CHANGING EPIDEMIOLOGY OF SYSTEMIC FUNGAL INFECTIONS AND THE POSSIBILITIES OF LABORATORY DIAGNOSTICS

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Advances in medical and surgical therapy over the past two decades have changed the nature of patient care during hospitalization. Recently developed technologies and therapies, involving bone marrow or solid-organ transplants and chemotherapeutic agents, have become common at many medical centers, resulting in the emergence of many immunocompromised individuals. In intensive care units (ICU) the use of invasive monitoring devices, parenteral nutrition, broad-spectrum antimicrobial agents, and assisted ventilation has helped in the treatment of patients suffering from previously devastating or fatal diseases and has provided opportunities for life to premature neonates previously thought to be non-viable [1]. However, these successes have resulted in the proliferation of a severely ill, immunocompromised, long-lasting hospitalized patient population. The AIDS epidemic has also added patients at risk to this growing population of immunocompromised individuals [2]. The immunocompromised patient is highly susceptible to nosocomial infections caused by organisms such as fungi that were previously considered to be of low virulence or "non-pathogenic" [3]. Besides the well-known endemic fungal pathogens (*Histoplasma capsulatum*, *Coccidioides immitis* and *Blastomyces dermatitidis*), opportunistic *Candida* species have been implicated most frequently in nosocomial fungal infections.

In the mid-1980s, many institutions, including cancer research, university and community hospitals, reported that fungi were becoming common pathogens in nosocomial infections [3, 4]. In addition, during the period 1980 to 1990, hospitals in the US providing data to the Center for Disease Control and Prevention (CDC) National Nosocomial Infections Surveillance (NNIS) system reported a steady increase in the rate of nosocomial fungal infections, from 2.0 to 3.8 per 1000 discharges [5]. The high rate of fungal infections was not limited to oncology wards and high-risk nurseries, but also occurred in cardiac surgery and burn and trauma wards. The data suggest that, although nosocomial fungal infections may be more common in large university-affiliated hospitals, the increase in incidence of these infections may be evident in all types of hospitals.

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The majority of nosocomial fungal infections are reported to be caused by *Candida* spp. Even today, *C. albicans* is by far the most common *Candida* species causing infections in humans, though the prevalence of other *Candida* spp, such as *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. lusitaniae* and *Torulopsis* (*Candida*) *glabrata,* has increased during recent years. Many institutions have reported newly recognized pathogenic fungi, previously thought to be non-pathogenic, including *Malasezzia* spp., *Fusarium* spp., *Trichosporon* spp., *Mucor* spp. and *Acremonium* spp. [6–8]. *Aspergillus* spp. may also be involved in infections of hematology and oncology patients [4].

C. albicans and the *Candida non-albicans* species may cause a similar spectrum of diseases, ranging from thrush to invasive diseases such as arthritis, osteomyelitis, endocarditis, endophthalmitis, meningitis or fungemia [1, 3].

Candidemia is the most extensively studied nosocomial invasive fungal infection. The presentation of fungemia may vary, with pyelonephritis, peritonitis, hepatosplenic abscesses, pneumonitis, myositis, macronodular skin lesions, osteomyelitis, endophthalmitis, meningitis and/or multiorgan involvement. Most serious nosocomial *Candida* infections are believed to be endogenous, acquired through prior colonization of the mouth, gastrointestinal tract, vagina or skin, which act as source [9]. The organs involved in disseminated candidiosis may vary with the route of infection. If a previously colonized gastrointestinal tract is the source of infection through a breakdown in mucosal or epithelial tissue, liver and splenic abscesses will probably result [10]. In contrast, if a colonized central venous catheter is the source, endocarditis or renal involvement is more likely [1]. Patients with candidemia and/or disseminated infection usually develop fever and leukocytosis, unless they are also on immunosuppressive medication. It has been proven that some infections caused by non-albicans *Candida* species such as *C. lusitaniae* or *C. parapsilosis* or by *Aspergillus* spp may be exogenous.

The differentiation between the exogenous and the endogenous acquisition of *Candida* infections is important for determining appropriate control measures to prevent the nosocomial transmission of *Candida* spp. Strain-typing studies are needed to avoid implicating an environmental source solely on the basis of the presence of a single species of *Candida.* Interstrain differentiation should be carried out. Strain-typing may also be important in less serious local infections, such as recurrent vulvovaginitis, to distinguish between recurrent or persistent infection. Various typing methods have been developed for this purpose [11, 12].

