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Original Article

Antifungal susceptibility of *Candida* spp. bloodstream isolates identified by real-time polymerase chain reaction: 2022-2023

Profilo di resistenza degli isolati di *Candida* spp. da emocoltura identificati mediante reazione a catena della polimerasi *real-time*: 2022-2023

Christian Leli,[1] Lidia Ferrara,[1] Paolo Bottino,[1] Luigi Di Matteo,[1] Marco Leone,[1] Camilla Furiani,[1] Elisabetta Scomparin,[1] Annalisa Roveta,[2] Antonio Maconi,[3] Andrea Rocchetti,[1] Daria Vay,[1] Franca Gotta,[1] Elisa Cornaglia[1]

[1]Microbiology Laboratory, Azienda Ospedaliero-Universitaria SS. Antonio e Biagio e Cesare Arrigo, Alessandria; [2]Research Laboratories, Research and Innovation Department (DAIRI), Azienda Ospedaliero-Universitaria SS. Antonio e Biagio e Cesare Arrigo, Alessandria; [3]Research Training Innovation Infrastructure, Research and Innovation Department (DAIRI), Azienda Ospedaliero-Universitaria SS. Antonio e Biagio e Cesare Arrigo, Alessandria, Italy

Key words: blood cultures, sepsis, *Candida*, Real-Time PCR, microbiology.

ABSTRACT

Background: here are described the antifungal susceptibility patterns of *Candida* spp. isolates identified by Real-Time Polymerase Chain Reaction (RT-PCR) from positive blood cultures collected from patients hospitalized at the Azienda Ospedaliero-Universitaria SS. Antonio e Biagio e Cesare Arrigo of Alessandria during the period 2022-2023.
Materials and Methods: all patients for whom clinical data were available and from whom blood cultures were collected and were positive only for yeasts, were included in the study. All blood cultures were processed by both Real-Time PCR and classic culture method.
Results: in our case series of 67 blood cultures positive only for yeasts, all isolates were susceptible to echinocandins and amphotericin B. Among the *Candida parapsilosis* and *Candida glabrata* isolates, 8/19 (42%) and 2/14 (14.3%), respectively, were resistant to fluconazole.
Conclusions: the rapid identification of the fungal isolate by means of RT-PCR along with the knowledge of the local susceptibility patterns can be of help in choosing the better antifungal therapy until the antimycogram is available.

Background: descrivere i pattern di suscettibilità ai farmaci antifungini degli isolati di *Candida* spp. identificati mediante reazione a catena della polimerasi *real-time* da emocolture positive prelevate da pazienti ricoverati presso l'Azienda Ospedaliero-Universitaria SS. Antonio e Biagio e Cesare Arrigo di Alessandria nel periodo 2022-2023.
Materiali e Metodi: sono stati inclusi nello studio tutti i pazienti dei quali erano disponibili dati clinici, che sono stati sottoposti a prelievo per emocoltura risultata positiva soltanto per lieviti. Tutte le emocolture incluse sono state processate sia mediante reazione a catena della polimerasi *real-time* che mediante esame colturale.
Risultati: nella nostra casistica di 67 emocolture positive soltanto per lieviti, tutti gli isolati sono risultati sensibili alle echinocandine e all'amfotericina B. Otto isolati su 19 (42%) di *Candida parapsilosis* e 2/14 (14.3%) di *Candida glabrata* sono risultati resistenti al fluconazolo.
Conclusioni: la rapida identificazione dell'isolato fungino mediante reazione a catena delle polimerasi *real-time* associata alla conoscenza dei pattern locali di suscettibilità ai farmaci può essere di aiuto nella scelta della migliore terapia antifungina, in attesa dell'antimicogramma.

