Phenotypic typing and epidemiological survey of antifungal resistance of *Candida* species detected in clinical samples of Italian patients in a 17 months' period

Margherita Scapaticci^{1,*}, Andrea Bartolini², Federica Del Chierico³, Cristel Accardi⁴, Francesco Di Girolamo⁵, Andrea Masotti⁶, Maurizio Muraca⁷, Lorenza Putignani⁸

Abstract

Introduction Yeast pathogens are emerging agents of nosocomial as well as community-acquired infections and their rapid and accurate identification is crucial for a better management of high-risk patients and for an adequate treatment.

Methods We performed a retrospective review of 156 yeast isolates collected during a 17 months' period of regular clinical practice at the Microbiology Department of San Camillo Hospital in Treviso, Italy and analyzed by the traditional culture-based method combined with matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS).

Results Out of all the samples collected MALDI-TOF MS was able to characterize with a MT score ≥ 1.7 (accurate result at species level) 12 different yeast and yeast-like species from 140 samples: *Candida albicans* (63.7%), *Candida glabrata* (13.6%), *Saccharomyces cerevisiae* (6.5%), *Candida parapsilosis* (5.7%), *Candida tropicalis* (2.1%), *Candida pararugosa* (2.1%), *Candida guilliermondii* (2.1%), *Candida kefyr* (1.4%), *Candida lusitanae* (0.7%), *Candida palmiophila* (0.7%), *Geotrichum silvicola* (0.7%), *Rhodotorula mucilaginosa* (0.7%). Susceptibility testing toward seven common antifungal agents showed a characteristic MIC distribution of *C. albicans* isolates for echinocandins: particularly we noticed that 72% and 46% of *C. albicans* showed an MIC value close to clinical breakpoint as defined by EUCAST, respectively for anidulafungin and micafungin.

Conclusion Accurate identification of microorganisms and the study of their antifungal susceptibility allow to understand the epidemiology of a particular area, permitting the choice of the most appropriate early antifungal treatment.

Keywords Yeast infection, MALDI-TOF MS, antifungal resistance

Introduction

Yeast pathogens are emerging agents of nosocomial and community-acquired infections, local or disseminated, particularly in

immunocompromised patients (intensive care unit, cancer, neutropenic patients) and their incidence has greatly increased in the last few years.¹

Received: 21 January 2018; revised: 11 March 2018; accepted: 12 March 2018.

¹PhD, Laboratory Medicine Department, San Camillo Hospital, Viale Vittorio Veneto 18, 31100, Treviso, Italy;

²MD, Laboratory Medicine Department, San Camillo Hospital, Viale Vittorio Veneto 18, 31100, Treviso, Italy;

³PhD, Unit of Human Microbiome, Children's Hospital and Research Institute Bambino Gesù, Piazza Sant'Onofrio 4, Rome, 00165, Italy; ⁴Unit of Human Microbiome, Children's Hospital and Research Institute Bambino Gesù, Piazza Sant'Onofrio 4, Rome, 00165, Italy; ⁵Unit of Human Microbiome, Children's Hospital and Research Institute Bambino Gesù, Piazza Sant'Onofrio 4, Rome, 00165, Italy;

⁶PhD, Gene Expression-Microarrays Laboratory, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4,

Rome 00165, Italy; ⁷MD, Department of Women's and Children's Health University of Padova, Via Giustiniani 3, Padova, Italy; ⁸PhD, Unit of Parasitology, Children's Hospital and Research Institute Bambino Gesù, Piazza Sant'Onofrio 4, Rome, 00165, Italy.

*Corresponding author: Margherita Scapaticci, PhD, Laboratory Medicine Department, San Camillo Hospital, Viale Vittorio Veneto 18, 31100, Treviso, Italy. scapaticci.m@gmail.com

Article downloaded from www.germs.ro

Published June 2018

© GERMS 2018

ISSN 2248 - 2997

ISSN - L = 2248 - 2997

Until recently, only few pathogenic species of *Candida* were known. However, recent years have seen an increase in the number of species responsible for human infections.² *C. albicans* currently represents the most commonly isolated species, while other species that cause superficial and/or invasive infections diversify into *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. kefyr* and the emerging multidrug resistant *Candida auris*.³