Prevention methods aimed at reducing identified risk factors for nosocomial fungal infection are being increasingly advocated. The usefulness of the prophylactic use of fluconazole among selected leukemia or bone marrow transplant patients is supported by recent studies. For the treatment of invasive *Candida* and *Aspergillus* infections, amphotericin B, its liposome-encapsulated form, flucytosine and fluconazole are more widely used. Amphotericin B is the most active and the most toxic antimycotic agent. However, about 80% of the *C. lusitaniae* strains display resistance to this agent. Flucytosine should be used in combination to prevent the early development of resistance. Fluconazole has a similar activity to that of amphotericin B in invasive fungal infections, with very favourable pharmacokinetics and low toxicity, but *C. glabrata* and *C. krusei* are resistant to fluconazole.

Laboratory methods available for diagnostics of fungal infections

A definitive laboratory diagnosis of invasive fungal infections can be made by the histopathologic demonstration of fungus invaded tissue, or by the isolation of *Candida* or other fungi from normally sterile body sites. Isolated *Candida* spp. should be identified at a species level, as some of the non-albicans *Candida* spp. proved to be resistant to antimycotic agents used in the empirical therapy of invasive fungal infection. Direct microscopic examination of the specimen (liquor) may help to identify encapsulated *Cryptococcus neoformans*.

For the primary culturing of fungi, Sabouraud dextrose agar is used most frequently. To ensure maximum recovery of fastidious fungi, brain heart infusion (BHI) agar supplemented with 5% sheep blood and media containing antibacterial agents can also be used with any specimen that might contain normal bacterial flora.

The identification of *C. albicans* can be based on germ tube formation in human serum, and the formation of chlamydospores on the rice agar. *C. albicans* screen plate (BioMerieux, Sanofi Diagnostics Pasteur) provide a rapid identification of *C. albicans* isolates directly in clinical material. Non-albicans species can be identified by the classical assimilation/fermentation method, which requires up to 28 days before the identification can be made [13]. For clinical purposes, more rapid tests are needed, which permit species determination on the same day or within 48–72 hours. Specific exoenzymes of the most frequent human pathogenic *Candida* species can be detected by using CHROMagar (Beckton Dickinson) for the direct culturing of specimens or for the subculturing of non-albicans isolates. The chromogenic substrates incorporated in the agar plate provide well-distinguishable coloured colonies of *C. albicans* (green), *C. tropicalis* (metallic blue), *T. glabrata* (purple) and *C. krusei* (pink). All other species which appear with white colonies can be identified by commercially available identification systems such as the ATB 32C, API 20 CAUX (BioMerieux) strip and the AUXACOLOR, and FUNGSCREEN (Sanofi Diagnostics Pasteur).

To diagnose invasive *Candida* infections, blood cultures may be used. Several blood culture systems are available for recovery of fungi. The lysis-centrifugation method provides a sensitive method, but several fully automated blood culture systems are also currently available (BacT/Alert, Organon Technica; Bactec, Becton Dickinson; Vital, BioMerieux). Positivity may be recorded after a few hours of incubation. Despite the fact that blood cultures remain the basic tool for the diagnosis of fungemia, this method may fail to detect as many as 50% of disseminated candidiasis cases. The detection of massive colonization with the same *Candida* species at different body sites (feces, urine, and oral cavity) may help in the prediction of the dissemination of the *Candida* infection. Even when blood cultures are positive, identification can be attempted several days after isolation. Serological diagnosis of fungemia is problematic, since it is neither sensitive nor specific. Direct antigen detection from serum samples may also be used for diagnosis of systemic fungal infections. Latex agglutination for *Candida*, *Aspergillus* and *Cryptococcus* (Sanofi Diangnostics Pasteur). However, the sensitivity of these tests is also low. ELISA method for detection of *Aspergillus* antigen from serum samples and from bronchoalveolar lavage was developed recently and have a sensitivity of 1 ng/ml (Platelis Aspergillus, Sanofi Diagnostics Pasteur). A PCR-based method can likewise be used to detect pathogenic fungi in human blood [14].

Over the past decade, routine susceptibility testing has played a minimal role in the management of nosocomial *Candida* infections. Many difficulties have arisen as concerning the establishment of a reliable and reproducible susceptibility testing method for *Candida* spp. Factors such as inoculum size, medium type, and temperature and time of incubation influence the results of such testing, and standardized methods have only recently been developed [15]. A comparison of the relative susceptibilities of *Candida* spp to antifungal agents, performed in a blinded fashion, revealed great intra- and interlaboratory variations in the results [16]. However, recent studies demonstrated a very good correlation between susceptibility tests involving Etest strips. The National Committee for Clinical Laboratory Standards (NCCLS) [17] provide new guidelines for the susceptibility testing of antifungal drugs for *Candida* spp.

The increasing number of severe infections caused by different yeasts and other fungi is a great challenge not only for the clinician, but also for the clinical microbiology laboratory. The provision of rapid and reliable methods for these pathogens that are difficult to culture and identify is very important, as at present the clinician's decision must be based on a synthesis of both laboratory and clinical data for the successful management of serious fungal infections.

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