Introduction

Candidemia is a pathological condition associated with high mortality, and patients who develop it often have comorbidities. Indeed, Battistolo *et al*.,[1] in 102 candidemia episodes during the period 2014-2017, found a 34% mortality, and some of the patients suffered from solid malignancies. Likewise, Muderris *et al*.[2] evaluated 163 adult candidemia and reported an overall 30-day mortality of 40.5%, with concurrent bacteremia as a strong predictor of mortality. Poissy *et al*.,[3] in a prospective multicenter matched case-control study, found as risk factors for candidemia: total parenteral nutrition, acute kidney injury, heart disease, prior septic shock, central venous catheter and exposure to glycopeptides, aminoglycosides or nitroimidazoles.

As for antibiotic therapy in bacterial sepsis, prompt antifungal therapy is the best approach to reducing the risk of death. Concerning this topic, Zhang *et al*.,[4] in a retrospective cohort

study including 1,981 patients with invasive fungal infections, found that empirical antifungal therapy is associated with a decreased mortality rate in Intensive Care Unit (ICU) patients, and the early detection of fungal infections could drastically reduce mortality rates.

Classic candidemia diagnosis is based on the collection of blood cultures, and once positive, a subculture on solid media is carried out.[5] Fungal isolates are thereafter identified, and an antimycogram is performed to estimate the probability of therapeutic success in the patient.[5] The identification from solid media by the biochemical method is time-consuming, sometimes needing up to 72 hours.[5] Other diagnostic approaches are nevertheless commercially available to directly identify microorganisms from positive blood cultures, such as matrix-assisted laser desorption ionization-time of flight mass spectrometry[6] and methods based on Nucleic Acid Amplification Techniques (NAATs).[7-9] Regarding NAATs, Mauri *et al*.,[10] in a study involving 136 positive blood cultures collected from critical patients, detected a significant number of yeasts (37/94 samples).

In some of these patients, antifungal therapy was set or adjusted.

For several years, Real-Time Polymerase Chain Reaction (RT-PCR) has been used at our institution for all blood cultures positive for fungi. The aim of this study was to describe the antifungal susceptibility patterns of *Candida* spp. isolates identified by RT-PCR from positive blood cultures at the Azienda Ospedaliero-Universitaria SS. Antonio e Biagio e Cesare Arrigo of Alessandria during the period 2022-2023.

Materials and Methods

Design of the study

This retrospective observational study evaluated the period from January 2022 to December 2023. Inclusion criteria: all patients hospitalized at the Azienda Ospedaliero-Universitaria SS. Antonio e Biagio e Cesare Arrigo of Alessandria with suspected sepsis, from whom blood cultures were collected, were positive only for yeasts at the RT-PCR and of whom clinical data were available, were included in the study. The clinical pictures of the patients with suspected sepsis were some of those described by Monti *et al*.,[11] such as arterial hypotension, impaired neurological status, either fever or hypothermia, tachypnea, oliguria or anuria along with laboratory test abnormalities such as either leukocytosis or leukopenia, C-reactive protein and creatinine increase, hyperlactatemia, disseminated intravascular coagulation.[11] The characteristics considered as risk factors for candidemia are those already described by other authors.[12]

Blood cultures

Blood culture processing at our laboratory has been described elsewhere.[13] Briefly, for each patient, at least two sets of blood cultures were collected and subsequently incubated. The blood cultures that flagged positive underwent microscopic examination of Gram-stained smears and subculture onto appropriate solid media. If the microscopic examination was consistent with yeasts, the blood cultures underwent processing by RT-PCR for rapid identification. The identification of fungi provided by the RT-PCR was subsequently confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry Vitek® MS (bioMérieux, Marcy l'Etoile, France).

Real-Time Polymerase Chain Reaction

The processing by RT-PCR has been described elsewhere.[13] Briefly, the commercially available FilmArray® Blood Culture Identification Panel (BioFire Diagnostics, Salt Lake City, UT, USA) was used according to the manufacturer's instructions.[14] Briefly, the pouch was rehydrated with 300 µl rehydration solution. A 200 µl aliquot of the positive blood culture was mixed into a syringe along with sample dilution buffer and then inoculated into the pouch. The pouch was loaded onto the FilmArray® device that subsequently performed amplification and detection.

