Journal of Infection 87 (2023) 428–437

Contents lists available at ScienceDirect

Journal of Infection

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

European candidaemia is characterised by notable differential epidemiology and susceptibility pattern: Results from the ECMM *Candida* III study

Maiken Cavling Arendrup [a,b,c,*,1], Sevtap Arikan-Akdagli [d,2], Karin Meinike Jørgensen [a], Aleksandra Barac [e,3], Jörg Steinmann [f,4], Cristina Toscano [g], Valentina Arsic Arsenijevic [h,5], Assunta Sartor [i,6], Cornelia Lass-Flörl [j,7], Axel Hamprecht [k,l,8], Tadeja Matos [m,9], Benedict R.S. Rogers [n,10], Inmaculada Quiles [o], Jochem Buil [p,q,r,11], Volkan Özenci [s,t,12], Robert Krause [u,v], Matteo Bassetti [w,x,13], Laura Loughlin [y,14], Blandine Denis [z], Anna Grancini [aa], P. Lewis White [ab,15], Katrien Lagrou [ac,ad,16], Birgit Willinger [ae], Riina Rautemaa-Richardson [af,ag,17], Petr Hamal [ah,18], Beyza Ener [ai,19], Tugce Unalan-Altintop [d,20], Ebru Evren [aj,21], Suleyha Hilmioglu-Polat [ak,22], Yasemin Oz [al,23], Ozlem Koyuncu Ozyurt [am,24], Faruk Aydin [an,25], Filip Růžička [ao,26], Eelco F.J. Meijer [p,q,r,27], Jean Pierre Gangneux [ap,28], Deborah E.A. Lockhart [aq,ar,29], Nina Khanna [as,30], Clare Logan [at,au,31], Ulrike Scharmann [av,32], Guillaume Desoubeaux [aw,33], Emmanuel Roilides [ax,34], Alida Fe Talento [ay,35], Karin van Dijk [az,36], Philipp Koehler [ba,bb,37], Jon Salmanton-García [ba,bb,38], Oliver A. Cornely [ba,bb,39], Martin Hoenigl [u,v,**,40]

[a] *Unit of Mycology, Statens Serum Institut, Copenhagen, Denmark*
[b] *Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark*
[c] *Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark*
[d] *Department of Medical Microbiology, Hacettepe University Medical School, Ankara, Turkey*
[e] *Clinic for Infectious and Tropical Diseases, University Clinical Center of Serbia, Belgrade, Serbia*
[f] *Institute for Clincal Hygiene, Medical Microbiology and Infectiology, Paracelsus Medical University, Klinikum Nürnberg, Nuremberg, Germany*
[g] *Microbiology Laboratory, Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal*
[h] *Faculty of Medicine University of Belgrade, Institute of Microbiology and Immunology, Medical Mycology Reference Laboratory (MMRL), Belgrade, Serbia*
[i] *SC Microbiology, Department of Laboratory Medicine, Friuli Centrale University Health Authority, Udin, Italy*
[j] *Institute of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria*
[k] *University of Cologne, University Hospital Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Cologne, Germany*
[l] *University of Oldenburg, Institute for Medical Microbiology and Virology, Oldenburg, Germany*
[m] *Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, Slovenia*
[n] *Department of Clinical Microbiology, University Hospitals of Leicester NHS Trust, Leicester, United Kingdom*
[o] *Department of Microbiology, La Paz University Hospital, Madrid, Spain*
[p] *Canisius Wilhelmina Hospital (CWZ), Medical Microbiology and Infectious Diseases, Nijmegen, the Netherlands*
[q] *Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, the Netherlands*
[r] *Center of Expertise in Mycology Radboudumc/CWZ, Nijmegen, the Netherlands*
[s] *Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Sweden*
[t] *Department of Clinical Microbiology, Karolinska University Hospital, Huddinge, Stockholm, Sweden*
[u] *Biotech Med, Graz, Austria*
[v] *Division of Infectious Diseases, Department of Internal Medicine, Medical University of Graz, Graz, Austria*
[w] *Infectious Diseases Unit, IRCCS San Martino Polyclinic Hospital, Genoa, Italy*
[x] *Department of Health Sciences (DISSAL), University of Genoa, Genoa, Italy*
[y] *Belfast Health and Social Care Trust, Belfast, United Kingdom*
[z] *Department of Infectious Diseases, Hôpital Saint-Louis, Fernand Widal, Lariboisière, AP-HP, Paris, France*

