International Journal of Antimicrobial Agents 44 (2014) 320–326

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Activity of echinocandins and triazoles against a contemporary (2012) worldwide collection of yeast and moulds collected from invasive infections

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ARTICLE INFO

Article history:
Received 19 December 2013
Accepted 3 June 2014

Keywords:
Triazoles
Echinocandins
fks
Surveillance

ABSTRACT

In this study, 1717 fungal clinical isolates causing invasive fungal infections were evaluated against nine antifungal agents using Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution methods. The isolates comprised 1487 *Candida* spp., 109 *Aspergillus* spp., 86 non-*Candida* yeasts (including 52 isolates of *Cryptococcus neoformans*) and 35 rare moulds obtained during 2012 from 72 hospitals worldwide. Echinocandin resistance among *Candida* spp. was low, and resistance rates to anidulafungin, caspofungin and micafungin varied from 0.0% to 2.8% among different species. Echinocandin-resistant *Candida glabrata* were shown to have *fks* mutations (*fks2* HS1 F659Y, F659del, S663F and S663P), and fluconazole resistance was also observed in those strains. One *Candida krusei* and one *Candida dubliniensis* had L701M or S645P *fks1* mutations, respectively. *Candida tropicalis* and *C. glabrata* had higher fluconazole resistance rates of 6.1% and 6.9%, respectively, compared with other *Candida* spp. Fluconazole-resistant *C. tropicalis* were collected in five countries (USA, China, Germany, Belgium and Thailand). Voriconazole was active against all *Candida* spp., inhibiting 91.2–99.7% of isolates using species-specific breakpoints. All agents except for the echinocandins and posaconazole were active against *Cr. neoformans*. Triazoles were active against other yeasts [MIC_{90} (minimum inhibitory concentration encompassing 90% of isolates tested), 2 μg/mL]. The echinocandins and the mould-active triazoles were active against *Aspergillus* [MIC/MEC_{90} (minimum effective concentration encompassing 90% of isolates tested) range, 0.015–2 μg/mL], but the activity of these agents was limited against uncommon mould species (MIC/MEC_{90} range, 4 μg/mL to >16 μg/mL).

1. Introduction

Invasive fungal infections (IFIs) constitute an ever-increasing cause of morbidity and mortality among immunocompromised individuals, those who have undergone intra-abdominal surgery, neonates and the elderly [1]. In a retrospective case–control study, Menzin et al. [2] used data from the 2004 Healthcare Cost and Utilization Project Nationwide Inpatient Sample to estimate the number, frequency, and excess costs and mortality of IFIs in the USA. The frequency of IFIs was estimated at 22 infections per 100 000 persons annually, representing 64 480 IFI cases per year and an excess mortality and length of stay in the hospital of 10% and 11 days, respectively. These IFIs accrued $29 281 more in medical costs per infection, contributing an estimated 735 000 additional hospital days and $1.89 billion in additional hospital costs. The most frequent infections were invasive candidiasis, other mycoses (e.g. pseudallescheriasis, fusariosis and other unspecified mycoses) and invasive aspergillosis. Notably, invasive candidiasis was associated with the highest attributable mortality (14%), excess length of stay (17.2 days) and excess costs ($45 616) among nine different types of IFI.

Concurrent with increasing numbers of IFI, surveillance programmes have become important in defining the species distribution and antifungal resistance profiles of the responsible pathogens [3–5] and thus are providing information necessary for appropriate empirical antifungal therapy [6]. Data from several sources show that mortality rates and resource utilisation significantly increase when therapy is delayed or inadequate (wrong dose, resistant isolate), further underscoring the importance of

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http://dx.doi.org/10.1016/j.ijantimicag.2014.06.007
0924-8579/

detailed epidemiological data [7,8]. Whereas fluconazole remains an important agent for the treatment of *Candida* and other yeasts, the introduction of yeast- and mould-active agents of the triazole (posaconazole and voriconazole) and echinocandin (anidulafungin, caspofungin and micafungin) classes has dramatically increased the options for prophylaxis and empirical and directed therapy of IFIs [9,10]. Although resistance to these agents among clinical isolates of opportunistic fungi is generally considered uncommon [3,5], both increased resistance and breakthrough infections have been reported among patients with long-term exposure to even the newest of antifungal agents [3,4,11,12]. These observations have prompted a call for enhanced surveillance efforts and an expanded role for antifungal susceptibility testing of *Candida* and *Aspergillus* spp. as well as of other opportunistic yeasts and moulds [11,13–16].