In addition, yeasts such as *Saccharomyces* are often considered the cause of serious infections in high-risk patients, while *Rhodotorula mucilaginosa* is widely distributed in the environment.⁴ Although it was once considered to be less virulent than *Candida* spp., *Rhodotorula* spp. is now considered a potential pathogen in patients treated with immunosuppressant drugs and bearers of central venous catheter.⁴ A late diagnosis and incorrect treatment can significantly contribute to the high mortality associated with invasive fungal infections. Even if the ESCMID recommendations for the therapeutic management of candidemia do not differentiate the therapeutic approach according to the species, meaning that as first line therapy a presumptive large spectrum antifungal drug (in practice echinocandins) can be used whatever is the causative species, rapid and accurate identification (ID) methods for fungal species causing invasive mycoses are crucial for a better management of high-risk patients and for an adequate treatment.⁵ Although the standardization of antifungal susceptibility testing associated a huge advance, some non-*albicans* species may be intrinsically resistant to first-line antifungal agents.^{6,7} For these reasons, in addition to the traditional ID methods, it is extremely important to apply new technologies and diagnostic pipelines able to provide specific information on properties of the pathogenic microorganisms during infection treatment, to aid clinicians in the therapeutic management of infections rather than to provide ordinary pathogen ID. Conventional phenotypic methods may fail to capture the growing diversity of fungal pathogens while offering inconclusive results, especially for unusual yeasts, because phenotypic

tests often require some hours or even days for only ID results.^{8,9}

In this regard, MS (mass spectrometry), particularly MALDI-TOF MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry), has been suggested as an effective alternative for microbial characterization thanks to the high efficiency of proteome profiling, allowing rapid and accurate ID of pathogenic microorganisms, including yeasts and filamentous fungi of medical interest.¹⁰⁻¹² One of the advantages of this technique is represented by the possibility to simultaneously and rapidly analyze complex proteins or peptide mixtures without a prior separation, or to analyze directly whole cells, with minimal sample preparation and negligible consumable costs.¹³⁻¹⁵

The aim of this study was to carry out an analysis of *Candida* spp. and yeasts isolated in a period of 17 months from clinical samples collected at the Microbiology Department of San Camillo Hospital in Treviso, using either traditional culture-based and MALDI-TOF MS-based techniques, and to investigate their susceptibility distribution to azoles, echinocandins, and amphotericin B, in order to give useful information for comparative epidemiological studies of antifungal resistance pattern in the Italian population.

Methods

This work is a retrospective review of laboratory results yielded in regular clinical practice during 17 months. A total of 156 clinical consecutive yeasts isolates from in- and out-patient samples (i.e., vaginal swab, urine, stools, nail fragments, skin swabs, bone fragments, oropharyngeal swabs) were collected at the Microbiology Unit of San Camillo Hospital of Treviso, Italy, from August 2013 to November 2014. Yeast isolates were processed using conventional ID methods based on biochemical characterization and also, they were identified by MALDI-TOF MS-based method at the Parasitology Unit of Bambino Gesù Hospital of Rome.

Phenotypic characterization by culture and biochemical assays

Sample cultures were performed on Sabouraud agar supplemented by chloramphenicol (BD Biosciences, Franklin Lakes, NJ, USA) for 48 hours at 37 °C in a dry incubator according to routine diagnostic conditions employed at the Microbiology Unit. Subsequently, colony morphology, texture, and color were examined on BBL CHROMagar *Candida* plates (BD Biosciences) for preliminary *C. albicans*, *C. tropicalis* and *C. krusei* ID.¹⁶ ATCC reference strains such as *C. albicans* ATCC 10231 and *C. krusei* ATCC 6258 (Oxoid, Thermo Fisher Scientific, Basingstoke, UK) were used as controls in phenotypic characterization.

