, such as abdominal surgery, haemodialysis, treatment with β-lactam antibiotics and concomitant presence of lipopolysaccharide due to Gram-negative bacteria.

Two tests, based on mannan antigen are being used to predict the onset of candidemia. Mannan antigen is located abundantly on the *Candida* cell wall surface and is known to be highly immunogenic for humans. A rise in the anti-mannan antibody titers is noted when *Candida* enters the bloodstream which can be detected by using several commercially available assays in sequential serum samples. Furthermore, to detect mannan antigen in blood samples commercial assay based on ELISA format is also available. The utility of this assay was evaluated for the diagnosis of invasive candidiasis in haematological and ICU patients with fair results. Furthermore, the overall performance of combined mannan antigen and the anti-mannan antibody testing is reported superior to either mannan or anti-mannan testing alone [18].

Molecular approaches to the diagnosis of fungal infection are proving as an attractive alternative to improve timely diagnosis of invasive fungal infection in high-risk patients. Various amplification-based assays have been developed to offer improvements in the detection of fungi, or when reliable results are not provided by conventional diagnostic approaches. Polymerase Chain reaction (PCR) primarily with primers targeted to gene sequences unique to fungi have been used most widely by the researchers. Species-specific identification of a fungus based on amplification followed by sequencing of the Internal Transcribed Spacer (ITS) region of the fungal rRNA operon has been tried with good results [19]. A variety of other rapid molecular testing method has been developed and tested over the years to supplement and to improve the performance of PCR. For example, Peptide Nucleic Acid Fluorescence In-situ Hybridization (PNA

FISH) assay can differentiate between *C. albicans* and *C. glabrata* more rapidly than conventional phenotypic method. This technique uses fluorescent probes to identify target areas in the genomes of microbial pathogens in samples, which is then detected by fluorescence microscopy. The other novel method MALDI-TOF has created a revolution for microbiological laboratories and has the potential to replace the conventional methods in diagnosing fungal infection in the next few years. MALDI-TOF MS is based, on protein finger-prints and has been exploited recently for rapid identification of *Candida* spp. isolates [20]. Although, these novel methods are a promising diagnostic prospect for various fungal pathogens, but require further development for clinical applications.

Management

For over three decades, amphotericin B with or without flucytosine was the only option for antifungal chemotherapy. With the changing spectrum of pathogens, the emergence of resistance, and the toxicity of commercially available antifungals have resulted in the need for an expanded arsenal of antifungal drugs. The currently available antifungal agents for systemic mycoses are polyene compounds (amphotericin B deoxycholate or liposomal forms), azoles (itraconazole, fluconazole, voriconazole, posaconazole, ravuconazole, isavuconazole), echinocandins (caspofungin, anidulafungin, micafungin) and flucytosine. The newer triazoles and the echinocandins have significantly changed the treatment options for fungal infections like aspergillosis and candidiasis.

CONCLUSION

The current gold standards for diagnosing IFI are of low sensitivity and lack rapidity. Delay in detection and treatment of fungal pathogens jeopardize the lives of patients in ICU. Thus, there is a need for the development of faster and more accurate diagnostic tests. The previous conventional diagnostic methods are being replaced by novel serologic and molecular methods for more accurate detection and identification of fungal pathogens. These novel methods look promising in their results, and have every potential to replace traditional diagnostic assays. However, these techniques need to undergo standardization before their use to detect and treat fungal pathogens in an effective and timely manner.

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