Journal de Mycologie Médicale 28 (2018) 574–584

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

General review/Revue générale

Epidemiology of antifungal susceptibility: Review of literature

I. Hadrich *, A. Ayadi

Laboratoire de biologie moléculaire parasitaire et fongique, faculté de médecine de Sfax, rue Magida-Boulila, 3029 Sfax, Tunisia

ARTICLE INFO

Article history:
Received 26 October 2017
Received in revised form 26 April 2018
Accepted 27 April 2018

Keywords:
Epidemiology
Susceptibility
Fungi
Yeast

ABSTRACT

Fungal infections are a major cause of morbidity and mortality despite the latest developments of diagnostic tools and therapeutic options. Early initiation of the appropriate antifungal therapy has been demonstrated to have a direct impact on the patient's outcome. Antifungal susceptibility testing methods are available to detect antifungal resistance and to determine the best treatment for a specific fungus. American and European standards have been developed, as well as equivalent commercial systems, which are more appropriate for clinical laboratories. These studies have allowed the development of interpretative breakpoints against the most frequent agents of fungal infections in the world. Surveillance of antifungal susceptibility patterns can provide the local drug resistance data to the clinicians, which can further aid better management of patients. Antifungal susceptibility tests have become essential tools to identify resistance to antifungals, to know the local and global disease epidemiology and to guide the treatment of fungal diseases. The distribution of species and the prevalence of antifungal resistance in fungi isolates varied among different areas. Here we summarize the epidemiology of antifungal susceptibility pattern of different fungal species.

Contents

* Corresponding author.
E-mail address: ineshadrich@yahoo.fr (I. Hadrich).

https://doi.org/10.1016/j.mycmed.2018.04.011
1156-5233/

1. Introduction

Fungal infections are a major cause of morbidity and mortality despite the latest developments of diagnostic tools and therapeutic options. Early initiation of the correct antifungal therapy has been demonstrated to have a direct impact on the patient's outcome [1,2].

Yet, treatment choices are restricted because of the scarce number of antifungal drug classes. Clinical management of fungal diseases is further compromised by the emergence of antifungal drug resistance, which eliminates available drug classes as treatment options. There are three main antifungal drug classes: the azoles, the echinocandins and the polyenes. Amphotericin B was used for the treatment of invasive fungal infections. There are several azoles, where fluconazole is mainly used for the treatment of *Candida* infections, second generation triazoles such as voriconazole, itraconazole, posaconazole and isavuconazole are primarily used for mould infections [3,4]. Caspofungin, anidulafungin and micafungin are the current licensed echinocandins used and serve as first line therapy of invasive *Candida* infections [3].

Antifungal drug susceptibility is normally quantified using the minimum inhibitory concentration (MIC). The MIC represents the lowest drug concentration that results in a notably reduction or complete lack of fungal growth. Antifungal resistance can be primary (intrinsic) or secondary (acquired). Primary resistance occurs naturally, without prior exposure to the drug. Primary resistance is found naturally among certain fungi without prior exposure to the drug and emphasizes the importance of identification of fungal species from clinical specimens. Examples include resistance of *Candida krusei* to fluconazole and of *Cryptococcus neoformans*. Although it is less common during antifungal therapy, acquired resistance in *Candida* spp. infections has also been reported. Most cases involve *C. glabrata* resistance to echinocandin although other species such as *C. albicans, C. tropicalis* and *C. krusei*, have also proven be able of developing secondary resistance [5,6].

Amphotericin B has limited activity against *A. terreus* [7] and *A. nidulans* [8], while *A. calidoustus* appears to be resistant to triazole compounds [9]. Furthermore, several species in the *A. fumigatus* complex (*A. lentulus, A. pseudofisheri* and *A. fumigatiaffinis*) appear to be intrinsically resistant to azoles, and in the case of *A. lentulus* and *A. fumigatiaffinis*, resistant to amphotericin B as well [10]. Secondary resistance is generated following exposure to an antifungal and may be associated with an altered gene expression [11].

