Journal de Mycologie Médicale xxx (2018) xxx–xxx

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EM|consulte
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General review/Revue générale

Non-culture based assays for the detection of fungal pathogens

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ARTICLE INFO

Article history:
Received 3 January 2018
Received in revised form 5 March 2018
Accepted 5 March 2018
Available online xxx

Keywords:
Fungal infection
Rapid detection
Conventional assays
Immunoassays
Molecular assays

ABSTRACT

Traditional, culture based methods for the diagnosis of fungal infections are still considered as gold standard, but they are time consuming and low sensitive. Therefore, in order to overcome the limitations, many researchers have focused on the development of new immunological and molecular based rapid assays that could enable early diagnosis of infection and accurate identification of fungal pathogens causing superficial and invasive infection. In this brief review, we highlighted the advantages and disadvantages of conventional diagnostic methods and possibility of non-culture based assays in diagnosis of superficial fungal infections and presented the overview on currently available immunochromatographic assays as well as availability of biomarkers detection by immunodiagnostic procedures in prompt and accurate diagnosis of invasive fungal infections. In addition, we presented diagnostic efficiency of currently available molecular panels and researches in this area.

Contents

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https://doi.org/10.1016/j.mycmed.2018.03.001
1156-5233/

1. Introduction

The primary task of microbiological/mycological diagnostics is to provide adequate information of the infection cause and its antimicrobial susceptibility in optimal period of time. Any delay in accurate pathogen identification and appropriate treatment initiation undoubtedly affect disease outcome.

Despite significant advances in medical technology in terms of rapid accurate diagnostic laboratory tests following by new therapeutics that have had a major effect in decreasing of morbidity and mortality of many fatal diseases, we are the witnesses that invasive fungal infections (IFIs) are still one of the biggest problems in medicine [1–3]. Increasing the number of immunocompromised patients with T-cell mediated immunity deficiencies, mucosal-cutaneous barrier or metabolic dysfunction, neutropenia, aging, excessive use of antibiotics, cytotoxic therapy and transplantation, as predispose risk factors and conditions, significantly influence the higher incidence of IFIs [4]. Moreover, the incidence and prevalence of nosocomial IFI are still high and *Candida* species represent the third most common blood isolate from hospitalized patients. Out of all IFI, 45% of invasive candidosis are from intensive care units (ICU). *Candida* bloodstream infection is prolonging stay in the ICU with mortality ranges from 40 to 71%. Also, it can cause complications during the clinical course of primary disease and increases treatment costs [5].

As opposed to IFI, superficial fungal infections (SFI) of the keratin rich structures are rare systemic and serious. These infections are caused commonly by dermatophytes, yeasts or non-dermatophytic molds and represent one of the dominant infections worldwide with global prevalence ranges from 22–25% [6,7]. The lack of rapid diagnostic methods for SFI consequently influence that only 3% of physicians in general practice and 40% dermatologists require mycological analyses before prescription of empiric treatment with systemic antifungals [8]. Additionally, these infections can affect the patient's quality of life as a potential cause of social, professional and emotional problems [9]. Thus, fungal infections of the skin, nail or hair could not be considered as a cosmetic problem of relatively minor significance. Given the fact that deep infections and dissemination of pathogen can be expected in immunodeficiency, some genetic abnormalities such as CARD9 mutation, and leukemia [10,11], the prompt diagnosis and treatment of SFIs could prevent complications and unwanted clinical outcomes.

So far, in the group of SFI, mucosal (oropharyngeal, vulvovaginal, intestinal) fungal infections have also been included. Today, the prevalence of oropharyngeal and vulvovaginal mucosal candidosis and *Candida*-overgrowth in intestines significantly rises up and the treatment of chronic/recurrent form is a big challenge for physicians. Also, frequent changes of local therapy by systemic, with the goal to improve the treatment, probably influences the emergence of resistant *Candida* species to azoles [12–16]. New, highly specific and sensitive rapid tests will disable misdiagnosis and unnecessary proscribing of antifungals, which will be in accordance with recently initiate appeal that systemic antifungals have to be kept for IFI [17].

Historically, it is very often highlighted that traditional/conventional methods for diagnosis of SFI and IFI are time consuming and low sensitive. The propagating/cultivating the causative agent is method with only deficiency in required time for isolation, but when we can conduct it, it is the unique analysis that provide antigen (Ag) detection, molecular, biochemical identification of etiological agents and antimicrobial susceptibility testing. On the other hand, histopathological examination of the tissue samples that is considered as a "gold standard" for IFI has a major shortcoming that lays in the complexity of the invasive biopsy procedure [18–20].

This review discusses currently available data on the advantages and disadvantages of some rapid assays for fungal detection, identification and the diagnosis of SFIs and IFIs.

2. Rapid assays for diagnosis of superficial fungal infections (SFIs)

2.1. Conventional microscopy examination (ME)

Conventional microscopic examination (ME) is cheap, easy-to use and fast method for detection of fungi in a patient's sample. Although staining procedures offer additional information concerning morphology of microorganisms, ME in a form of wet mount technique provides rapid detection of fungal blastoconidia-yeast and pseudohyphal-hyphal forms in patient's material (Fig. 1) [21]. Wet mount with chlorlactophenol or KOH as reagents is still irreplaceable technique for screening of patients with suspected SFI of the skin, hair and nail. Additionally, diagnosis of vaginal discharge by wet mount microscopy is useful for the diagnosis of *Candida* vaginitis - one of the most prevalent genital infections in women [22,23]. Nowadays, specific staining with chemicals or fluorescent dyes (acridin-orange, calcofluor wight, blankoflor) can improve visualization and detection of fungi [18,24,25].

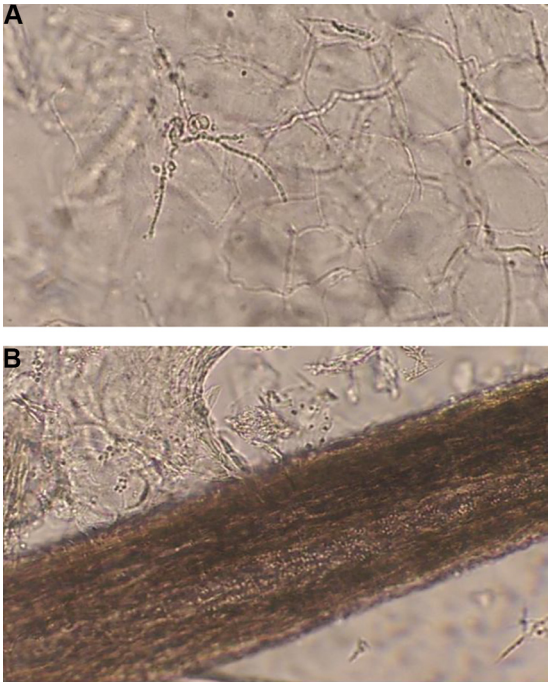

Fig. 1. A. Fungal mycelia in patients material. B. Multiple fungal spores in hair.

Please cite this article in press as: Otašević S, et al. Non-culture based assays for the detection of fungal pathogens. Journal De Mycologie Médicale (2018), https://doi.org/10.1016/j.mycmed.2018.03.001