Anti-fungal susceptibility testing

Anti-fungal susceptibility tests were performed by using Sensititre® YeastOne® YO10 cards, according to the manufacturer's instructions (TREK Diagnostic Systems, Cleveland, OH, USA). The test used broth microdilution method to provide qualitative (S=sensitive, I=intermediate or R=resistant) and quantitative results, related to the minimum inhibitory concentration (MIC), by a 96 wells' plate containing antimicrobial agents in dehydrated form and in appropriate dilutions. The antifungal agents assayed were: amphotericin B (AMB), itraconazole (ITC), voriconazole (VRC), posaconazole (POS), fluconazole (FLU), caspofungin (CAS), micafungin (MFG), anidulafungin (AFG).^{6,17}

Susceptibility profiles were optically evaluated with strain growth associated to blue chromogen (S) or rose-red (R) indicator change. All MIC values were evaluated with EUCAST clinical breakpoint tables for *Candida* spp. and *Aspergillus* spp. Statistical and epidemiological analysis of antifungal susceptibility was reevaluated according to the latest version of EUCAST guidelines (v.9.0) to make the data applicable to the current epidemiological reality.¹⁸

Spectra generation and interpretation

After growth of fungal isolates three or four colonies from each sample were mixed

thoroughly in 300 mL double-distilled water. Absolute ethanol (900 µL) (Sigma-Aldrich, Milan, Italy) was added, tube contents carefully mixed, centrifuged at 14,000 g for 2 min at room temperature (RT), the supernatant discarded and the pellet air dried. The pellet was mixed thoroughly with 50 µL of formic acid (70%) (Sigma-Aldrich), before addition of an equivalent volume of acetonitrile (Sigma-Aldrich). The mixture was centrifuged at 14,000 g for 2 min, and 1.5 µL of the supernatant from each sample (i.e., two spots for each) were placed onto a MSP 96 polished steel target (Bruker Daltonik GmbH, Bremen, Germany) and allowed to dry at RT. Each sample spot was overlaid with 1 µL of matrix, which consisted of a saturated solution of α -cyano-4 hydroxycinnamic acid (HCCA) in 50% acetonitrile- and 2.5% trifluoroacetic acid (Sigma-Aldrich) (final concentration 10 mg HCCA/mL) and air-dried at RT.

Measurements were performed with a Microflex LT mass spectrometer (Bruker Daltonik GmbH) using FlexControl software (version 3.0, Bruker Daltonik GmbH). Spectra were recorded in the linear positive mode at a laser frequency of 20 Hz (ion source 1 voltage, 20 kV; ion source 2 voltage, 18.4 kV; lens voltage, 9.1 kV) within a mass range from 2,000 to 20,137 Da. Each sample was tested in duplicate to assess spectra reproducibility. Two hundred laser shots per sample spot were acquired employing Flex Control software package. Spectra were internally calibrated by using an *Escherichia coli* ribosomal proteins BTS (Bacterial Test Standard, Bruker Daltonik GmbH) according to the manufacturer's instructions. All the produced spectra were visually inspected before statistical analysis into BioTyper software (version 3.0; Bruker Daltonik GmbH) that provided the pattern matching with default settings. Results of the pattern-matching process were expressed with MT scores ranging from 0 to 3.0 as proposed by the manufacturer. For each isolate, the highest score of a match against a spectrum in the customized database was used for identification. Scores below 1.7 were considered not to have generated a reliable

identification, a score ≥ 1.7 was used for reliable species identification.

Statistical analysis

Data were analyzed using Pearson's Chi-squared test and Mann-Whitney U test. Furthermore, the degree of concordance between results obtained by traditional methods and MALDI-TOF MS was calculated using Cohen's kappa coefficient. Statistical significance was defined as a two-tailed $p < 0.05$ for all analyses which were carried out using the Stata 12 software package (College Station, TX, USA).

and from May (N=24) to June 2014 (N=13) (Figure 1A), even if the Mann-Whitney U Test did not show any statistical association ($p > 0.05$) between yeast isolation and their seasonal-driven occurrence.

Out of the 156 fungal isolates, 75 were isolated from vaginal swabs (48%), 30 from urine (19%), 26 from feces (17%), and 25 from other sites (16%) (Figure 1B). One-hundred and seventeen of 156 samples derived from outpatients (75%), the remaining 39 belonged to hospitalized patients, specifically 32 were isolated from patients admitted to the medical wards (82%) and seven from patients admitted to surgical wards (18%) (Figure 1C).

Figure 1. Graphical distribution of *Candida* spp. isolates collected from August 2013 to November 2014 (N=156)

The strains are grouped on the basis of collection date (Panel A), sample type (Panel B), ward distribution (Panel C), and species distribution by traditional ID methods (Panel D).