Antifungal susceptibility test

The antifungal susceptibility test was performed using the Micronaut-AM Antifungal Agents MIC (MERLIN Diagnostika GmbH, Bornheim, Germany), a commercially available ready-to-use plate testing of up to 9 antimycotics in up to 11 concentrations following the Minimum Inhibitory Concentrations (MIC) procedure:[15] (anidulafungin: 0.002-8 mg/L, micafungin: 0.002-8 mg/L, caspofungin: 0.002-8 mg/L, fluconazole: 0.002-128 mg/L, posaconazole: 0.0078-8 mg/L, voriconazole: 0.0078-8 mg/L, itraconazole: 0.031-4 mg/L, amphotericin B: 0.031-16 mg/L, and 5-flucytosine: 0.0625-32 mg/L). The assay was performed according to the manufacturer's instructions.[15] Briefly, 0.5 McFarland yeast suspension was prepared in NaCl 0.9%, then 10 µL were transferred into 11.5 mL of MICRONAUT-RPMI-1640 Medium, appropriately supplemented with indicators.[15] Subsequently, 100 µL of the solution was dispensed into each well using an Opentrons OT2 pipetting robot.[16] Readings were performed photometrically with the Skan device and evaluated with the MICRONAUT Software.[15] The antifungal agents tested in this study were those published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and MIC results were interpreted according to EUCAST breakpoint tables for interpretation of MICs for antifungal agents, version 10.0, 2020.[17]

Laboratory and clinical data

The laboratory data were extracted by our Laboratory Information System Concerto (Dedalus Healthcare Systems Group SpA, Florence, Italy). Clinical data were obtained from the hospital administration system TRAKCare® (InterSystems).

Statistical methods

Continuous variables were expressed as median and Interquartile Range (IQR). Categorical variables were expressed as absolute numbers and percentages. The MIC values were expressed in mg/L on the X-axis and the frequency as absolute numbers on the Y-axis. Median values were compared using the Kruskal-Wallis test. Frequencies were compared by the Freeman-Halton extension of Fisher's exact test. Microsoft Excel 2010 (Microsoft Corporation, Redmond, USA) and IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, New York, USA) were used for the analysis. The significance level was set at $p \leq 0.05$.

Results

Demographic and clinical data

From a total of 495 Real-Time PCR tests performed on the same number of positive blood cultures during the interval considered, 71

were positive only for yeasts, but of 4/71 patients, data about antifungal therapy were not available. Therefore only 67 were included in the study. The median age was 71 years (IQR: 59-77), and 45/67 (67.2%) were males. The clinical characteristics of the patients evaluated are described in Table 1. More than half of the patients suffered from septic shock and/or had a central line, while more than one-third had a hematological or solid malignancy and/or suffered from heart disease.

Candida spp. identified

Table 2 summarizes the *Candida* spp. identified by RT- PCR. *Candida albicans* was the species most frequently identified. Seven out of 67 (10.4%) blood cultures were polymicrobial. The RT-PCR results were available to clinicians about 1 hour after the Gram stain report, while the identification by Vitek® MS from standard culture was available after around 24 hours. Vitek® MS identification of all *Candida* spp. recovered by culture [33/33 (100%) *C. albicans*, 19/19 (100%) *Candida parapsilosis*, 14/15 (93.3%) *Candida glabrata*, 5/7 (71.4%) *Candida tropicalis*] confirmed the results obtained by RT-PCR.

Bivariate analysis between demographic/clinical characteristics and *Candida* spp.

Table 3 describes the association between demographic/clinical characteristics and the species of *Candida* identified. No significant association between *C. albicans*, *Candida* non-albicans, or a mixed infection and any of the variables considered was found.

Antifungal susceptibility profiles and therapy

The antifungal susceptibility patterns of the *Candida* spp. isolates are described in Figures 1 and 2. Caspofungin MIC values were interpreted according to EUCAST guidelines and available literature; therefore isolates susceptible to anidulafungin as well as micafungin were considered susceptible to caspofungin.[18,19] Not always all the isolates identified by RT-PCR were recovered by culture, and likewise, it was not always possible to test all isolates for the same antifungal agent. All isolates were susceptible to echinocandins and amphotericin B. In particular, the only *Candida krusei* isolate had MIC values of 0.015 mg/L for anidulafungin and 0.5 mg/L for amphotericin B. Among the *Candida parapsilosis* and *Candida*

Table 1. Characteristics of the patients evaluated (N=67).