[*] Correspondence to: Statens Serum Institut, Artillerivej 5, Unit for Mycology (Building 45-112), DK-2300 Copenhagen S, Denmark.
[**] Correspondence to: Division of Infectious Diseases, Department of Internal Medicine, Medical University of Graz, Auenbruggerplatz 15, 8036 Graz, Austria.
E-mail addresses: maca@ssi.dk (M.C. Arendrup), hoeniglmartin@gmail.com, martin.hoenigl@medunigraz.at (M. Hoenigl).

https://doi.org/10.1016/j.jinf.2023.08.001
0163-4453/© 2023 The Author(s). Published by Elsevier Ltd on behalf of The British Infection Association. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

[aa] U.O.S Microbiology – Analysis Laboratory, IRCCS Foundation, Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[ab] Public Health Wales Microbiology Cardiff and Cardiff University School of Medicine, United Kingdom
[ac] Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium
[ad] Department of Laboratory Medicine and National Reference Center for Mycosis University Hospitals Leuven, Leuven, Belgium
[ae] Division of Clinical Microbiology, Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria
[af] Mycology Reference Centre Manchester and Department of Infectious Diseases, Wythenshawe Hospital, Manchester University NHS Foundation Trust, Manchester, United Kingdom
[ag] Division of Evolution, Infection and Genomics, Faculty of Biology, Medicine and Health, University of Manchester, United Kingdom
[ah] Department of Microbiology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic
[ai] Department of Medical Microbiology, Bursa Uludağ University Medical School, Bursa, Turkey
[aj] Department of Medical Microbiology, Ankara University Medical School, Ankara, Turkey
[ak] Department of Medical Microbiology, Ege University Medical School, Izmir, Turkey
[al] Department of Medical Microbiology, Eskisehir Osmangazi University Medical School, Eskisehir, Turkey
[am] Department of Medical Microbiology, Akdeniz University Medical School, Antalya, Turkey
[an] KTÜ Tıp Fakültesi Tıbbi Mikrobiyoloji AbD, Trabzon, Turkey
[ao] Masaryk University, Faculty of Medicine and St. Anne's Faculty Hospital, Department of Microbiology, Brno, Czech Republic
[ap] Univ Rennes, CHU Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail), UMR_S 1085, F-35000 Rennes, France
[aq] Department of Medical Microbiology, Aberdeen Royal Infirmary, Foresterhill, Aberdeen AB25 2ZN, United Kingdom
[ar] Institute of Medical Sciences, School of Medicine Medical Sciences & Nutrition, University of Aberdeen, Aberdeen AB25 2ZD, United Kingdom
[as] Division of Infectious Diseases and Hospital Epidemiology, University Hospital of Basel, Basel, Switzerland
[at] Clinical Infection Unit, St Georges University NHS Hospital Foundation Trust, Blackshaw Road, London, United Kingdom
[au] Institute of Infection & Immunity, St Georges University London, Cranmer Terrace, London, United Kingdom
[av] Institute of Medical Microbiology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany
[aw] Department of Parasitology-Mycology-Tropical medicine, CHRU Tours, Tours, France
[ax] Hippokration General Hospital, Infectious Diseases Department, Medical School, Aristotle University of Thessaloniki, Greece
[ay] Department of Microbiology, Beaumont Hospital, Dublin, Ireland
[az] Department of Medical Microbiology and Infection Control, Amsterdam University Medical Centers, Academic Medical Center, Amsterdam Infection and Immunity Institute, Amsterdam, the Netherlands
[ba] University of Cologne, Faculty of Medicine and University Hospital Cologne, Department I of Internal Medicine, and Excellence Center for Medical Mycology (ECMM), Cologne, Germany
[bb] University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Institute of Translational Research, Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Cologne, Germany