Results

One-hundred and fifty-six yeast isolates were collected at the Microbiology Unit of San Camillo Hospital of Treviso from August 2013 to November 2014. The median number of yeast isolations was eight per month, but we observed an increment in the isolation number in November 2013 (N=13), March 2014 (N=18)

Using conventional microbiological ID methods, it was possible to identify at species level only three types of *Candida* spp. (*C. albicans*, *C. tropicalis* and *C. krusei*), while for the remaining samples the identification was limited to genus *Candida*. Particularly, 101 out of 156 strains were identified as *C. albicans* (64.7%),

Figure 2. MALDI-TOF MS spectra of the 12 yeast and yeast-like species identified by MT ID originated by MALDI Biotyper Software analysis

The m/z values are expressed in Da and the amplitudes are reported in a scale of intensity arbitrary units (a.u.). The MT IS score was in all cases >1.7 .

two out of 156 as *C. tropicalis* (1.2%) and one out of 156 as *C. krusei* (0.6%) (Figure 1D).

To compare and refine on conventional yeast identification, MALDI-TOF MS biotyper was applied to all strains, allowing the accurate identification at species level (MT score ≥ 1.7) of 12 different yeast and yeast-like species from 140 samples: *C. albicans* (63.7%), *C. glabrata* (13.6%), *Saccharomyces cerevisiae* (6.5%), *C. parapsilosis* (5.7%), *C. tropicalis* (2.1%), *C. pararugosa* (2.1%), *C. guilliermondii* (2.1%), *C. kefyr* (1.4%), *C.*

lusitaniae (0.7%), *C. palmiophila* (0.7%), *Geotrichum silvicola* (0.7%), *R. mucilaginosa* (0.7%) (MS data are reported in Figure 2). One-hundred and thirteen strains of the 140 (80.7%) showed a best MT score ≥ 2.0 .

By MALDI-TOF MS we obtained 91 identifications (65.0%) in agreement with the traditional identification method, with a modest degree of concordance (Cohen's kappa coefficient of 0.281). Three strains (2.2%) showed discrepancy between the two methods: a

presumptive *C. krusei* was identified as *S. cerevisiae* by MALDI TOF MS, while two strains identified as *C. albicans* by traditional methods were *C. kefyr* and *C. parapsilosis*. Moreover, for 46 strains (32.8%), previously identified as *Candida* spp., the MALDI-TOF MS method overcame the traditional one, since this method was able to give an identification at the species level. The susceptibility distribution toward seven common antifungal agents were assessed for the 140 samples identified by MALDI-TOF. Susceptibility breakpoints and MIC categorization for antifungal susceptibility analysis were performed by applying interpretative criteria as published by EUCAST (Table 1).¹⁸

As showed in Table 1, all *C. albicans* isolated were sensitive to fluconazole (FLC), voriconazole (VRC), posaconazole (POS) and amphotericin B (AMB), while 20.2% of them showed resistance to micafungin (MCF) and anidulafungin (ANF) with MIC values very close to the clinical breakpoint (CB) (Figure 3). Overall, 5.3% of *C. glabrata* showed resistance to MCF and 10.5% to ANF, while all of them showed sensitivity against AMB and intermediate sensitivity to FLC; 12.5% and 25% of *C. parapsilosis* showed resistance to itraconazole (ITC) and POS, respectively. Finally, 66.7% of *C. tropicalis* were I to FLC and 33.3% were R to ANF. For all the other yeasts and *Candida* spp. isolated we found that 25% were I and 33% R to FLC. No strain showed resistance against VRC and AMB.

Moreover, analyzing resistance to antifungals based on the type of yeast and on the type of samples we found a significant association only between resistance to anidulafungin and *C. albicans* isolates collected from genital samples (vaginal swabs, urethral swabs and vulvar swabs) with a $p=0.013$, calculated by Pearson's Chi-squared test.