In the present study, Clinical and Laboratory Standards Institute (CLSI) broth microdilution (BMD) methods [17,18] and newly developed clinical breakpoints (CBPs) and epidemiological cut-off values (ECVs) were used to examine the rates of resistance and non-susceptibility among anidulafungin, fluconazole, voriconazole and comparator agents tested against 1717 clinical isolates of *Candida* (1487 isolates; 20 species), non-*Candida* yeasts (86 isolates; 11 species), *Aspergillus* spp. (109 isolates, 10 species) and non-*Aspergillus* moulds (35 isolates; 24 species). These isolates were collected from patients with IFI during 2012 from 72 medical centres in a global antifungal surveillance programme. Isolates of the less common species of *Candida*, the non-*Candida* yeasts and all filamentous fungi were identified using molecular methods, and isolates of *Candida* spp. showing resistance to one or more echinocandins were examined for mutations in hotspot (HS) regions of *fks1* and *fks2*.

2. Materials and methods

2.1. Organisms

A total of 1717 clinical isolates from patients with IFI were collected during 2012 from 72 laboratories located in North America (445 isolates; 12 sites), Europe (706 isolates; 30 sites), Latin America (217 isolates; 10 sites) and the Asia-Pacific Region (349 isolates; 20 sites) as part of a global surveillance programme. In each case, collection was approved by the appropriate institutional review board. Each participating centre recovered consecutive unique isolates from patients with bloodstream infections (1066 isolates), from normally sterile body fluids, tissues and abscesses (95 isolates), respiratory tract specimens (228 isolates) and other/unknown sites (328 isolates).

Isolates were identified at participating institutions using methods routinely employed at the submitting laboratory, including VITEK, MicroScan, API and AuxaColor supplemented by classical methods for yeast and mould identification [19,20]. Isolates were submitted to JMI Laboratories (North Liberty, IA), where the identity was confirmed by morphological, biochemical and molecular methods as previously described [14,21].

Among the 1487 isolates of *Candida*, there were 711 *Candida albicans*, 274 *Candida glabrata*, 245 *Candida parapsilosis*, 131 *Candida tropicalis*, 36 *Candida krusei* and 90 miscellaneous *Candida* spp. (25 *Candida lusitaniae*, 14 *Candida dubliniensis*, 13 *Candida guilliermondii*, 12 *Candida kefyr*, 2 *Candida fermentati*, 2 *Candida lipolytica*, 3 *Candida orthopsilosis*, 1 *Candida catenulata*, 1 *Candida haemulonii*, 1 *Candida inconspicua*, 2 *Candida fabianii*, 1 *Candida bracarensis*, 1 *Candida thasaenensis*, 1 *Candida fluviatilis*, 8 *Candida pelliculosa* and 2 unspeciated *Candida*). The collection also included *Cryptococcus neoformans* (52 isolates), *Cryptococcus gattii* (2 isolates), *Cryptococcus laurentii* (1 isolate), *Trichosporon asahii* (13 isolates), *Saccharomyces cerevisiae* (6 isolates), *Rhodotorula mucilaginosa* (4 isolates), *Dipodascus capitatus* (3 isolates) and 1 isolate each of *Trichosporon mycotoxinivorans*, *Lodderomyces elongisporus*, *Geotrichum clavatum*, *Malassezia pachydermatis* and unspeciated yeast. Moulds included 68 *Aspergillus fumigatus* sensu stricto, 41 miscellaneous *Aspergillus* spp. [13 *Aspergillus flavus* species complex (SC), 10 *Aspergillus niger* SC, 7 *Aspergillus terreus* SC, 2 *Aspergillus alabamensis*, 3 *Aspergillus nidulans*, 3 *Aspergillus sydowii* and 1 each of *Aspergillus clavatus*, *Aspergillus tubingensis* and *Aspergillus nomius*] and 35 other moulds (6 *Fusarium* spp., 1 *Epicoccum nigrans*, 1 *Scedosporium apiospermum*, 1 *Penicillium* subgenus Aspergilloides, 2 *Penicillium* subgenus Terverticillata, 3 *Alternaria* spp., 1 *Purpureocillium* (*Paecilomyces*) *lilacinum*, 1 *Saroccladium* [*Acremonium*] *kiliense*, 1 *Lichtheimia ramosa*, 1 *Scedosporium aurantiacum*, 1 *Scedosporium prolificans*, 1 *Scopulariopsis brevicaulis*, 1 *Trichoderma longibrachiatum*, 2 *Rhizopus microsporus* group, 2 *Curvularia* spp., 1 *Hamigera* sp., 1 *Microascus* sp., 1 *Penicillium* sp., 3 *Cochliobolus* spp., 1 *Coprinellus* sp., 2 *Cunninghamella* spp. and 1 *Phoma* sp.).