Variable	N	%
Diabetes mellitus	13	19.4
Total parenteral nutrition	6	8.9
Renal failure	14	20.9
Heart disease	23	34.3
Septic shock	37	55.2
Hematological or solid malignancy	25	37.3
Abdominal surgery	8	11.9
Central line	34	50.7
Exposure to aminoglycosides	0	0
Exposure to nitroimidazoles	0	0
Exposure to glycopeptides	1	1.5
Prolonged corticosteroid therapy	2	2.9
Usage of immunosuppressive agents	7	10.4

Table 2. Candida spp. identified by Real-Time Polymerase Chain Reaction (RT-PCR).

Variable	N	%
Candida albicans	28	41.8
Candida parapsilosis	16	23.9
Candida glabrata	11	16.4
Candida tropicalis	4	6.0
Candida krusei	1	1.5
Candida albicans + *Candida glabrata*	3	4.4
Candida albicans + *Candida parapsilosis*	1	1.5
Candida glabrata + *Candida tropicalis*	1	1.5
Candida parapsilosis + *Candida tropicalis*	1	1.5
Candida albicans + *Candida parapsilosis* + *Candida tropicalis*	1	1.5
Total	67	100

Table 3. Bivariate analysis between demographic/clinical characteristics and *Candida* spp.

Variable	*Candida albicans*	*Candida* non-albicans	Mixed infection	P
Age	72.6 (64.5-78.8)	67.1 (55.9-75.4)	58.9 (49.9-61.7)	0.061
Diabetes mellitus	7 (53.8)	5 (38.5)	1 (7.7)	0.740
Total parenteral nutrition	1 (16.7)	4 (66.7)	1 (16.7)	0.353
Renal failure	9 (64.3)	5 (35.7)	0 (0)	0.117
Heart disease	13 (56.5)	10 (43.5)	0 (0)	0.053
Septic shock	18 (48.6)	14 (37.8)	5 (13.5)	0.187
Hematological or solid malignancy	13 (52)	9 (36)	3 (12)	0.292
Abdominal surgery	4 (50)	3 (37.5)	1 (12.5)	0.760
Central line	14 (41.2)	17 (50)	3 (8.8)	0.940
Exposure to aminoglycosides	0 (0)	0 (0)	0 (0)	-
Exposure to nitroimidazoles	0 (0)	0 (0)	0 (0)	-
Exposure to glycopeptides	1 (100)	0 (0)	0 (0)	0.522
Prolonged corticosteroid therapy	1 (50)	0 (0)	1 (50)	0.098
Usage of immunosuppressive agents	2 (28.6)	4 (57.1)	1 (14.3)	0.617

Continuous variables are expressed as median (Interquartile Range, IQR). Categorical variables are expressed as absolute numbers (row percentage).

glabrata isolates, 8/19 (42%) and 2/14 (14.3%), respectively, were resistant to fluconazole. With regard to therapy administered to patients with candidemia, it was started or modified within 24 hours from RT-PCR reporting: 61/67 (91%) were treated with caspofungin, 5/67 (7.5%) with anidulafungin and 1/67 (1.5%) with amphotericin B. The antimycogram was available after an average of 48 hours from RT-PCR reporting.

Clinical outcome

With regard to clinical outcome, data on the 30-day in-hospital mortality were available, and 19/67 (28.4%) of the patients died within 30 days from diagnosis of candidemia.

Discussion

Concerning demographic and clinical characteristics, the results of this study substantially match those of Mareković *et al*.,[20] who in a study on 160 patients with candidemia found a prevalence of males, more than 80% had a central line and around one fourth underwent abdominal surgery. Also, Bourassa-Blanchette *et al*.,[21] in a study involving 455 individuals with 466 episodes of candidemia, found a similar percentage of risk factors such as the presence of diabetes, cardiovascular disease, chronic kidney disease, and recent history of neoplasm.

