Diagnostic Microbiology and Infectious Disease 81 (2015) 34–40

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Sentinel surveillance of invasive candidiasis in Spain: epidemiology and antifungal susceptibility

M.C. Nieto *, O. Tellería, R. Cisterna

Clinical Microbiology and Infection Control Department, Basurto University Hospital, Avda. Montevideo 18, 48013, Bilbao, Spain

ARTICLE INFO

Article history:
Received 7 November 2013
Received in revised form 24 May 2014
Accepted 27 May 2014
Available online 5 June 2014

Keywords:
Invasive candidiasis
Echinocandins
Epidemiology
Candidemia
Susceptibility
Antifungals

ABSTRACT

In order to know the epidemiology and the changes of antifungal resistance in invasive candidiasis (IC) we carried out this prospective study of Candida strains belonging to patients admitted to 26 Spanish hospitals from June 2011 to June 2012 diagnosed with IC. Clinical information and the identity of the Candida species were collected and antifungal susceptibility was tested using broth microdilution in five agents: amphotericin B, fluconazole, voriconazole, caspofungin and anidulafungin. A total of 705 cases-isolates were documented. Most of the patients suffered from candidemia and several underlying diseases and more than half of them were neutropenic or under immunosuppressive therapy, factors associated with higher mortality. Thirty percent of global mortality was documented. *C. albicans* was the most frequently isolated species, although an increase of non-*C. albicans* species was observed. Resistance to fluconazole was of 3.4%, lower than in previous years (6.3%). *C. parapsilosis* presented a higher MIC_{90} of echinocandins compared to other species.

1. Introduction

Candida is the most common fungal pathogen in humans and the leading cause of invasive candidiasis (IC) characterized by significant morbidity and mortality in critically ill patients (Cornely et al., 2012; Lionakis and Netea, 2013). The incidence of invasive Candida infections has increased along with the augmented use of intravenous catheters, previous administration of broad-spectrum antibiotics, parenteral nutrition, immunosuppressive therapy, solid organ transplantation and invasive procedures (Mayr et al., 2011). Furthermore, in recent years, geographical differences are emerging in epidemiology, demonstrating an alteration towards to the isolation of non-albicans species. This change has been correlated with routine prophylaxis with fluconazole administered to some patients, and intrinsic or acquired resistance to azoles by some species of Candida. Noteworthy, that these details are influencing both the choice of an appropriate empirical treatment and the prophylactic decision making (Concia et al., 2009).

On the other hand, treatment results also depend largely on the adequate use of the available treatment options. Compared to other bloodstream infections, IC appears to be associated with a particularly high rate of inappropriate frontline treatment, mainly in the form of delay or even failure of the antifungal therapy. This fact is required to be underscored, as several studies have confirmed that early and suitable treatment of IC considerably improves patient survival (Concia et al., 2009).

The relatively high incidence of IC, increased associated mortality and the growing prevalence of resistance to fluconazole suggest the need for a more effective infection control. In this regard, the echinocandins (anidulafungin, caspofungin and micafungin) seem particularly efficient (Concia et al., 2009).

Echinocandins are cyclic hexapeptides able to inhibit the enzyme responsible for the biosynthesis of the major cell wall biopolymers, (1-3) - D-glucan synthase, as an excellent molecular target with no presence in human cells (Ruiz-Camps and Cuenca-Estrella, 2009). These drugs possess fungicidal activity against most species of *Candida*, including azole-resistant species (Deresinski and Stevens, 2003). Caspofungin and anidulafungin have been approved by the U.S. Food and Drug Administration for the treatment of IC, including candidemia. These agents all provide excellent clinical efficacy together with low toxicity for the treatment of serious Candida infections (Pfaller and Diekema, 2007). Echinocandins are also being used in combination with triazole antifungal agents, such as voriconazole, as the primary treatment against yeasts. Although strict correlation between values of the minimum inhibitory concentration (MIC) and clinical outcome has not been established yet, it is expected that exposure of the patient to echinocandins will increase the number of strains with reduced susceptibility or resistance to echinocandins.

In order to assess the frequency of IC, species distribution, trends in antifungal resistance and IC risk factors, the project named as "Surveillance of Susceptibility in IC" was launched with the collaboration between a central laboratory, the Basurto University Hospital (Bilbao), and 26 sentinel tertiary hospitals in Spain.

* Corresponding author. Tel.: +34-944-006-003.
E-mail address: mariacarmen.nietotoboso@osakidetza.net (M.C. Nieto).

http://dx.doi.org/10.1016/j.diagmicrobio.2014.05.021
0732-8893/

2. Materials and methods

2.1. Surveillance and case definition

The prospective, nationwide study was performed in an established period of one year, from June 2011 to June 2012, in the Basurto University Hospital (Bilbao) with the participation of 26 tertiary, sentinel hospitals which regions are listed in the Table 1. The collaborative hospitals were selected by a national coordinator on the basis of their capacity to document all episodes of IC occurring during the study period. A case of IC was defined as the incident isolation of a *Candida sp*. from blood culture or other sterile, clinical samples of patients with temporally related clinical signs and symptoms. Only the first episode of IC was reported per patient with recurrent or subsequent episodes of infection. Patients whose cultures grew >1 documented species of Candida were excluded from the analysis. Neutropenia was defined as an absolute count of <500 neutrophils/mm^3. Neonates were defined as patients with age ≤28 days, children as patients 1-18 years old, adults as patients 19-65 years old, and elderly as patients >60 years.