Two unusual in vitro testing phenotypes were observed during antifungal susceptibility testing: the trailing effect (TE) and paradoxical growth (PG). TE is characterized by a reduced but persistent growth at concentrations above the MIC. PG is characterized by growth in the presence of low concentrations, no growth at intermediate concentrations, and growth resuming at higher concentrations [12].

These two phenomena interfere with the determination of the MIC. The clinical impact of TE and PG was studied for *Candida* [13,14] and *Aspergillus* [15,16].

2. Methods of antifungal susceptibility testing

Antifungal susceptibility testing methods are available to detect antifungal resistance and to determine the best treatment for a specific fungus. Clinical microbiology relies on these methods to select the agent of choice for a fungal infection, and to know the local and the global epidemiology of antifungal resistance.

Microdilution methods are the gold standard or reference techniques. Two organizations, the European Committee on Antibiotic Susceptibility Testing (EUCAST) and the Clinical Laboratory Standards Institute (CLSI) have standardized methods to perform antifungal susceptibility testing. Differences between these two methods have been widely discussed in several reports. However, their results have demonstrated to be comparable and are used worldwide [17–19]. Furthermore, EUCAST has set a species-specific approach for the most prevalent pathogenic yeasts (E.Def 7.2) [20] and moulds (E.Def 9.2) [21], recently updated [22] and established clinical breakpoints. In spite of this, data collection on MICs of antifungal agents against moulds still provides a valuable tool to guide clinicians in prescribing the best antifungal treatment.

The EUCAST broth microdilution standard (Clinical Laboratory Standard Institute 2008) differs from CLSI (Clinical Laboratory Standard Institute 2011) in the usage of microplate well shape, content of sugar of the RPMI broth (2 and 0.2% dextrose), inoculum concentrations (10^5 CFU/mL adjusted by conidial counting and 10^4 CFU/mL adjusted by spectrophotometer) and final DMSO concentration (0.5 and 1%).

Clinical laboratories can determine susceptibility to antifungals through a series of commercially available systems, including the Sensititre YeastOne® panel (ThermoFisher, Cleveland, USA) and the Vitek 2 system, both based on microdilution methods, or agar-based assays, e.g. test strips (E-Test®, bioMérieux; MIC®, Oxoid) and discs impregnated with a single antifungal agent. But, most of these tests are not validated for *Aspergillus* antifungal susceptibility testing. Numerous in vitro factors such as media, buffer, inoculum, incubation and endpoint criteria can affect results significantly [23,24].

The microdilution methods seem to be restricted to reference laboratories because they are laborious.

Vitek 2 yeast susceptibility test (bioMérieux) is an automated method of yeast species identification and antifungal susceptibility testing through the analysis of yeast growth. The spectrophotometric approach to antifungal susceptibility testing has been shown to be feasible for use in the clinical laboratory [25–27]. The system provides 64-well cards containing aliquots of amphotericin B, fluconazole, flucytosine, posaconazole, caspofungin, micafungin and voriconazole in a miniaturized version of the broth dilution method. The system integrates a software program which validates and interprets susceptibility test results according to CLSI clinical breakpoints based on the drug MIC values.

For commercial agar-based methods, commercially prepared strips are available from bioMérieux (Etest®) and Liofilchem Diagnostici (MIC Test Strip®). The method consists of a predefined gradient of antifungal drug concentrations on a plastic strip that is used to determine the MIC. When the strip is applied on an inoculated agar surface, the antifungal agent is immediately transferred to the agar matrix and after an incubation time, an inhibition ellipse centered along the strip is formed.

Moreover, several automated or semi-automated commercial methods based on agar diffusion or the use of colorimetric indicators in Etest, Sensititre YeastOne, Fungitest or Vitek have been designed for routine daily practice. Disk and strip diffusion methodologies are simple, rapid, cost-effective and produce similar results to the reference methods for yeasts. Automated systems significantly reduce the biologist hands-on time, turn-around time, and variability due to the standardized format. Evaluation of these methodologies requires the determination of break point category agreements with reference methods.

3. Epidemiology of antifungal susceptibility

3.1. Candidiasis

Candida species, as opportunistic organisms, can cause various clinical manifestations, ranging from mild cutaneous infections to