With regard to the species of Candida identified in the present study, also Mareković *et al*.,[20] found a greater prevalence of *C. albicans* followed by *C. parapsilosis* and *C. glabrata*. In this study, we observed a higher prevalence of mixed infections, possibly due to the use of a molecular method. With respect to the possible association between age and *Candida* spp., even if it did not reach statistical significance, from Table 3 it can be seen how candidemia caused by *C. albicans* corresponded to oldest patients and those suffering from heart disease compared to *Candida* non-albicans. Concerning age, these results are in contrast with the trend observed by Pfaller *et al*.[22] in a study on 2,047 bloodstream infections due to *Candida* spp. Nevertheless, in that study, patients ≥65 years were 30% of the total, whereas in this study were twice as many (39/67; 58%), and isolates were identified by the routine method in use at each laboratory, not necessarily by a RT-PCR as in our study. With respect to heart disease, a more in-depth study will be needed, since several cardiovascular diseases are associated with candidemia, such as the presence of cardiovascular implantable electronic devices,[23] *Candida* infective endocarditis[24] and open-heart surgery.[25]

Concerning the susceptibility profiles found in this study (Figures 1 and 2), an interesting bimodal distribution for fluconazole against *C. parapsilosis* was observed. This finding will be the subject of a future study. Looking at the findings of other Authors, some differences were observed. Indeed, comparing our findings with Mareković *et al*.,[20] we did not find strains of *C. albicans* resistant to echinocandins and we observed a lower rate of resistance to fluconazole for *C. parapsilosis*. Comparing our results with those of Bourassa-Blanchette *et al*.,[21] we observed lower median MIC values of azoles against both *C. albicans* and *Candida tropicalis*, while higher median MIC values of fluconazole were observed against *C. parapsilosis*. Comparable results for *C. glabrata* were found. Finally, also in a retrospective study on a total of 532 patients with candidemia by Alhatmi *et al*.,[26] resistant strains to echinocandins and to azoles other than fluconazole were found, in contrast to the findings of this study. These differences highlight the need for periodic reporting of the local antimicrobial resistance profiles, for fungi as well as for bacteria. In light of the foregoing, the empirical antifungal therapy administered at the time of the identification by RT-PCR was appropriate in all cases and was started an average of 48 hours earlier than the time the antimycogram was available.

The 30-day in-hospital mortality found in this study is lower than that reported by Koehler *et al*.,[27] and Muderris *et al*.[2] This finding is of interest, nevertheless, the effect of possible confounding variables such as the comorbidities of the patients included and the local epidemiology must be considered.

This study has indeed limitations. First, this is a retrospective observational study, therefore there is risk of selection bias along with the limitation of a small sample size. Second, the local prevalence and antifungal susceptibility found do not necessarily apply to other settings.