[1] **ORCID ID:** 0000-0002-4747-0144
[2] **ORCID ID:** 0000-0001-9807-6764
[3] **ORCID ID:** 0000-0002-0132-2277
[4] **ORCID ID:** 0000-0002-3181-3667
[5] **ORCID ID:** 0000-0001-8132-3300
[6] **ORCID ID:** 0000-0003-3766-6765
[7] **ORCID ID:** 0000-0002-2946-7785
[8] **ORCID ID:** 0000-0003-1449-5780
[9] **ORCID ID:** 0000-0002-5696-1412
[10] **ORCID ID:** 0000-0002-7041-6744
[11] **ORCID ID:** 0000-0003-4031-0778
[12] **ORCID ID:** 0000-0002-8069-4027
[13] **ORCID ID:** 0000-0002-0145-9740
[14] **ORCID ID:** 0000-0002-7367-3287
[15] **ORCID ID:** 0000-0003-3056-4205
[16] **ORCID ID:** 0000-0001-8668-1350
[17] **ORCID ID:** 0000-0002-1071-6040
[18] **ORCID ID:** 0000-0002-5361-8125
[19] **ORCID ID:** 000-0002-4803-8206
[20] **ORCID ID:** 0000-0001-5318-2942
[21] **ORCID ID:** 0000-0001-7615-0521
[22] **ORCID ID:** 0000-0001-8850-2715
[23] **ORCID ID:** 0000-0003-2243-7644
[24] **ORCID ID:** 0000-0003-1260-0671
[25] **ORCID ID:** 0002-0139-908X
[26] **ORCID ID:** 0000-0001-5679-0513
[27] **ORCID ID:** 0000-0002-0226-024X
[28] **ORCID ID:** 0000-0002-4974-5607
[29] **ORCID ID:** 0000-0002-4262-3842
[30] **ORCID ID:** 0000-0002-2642-419X
[31] **ORCID ID:** 0000-0003-3037-8314
[32] **ORCID ID:** 0000-0001-7689-7799
[33] **ORCID ID:** 0000-0001-7945-9890
[34] **ORCID ID:** 0000-0002-0202-364X
[35] **ORCID ID:** 0000-0003-1271-2550
[36] **ORCID ID:** 0000-0002-2054-2857
[37] **ORCID ID:** 0000-0002-7386-7495
[38] **ORCID ID:** 0000-0002-6766-8297
[39] **ORCID ID:** 0000-0001-9599-3137
[40] **ORCID ID:** 0000-0002-1653-2824

ARTICLE INFO

Article history:
Accepted 3 August 2023
Available online 6 August 2023

Keywords:
Candida
C. parapsilosis
Fluconazole resistance
Echinocandin resistance
Fks1
EUCAST

SUMMARY

The objectives of this study were to assess *Candida* spp. distribution and antifungal resistance of candidaemia across Europe. Isolates were collected as part of the third ECMM *Candida* European multicentre observational study, conducted from 01 to 07-07-2018 to 31-03-2022. Each centre (maximum number/country determined by population size) included ~10 consecutive cases. Isolates were referred to central laboratories and identified by morphology and MALDI-TOF, supplemented by ITS-sequencing when needed. EUCAST MICs were determined for five antifungals. *fks* sequencing was performed for echinocandin resistant isolates. The 399 isolates from 41 centres in 17 countries included *C. albicans* (47.1%), *C. glabrata* (22.3%), *C. parapsilosis* (15.0%), *C. tropicalis* (6.3%), *C. dubliniensis* and *C. krusei* (2.3% each) and other species (4.8%). Austria had the highest *C. albicans* proportion (77%), Czech Republic, France and UK the highest *C. glabrata* proportions (25–33%) while Italy and Turkey had the highest *C. parapsilosis* proportions (24–26%). All isolates were amphotericin B susceptible. Fluconazole resistance was found in 4% *C. tropicalis*, 12% *C. glabrata* (from six countries across Europe), 17% *C. parapsilosis* (from Greece, Italy, and Turkey) and 20% other *Candida* spp. Four isolates were anidulafungin and micafungin resistant/non-wild-type and five resistant to micafungin only. Three/3 and 2/5 of these were sequenced and harboured *fks*-alterations including a novel L657W in *C. parapsilosis*. The epidemiology varied among centres and countries. Acquired echinocandin resistance was rare but included differential susceptibility to anidulafungin and micafungin, and resistant *C. parapsilosis*. Fluconazole and voriconazole cross-resistance was common in *C. glabrata* and *C. parapsilosis* but with different geographical prevalence.