Discussion

In order to correctly and quickly identify the causative agent of yeast infections and to promptly choose the correct therapy it is necessary to set up fast and reliable ID methods that can help to better understand yeast and

yeast-like species-specific susceptibility patterns. Chromogenic agar media and biochemical or enzymatic panels may be occasionally disadvantageous due to their limited range of species identification profiles.¹⁹ In fact, using only conventional culture-based ID methods, some less common species (i.e, strains of *S. cerevisiae*, *C. parapsilosis*, *C. kefyr*, *C. lusitanae*, and *R. mucilaginosa*) can be often misidentified or, simply, cannot be identified at all. For this reason, microbiologists are forced to deal with not-identified or misidentified yeast strains with the consequent clinical risk of ineffective treatments for several microorganisms.²⁰

Since the advent of ionization techniques, MS has become a standard method of protein analysis and TOF instruments have been used for proteomics applications, specifically in combination with MALDI procedures.^{21,22} In fact, MALDI-TOF MS appears as an efficient strain ID/epidemiological typing tool, generating data with negligible cost, and allowing the elimination of inappropriate biochemical tests.

Although MALDI TOF MS is actually used predominantly as a rapid identification method and it does not provide information about susceptibility to antimicrobial agents, its contribution to targeted therapy could be essential because a correct identification allows the selection of antifungals based on the knowledge of local epidemiological data regarding antifungal susceptibility of different fungal genera or species and may help in decreasing hospital length of stay and total care costs; for example they could be crucial in clinical management of new emerging multi-drug resistant *Candida* spp. such as *C. auris* that is currently causing outbreaks in healthcare settings worldwide.^{3,23}

In addition to species-specific antifungal resistance patterns (Table 1), in our survey we detected a characteristic MIC distribution of *C. albicans* isolates for echinocandins: particularly, for both anidulafungin and micafungin, we noticed that 64 isolates out of 89 (71.9%) and 41 strains out of 89 (46.1%), respectively, showed a MIC value close to clinical breakpoint (Figure 3) and in this context it is important to underline

Table 1. Antifungal susceptibility of *Candida* spp. isolates (N=140)

Species (no. tested/detected)	FLC	VRC	ITC	POS	MCF	ANF	AMB
<i>C. albicans</i> (n=89/89)							
Range (mg/L)	≤0.12 – 1	≤0.008 – 0.06	≤0.015 – 0.25	≤0.008 – 0.06	≤0.008 – 0.12	≤0.015 – 0.06	≤0.12 – 1
S (%)	100	100	95.5	100	79.8	79.8	100
I (%)	0	–	–	–	–	–	–
R (%)	0	0	4.5	0	20.2	20.2	0
<i>C. glabrata</i> (n=19/19)							
Range (mg/L)	0.5 – 32	≤0.008 – 0.25	0.06 – 0.5	0.06 – 1	≤0.008 – 0.06	0.03 – 0.12	≤0.12 – 0.5
S (%)	0	IE	IE	IE	94.7	89.5	100
I (%)	100	IE	IE	IE	–	–	–
R (%)	0	IE	IE	IE	5.3	10.5	0
<i>C. parapsilosis</i> (n=8/8)							
Range (mg/L)	0.25 – 8	≤0.008 – 0.12	0.03 – 0.5	0.015 – 0.25	0.25 – 2	0.25 – 2	≤0.12 – 0.25
S (%)	62.5	100	87.5	75	0	0	100
I (%)	25	–	–	–	100	100	–
R (%)	12.5	0	12.5	25	0	0	0
<i>C. tropicalis</i> (n=3/3)							
Range (mg/L)	1 – 4	0.12	0.25	0.12 – 0.25	0.015 – 0.03	0.03 – 0.25	≤0.12 – 1
S (%)	33.3	100	0	0	IE	66.7	100
I (%)	66.7	–	–	–	IE	–	–
R (%)	0	0	100	100	IE	33.3	0
<i>Candida</i> spp. and others ^a (n=15/21)							
Range (mg/L)	0.25 – 16	≤0.008 – 0.25	0.03 – 0.5	≤0.008 – 0.5	≤0.008 – 8	≤0.015 – 4	≤0.12 – 1
S (%)	42 ^b	IE	IE	IE	IE	IE	IE
I (%)	25 ^b	IE	IE	IE	IE	IE	IE
R (%)	33 ^b	IE	IE	IE	IE	IE	IE

AMB – amphotericin B; ANF – anidulafungin; FLC – fluconazole; I – intermediate; ITC – itraconazole; MCF – micafungin; POS – posaconazole; S – susceptible; R – resistant; VRC – voriconazole.