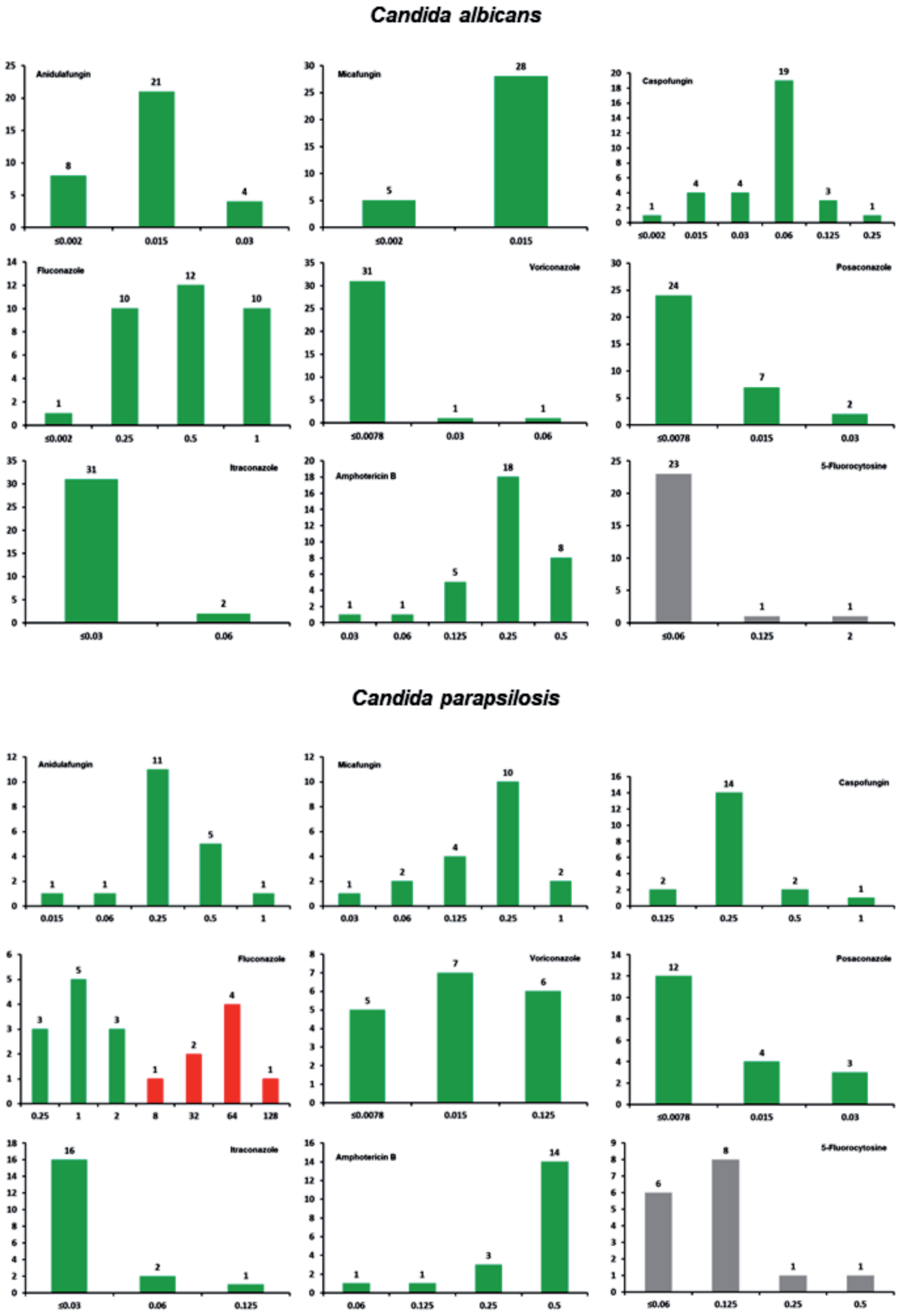

Figure 1. Minimum Inhibitory Concentration (MIC) distributions of the main antifungal agents against *Candida albicans* and *Candida parapsilosis*. MIC values are displayed on the X-axis as mg/L, on the Y-axis the frequency is described. Green: susceptible, standard dosing regimen; yellow: susceptible, increased exposure; red: resistant; gray: no clinical breakpoints available in EUCAST.