2.2. Demographic and clinical data

Information about the received strains and affected patients was collected through an on-line database, completed prospectively by each institution when an IC case was identified. The report form contained the following information: age, gender, date of admission, date of analysis, exposure to invasive medical procedures, underlying medical conditions, prior use of antibiotics, antifungals or corticosteroids and analysis outcome. The clinical case report list in the database was compared with the isolates received at the Basurto University Hospital. The study was approved by the local institutional review board of each site. Audits of medical records to verify accuracy of data and completeness were performed in 25% of cases.

2.3. Underlying diseases

To assess life expectancy of the patients, the outcome of the underlying disease prior to IC was scored according to McCabe system. This score is classified into several categories: “nonfatal disease” (life expectancy greater than 5 years), “ultimately fatal disease” (life expectancy between 1 and 4 years); “rapidly fatal disease” (life expectancy less than 1 year) (McCabe and Jackson, 1962). If the death of the patient was considered to be related to the infection, it was designated as "death related to IC". If the death was associated to the underlying disease or other medical or surgical complications, it was classified as "death not related to IC". The death of the patient was taken into consideration when it took place during hospitalization.

Table 1
Number of received strains by institution and by geographic area.

Geographic Area	Autonomous community	Institution	No. strains
North	Aragon	1	45
	Asturias	1	17
	Galicia	4	119
	Navarra	1	26
	Basque Country	1	23
South	Andalusia	1	46
	Extremadura	2	36
	Canary Islands	1	72
East	Catalonia	1	21
	Region of Valencia	3	36
	Balearic Islands	2	31
	Murcia	1	42
Center	Castilla y León	4	69
	Castilla la Mancha	1	24
	Region of Madrid	2	98
Overall		26	705

2.4. Yeast identification

The strains were maintained on Sabouraud Dextrose Agar with Chloramphenicol (Becton Dickinson GmbH, Heidelberg, Germany) medium and subcultured onto ChromID™ Candida plates (BioMérieux, Marcy l’Etoile, France) at 37ºC for 24 h in order to analyze the colony morphology and ensure the purity of the subculture. Identity of all the clinical isolates was confirmed by using the api® 20 C AUX, biochemical gallery (bioMérieux® SA), according to the manufacturer’s instructions. The strains not reliably identified by api® 20 C AUX techniques, were verified by nucleic acid amplification and subsequent sequencing (CLSI 2008a,b).

2.5. In vitro susceptibility testing

Antifungal susceptibility testing was performed using broth microdilution assay according to the CLSI guidelines (CLSI M27-S3 document, 2008). Five antifungal agents were supplied by the manufacturers as pure standard powders to be tested. Amphotericin B was purchased from the Sigma Chemical Corporation, St. Louis, MO, caspofungin by Merck & Co., Inc., Whitehouse Station, NJ, and fluconazole, voriconazole, and anidulafungin from Pfizer Incorporated, New York, NY. Stock solutions of fluconazole and caspofungin were prepared in distilled water and stock solutions of amphotericin B, voriconazole and anidulafungin were prepared in 100% dimethyl sulfoxide (DMSO) (Sigma-Aldrich St. Louis). Then, those stock solutions were diluted in RPMI 1640, with L-glutamine, without bicarbonate, and buffered at pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) (Sigma-Aldrich), with the final concentration range from 0.015 to 8 μg/ml for amphotericin B, 0.125 to 64 μg/ml for voriconazole, and 0.03 to 16 μg/ml for fluconazole, caspofungin and anidulafungin. The yeast inoculum suspension was prepared by using a nephelometer to obtain a final yeast concentration of 0.5 x 10^3 cells/ml, to reach 2.5 x 10^3 cells/ml in each well of the microtiter plate. The plates were incubated at 35°C and the end points were read visually at 24 hours in the case of echinocandins and 48 hours in the case of amphotericin B and azole antifungals. Quality control strains (Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258; American Type Culture Collection, Manassas, VA) were included each day of assay. The MIC for amphotericin B was considered the lowest drug concentration able to prevent any discernible growth and the MICs for echinocandins and azole antifungals were considered the lowest tested drug concentrations causing a significant reduction in growth (approximately 50 %) relative to the drug-free growth control.

2.6. Statistical analysis

Data were analyzed by the statistics software SPSS 20.0 and the categorical data using the chi-square test or Fisher's exact test when the expected frequency was less than 5 cases. The continuous variables were compared by the K-S of the Kolmogorov-Smirnov test for variables that met the criteria of normality and Wilcoxon test or Mann-Whitney test for variables that did not meet the criteria of normality. Multivariate analysis was performed in order to detect those factors related to mortality for variables that had significant p values (<0.05) by univariate analysis. The multivariate analysis comprised of a logistic regression, where the death of the patient was considered as the dependent variable and all those variables statistically associated with a higher mortality rate in the bivariate analysis were included as independent variables, with sequential elimination of insignificant variables. Spearman's rank correlation was used to measure the relationship between the MICs of fluconazole and voriconazole, and anidulafungin and caspofungin.