IE indicates that there is insufficient evidence that the species in question is a good target for therapy with the drug. EUCAST suggest that a MIC value with a comment but without an accompanying S, I or R categorization may be reported.

^a*Saccharomyces cerevisiae* (n=9), *C. guilliermondii* (n=3), *C. parapsilosis* (n=3), *C. kefyr* (n=2), *C. lusitanae* (n=1), *C. palmiophila* (n=1), *Geotrichum silvicola* (n=1), *Rhodotorula mucilaginosa* (n=1).

^bInterpretation, according to EUCAST, available for 12/15 isolates.

that CB is equivalent to epidemiological cut-off (ECOFF) as defined by EUCAST.^{18,24} Comparing our study with those of other Italian authors it is interesting to note that we found a higher

percentage of *C. albicans* resistant to echinocandins (according to EUCAST).²⁵

Our study has the limitation of being an observational laboratory-based survey, without clinical evidences about type and duration of

Figure 3. MIC distribution of echinocandins for *Candida albicans* isolates (N=89)

The arrows indicate EUCAST Clinical Breakpoint (CB) and epidemiological cut-off values (ECOFF) for micafungin (**Panel A**) and anidulafungin (**Panel B**).

antifungal therapy, and molecular resistance mechanisms were not actually investigated; for these reasons further studies are needed.

Conclusion

An accurate identification of microorganisms and the study of their antimicrobial susceptibility allow the understanding of the epidemiology of a particular area, permitting the most appropriate choice of early antifungal treatment. Until now, in Italy, studies about in vitro susceptibility of echinocandins against *Candida* isolates using EUCAST methods are rarely reported.²⁵

MS-based diagnostic methods, coupled with reasoned antimicrobial strategy, may provide a substantial alternative to conventional laboratory methods allowing advanced clinical management of patients.

Authors' contributions statement: MS designed the study, performed the laboratory experiments, collected the data, interpreted the findings and wrote the paper. AB collected the data, interpreted the findings and wrote the paper. FDC, CA, FDG, AM helped interpret the findings. MM, LP coordinated the study, supervised the laboratory experiments and helped interpret the findings. All authors read and approved the final manuscript.

Conflicts of interest: All authors – none to disclose.

Funding: None to declare

References

1. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis 2009;48:503-35. [\[Crossref\]](#)
2. Sullivan DJ, Coleman DC. *Candida dubliniensis*: characteristics and identification. J Clin Microbiol 1998;31:329-34.