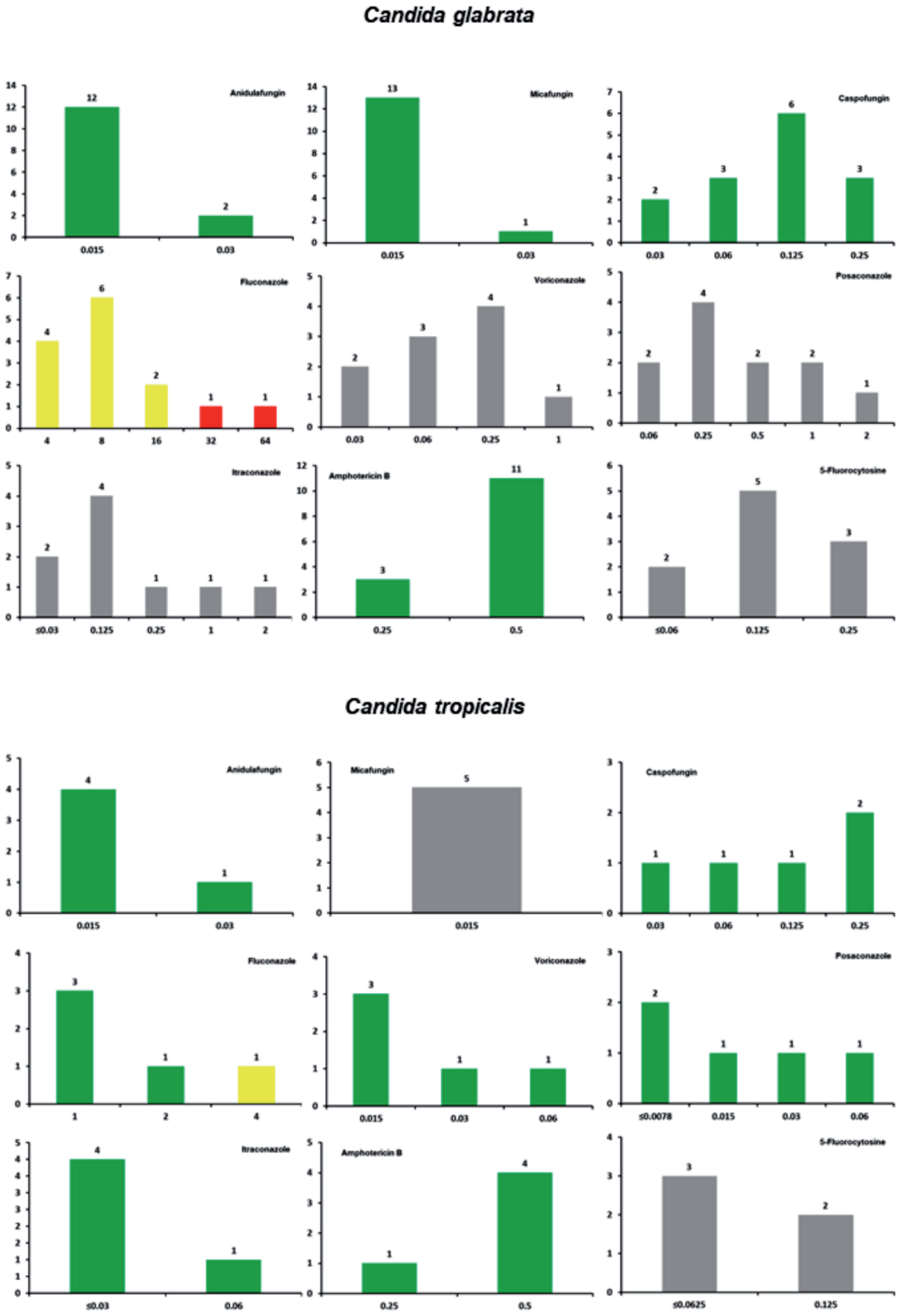

Figure 2. Minimum Inhibitory Concentration (MIC) distributions of the main antifungal agents against *Candida glabrata* and *Candida tropicalis*. MIC values are displayed on the X-axis as mg/L, on the Y-axis the frequency is described. Green: susceptible, standard dosing regimen; yellow: susceptible, increased exposure; red: resistant; gray: no clinical breakpoints available in EUCAST.

Conclusions

This study showed how a rapid identification of an invasive fungal isolate, along with the local antifungal susceptibility patterns of the isolates, could be of help in choosing an appropriate antifungal therapy during candidemia with an ongoing antimycogram. These findings, when confirmed by further studies with larger sample size, could be considered in antifungal stewardship programs and clinical practice guidelines.

Correspondence:Christian Leli, Microbiology Laboratory, Azienda Ospedaliero-Universitaria SS. Antonio e Biagio e Cesare Arrigo, via Venezia 16, 15121 Alessandria, Italy.
Tel. +39 0131 207440; Fax +39 0131 206854.
E-mail: christian.leli@ospedale.al.it

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