2.2. Antifungal susceptibility testing

All yeast isolates were tested for in vitro susceptibility to the echinocandins (anidulafungin, caspofungin and micafungin) and triazoles (fluconazole, itraconazole, posaconazole and voriconazole) using the CLSI BMD method [17]. Minimum inhibitory concentration (MIC) results for all agents were read following 24 h of incubation when tested against *Candida* spp., whereas MIC endpoints for the triazoles were read after 48 h when tested against non-*Candida* yeasts. In all instances, MICs were determined visually as the lowest concentration of drug that caused significant diminution of growth levels [17,22].

In vitro susceptibility testing of *Aspergillus* spp. and other moulds against the echinocandins and triazoles (itraconazole, posaconazole and voriconazole) was performed using the CLSI BMD method [18]. Triazole MICs and echinocandin minimum effective concentrations (MECs) were determined as described in the CLSI reference method [18].

The recently revised CLSI CBPs were used to identify strains of the five most common species of *Candida* (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*) [23]. The CLSI has not assigned CBPs for voriconazole and *C. glabrata* and recommends the ECV of 0.5 μg/mL to be used to differentiate wild-type (WT) from non-WT strains of this species [24].

CBPs have not been established for any antifungal agent and the less common species of *Candida*, non-*Candida* yeasts, *Aspergillus* spp. or the non-*Aspergillus* moulds; however, ECVs have been established for echinocandins and triazoles and six species of *Candida* that are encountered less frequently (*C. lusitaniae*, *C. guilliermondii*, *C. dubliniensis*, *C. kefyr*, *C. orthopsilosis* and *C. pelliculosa*) [24]. ECVs have been derived for *Cr. neoformans* and fluconazole (8–16 μg/mL), itraconazole (1 μg/mL), posaconazole (0.5 μg/mL) and voriconazole (0.25 μg/mL) [25]. ECVs have also been developed for *A. fumigatus*, *A flavus*, *A. terreus* and *A. niger* and itraconazole, posaconazole and voriconazole [26]: itraconazole and voriconazole MICs of >1 μg/mL were considered non-WT for *A. fumigatus*, *A. flavus* and *A. terreus*; itraconazole MICs of >1 μg/mL and voriconazole MICs of >2 μg/mL were considered non-WT for *A. nidulans*, whereas itraconazole and voriconazole MICs of >2 μg/mL were non-WT for *A. niger*. Posaconazole MICs of >0.5 μg/mL were considered non-WT for *A. fumigatus*, *A. terreus* and *A. niger*, and MICs of >0.25 μg/mL were non-WT for *A. flavus*; posaconazole MICs of >1 μg/mL were non-WT for *A. nidulans*. Isolates of these *Aspergillus* spp. for which triazole MIC results exceed the ECV are considered to be non-WT and may harbour acquired mutations in the *cyp51A* gene [27].

Quality control was performed as recommended by the CLSI [17,18] using *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.