3. Ben-Ami R, Berman J, Novikov A, et al. Multidrug-resistant *Candida haemulonii* and *C. auris*, Tel Aviv, Israel. *Emerg Infect Dis* 2017;23:195-203. [\[Crossref\]](#)
4. Fell JW, Boekhout T, Fonseca A, Scorzetti G, Statzell-Tallman A. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. *Int J Syst Evol Microbiol* 2000;50 Pt3:1351-71. [\[Crossref\]](#)
5. Cornely OA, Bassetti M, Calandra T, et al. ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: non-neutropenic adult patients. *Clin Microbiol Infect* 2012;18 Suppl 7:19-37. [\[Crossref\]](#)
6. Cuenca-Estrella M, Gomez-Lopez A, Alastruey-Izquierdo A, et al. Comparison of the Vitek 2 Antifungal Susceptibility System with the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution reference methods and with the Sensititre YeastOne and Etest techniques for in vitro detection of antifungal resistance in yeast isolates. *J Clin Microbiol* 2010;48:1782-6. [\[Crossref\]](#)
7. Rodriguez-Tudela JL, Alcazar-Fuoli L, Cuesta I, et al. Clinical relevance of resistance to antifungals. *Int J Antimicrob Agents* 2008;32 Suppl 2:S111-3. [\[Crossref\]](#)
8. Posteraro B, Torelli R, De Carolis E, Posteraro P, Sanguinetti M. Update on the laboratory diagnosis of invasive fungal infections. *Mediterr J Hematol Infect Dis* 2011;3:e2011002. [\[Crossref\]](#)
9. Risch M, Radjenovic D, Han JN, Wydler M, Nydegger U, Risch L. Comparison of MALDI TOF with conventional identification of clinically relevant bacteria. *Swiss Med Wkly* 2010;140:w13095. [\[Crossref\]](#)
10. Dworzanski JP, Snyder AP. Classification and identification of bacteria using mass spectrometry-based proteomics. *Expert Rev Proteomics* 2005;2:863-78. [\[Crossref\]](#)
11. Claydon MA, Davey SN, Edwards-Jones V, Gordon DB. The rapid identification of intact microorganisms using mass spectrometry. *Nat Biotechnol* 1996;14:1584-6. [\[Crossref\]](#)
12. Marinach-Patrice C, Fekkar A, Atanasova R, et al. Rapid species diagnosis for invasive candidiasis using mass spectrometry. *PLoS One* 2010;25;5:e8862. [\[Crossref\]](#)
13. Dhiman N, Hall L, Wohlfiel SL, Buckwalter SP, Wengenack NL. Performance and cost analysis of matrix-assisted laser desorption ionization-time of flight mass spectrometry for routine identification of yeast. *J Clin Microbiol* 2011;49:1614-6. [\[Crossref\]](#)
14. Bizzini A, Durussel C, Bille J, Greub G, Prod'hom G. Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. *J Clin Microbiol* 2010;48:1549-54. [\[Crossref\]](#)
15. Hsieh SY, Tseng CL, Lee YS, et al. Highly efficient classification and identification of human pathogenic bacteria by MALDI-TOF MS. *Mol Cell Proteomics* 2008;7:448-56. [\[Crossref\]](#)
16. Pfaller MA, Huston A, Coffman S. Application of CHROMagar *Candida* for rapid screening of clinical specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida (Torulopsis) glabrata*. *J Clin Microbiol* 1996;34:58-61.
17. Arendrup MC, Meletiadis J, Mouton KW, et al. Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts. (2017). EUCAST definitive document E.DEF 7.3.1.
18. The European Committee on Antimicrobial Susceptibility Testing (EUCAST): Clinical Breakpoints. Accessed on: 26 May 2018. Available at: http://www.eucast.org/clinical_breakpoints/
19. Murray CK, Beckius ML, Green JA, Hospenthal DR. Use of chromogenic medium for the isolation of yeasts from clinical specimens. *J Med Microbiol* 2005;54:981-5. [\[Crossref\]](#)
20. Zaragoza O, Mesa-Arango AC, Gómez-López A, Bernal-Martinez L, Rodríguez-Tudela JL, Cuenca-Estrella M. Process analysis of variables for standardization of antifungal susceptibility testing of nonfermentative yeasts. *Antimicrob Agents Chemother* 2011;55:1563-70. [\[Crossref\]](#)
21. Hillenkamp F, Karas M, Beavis RC, Chait BT. Matrix-assisted laser desorption/ionization mass spectrometry of biopolymers. *Anal Chem* 1991;63:1193A-2203A. [\[Crossref\]](#)
22. Amiri-Eliasi B, Fenselau C. Characterization of protein biomarkers desorbed by MALDI from whole fungal cells. *Anal Chem* 2001;73:5228-31. [\[Crossref\]](#)
23. Perez KK, Olsen RJ, Musick WL, et al. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. *Arch Pathol Lab Med* 2013;137:1247-54. [\[Crossref\]](#)
24. The European Committee on Antimicrobial Susceptibility Testing (EUCAST): MIC distributions and ECOFFs. Accessed on: 28 August 2017. Available at: http://www.eucast.org/mic_distributions_and_ecoffs/
25. Montagna MT, Lovero G, Coretti C, et al. Susceptibility to echinocandins of *Candida* spp. strains isolated in Italy assessed by European Committee for Antimicrobial Susceptibility Testing and Clinical Laboratory Standards Institute broth microdilution methods. *BMC Microbiol* 2015;15:106. [\[Crossref\]](#)

Please cite this article as:

Scapaticci M, Bartolini A, Del Chierico F, Accardi C, Di Girolamo F, Masotti A, Muraca M, Putignani L. Phenotypic typing and epidemiological survey of antifungal resistance of *Candida* species detected in clinical samples of Italian patients in a 17 months' period. *GERMS* 2018;8(2):58-66. doi: 10.18683/germs.2018.1132.