© 2023 The Author(s). Published by Elsevier Ltd on behalf of The British Infection Association. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Introduction

The epidemiology of candidaemia has changed over the past decades. The proportion of *C. albicans* has declined from 70% to 80% in the 1980–90s[1,2] to ~40–60% in recent population based European reports.[3–6] In parallel, the proportion of *C. glabrata* has increased, particularly in Northern Europe, the US and Australia,[4–7] and the proportion of *C. parapsilosis*, particularly in Southern Europe, China and Latin America.[8–11] Moreover, *C. auris* has emerged globally over the past 13 years but with a highly uneven burden in different European countries and centres due to its unique ability to cause nosocomial transfer and difficulty to control outbreaks.[12–14]

Acquired antifungal resistance in *Candida* is diverse with respect to agents, underlying mechanisms, magnitude of minimal inhibitory concentration (MIC) elevation and frequency.[15] Amphotericin B resistance is extremely rare. Echinocandin resistance is mediated by hotspot mutations in the *fks1* target gene and for *C. glabrata* also *fks2*. It arises after a median of 30 days of treatment and more readily where sub-therapeutic drug levels and biofilm occur, such as infected foci and on the mucosal surfaces, compared to the bloodstream, which hampers timely detection.[16–18] Azole resistance on the contrary, is most often multifactorial with target gene mutations, target gene upregulation and efflux pumps acting in concert and typically evolves after long-term (months) therapy.[15,19] Although a recent study for the first time demonstrated horizontal transfer of chromosomal and plasmid located azole resistance in *A. fumigatus* under laboratory conditions, transfer of antifungal resistance genes has not been demonstrated for *Candida* isolates and is not regarded a significant driver of resistance.[20] Hence, resistance is most commonly due to an intrinsically resistant species (thus predictable from the species identification) or acquired in the individual patient during the course of antifungal therapy. Consequently, there has been a low level of suspicion for acquired resistance in the antifungal drug naïve patients. However, in-ward patient to patient transfer of fluconazole resistant clonal *C. glabrata* isolates and outbreaks of fluconazole resistant *C. parapsilosis* isolates in paediatric and adult settings have been reported undermining species identification as means of predicting the appropriate therapy in the antifungal naïve patient.[21–26] They illustrate the importance of contemporary and localized epidemiological data for the appropriate initial management of candidaemia, to monitor the emergence of acquired resistance and predict future challenges and the importance of antifungal stewardship.

Based on this background, the European Confederation of Medical Mycology (ECMM) *Candida* III European multicentre observational study commenced in 2018.[27] The objectives were to assess epidemiology, adherence to guideline recommendations and associated outcomes of candidaemia across Europe.[28] This work analysed isolates that were collected as part of this multicentre study and reports species distribution and susceptibility patterns across Europe.

Materials and methods

Isolates

To provide a balanced and representative picture of candidaemia in Europe, the number of eligible centres per ECMM country included in the ECMM *Candida* III European multicentre observational study was determined by population size. As general guidance, the maximum number of included hospitals per country were: eight for each of the six countries with populations > 50 million (i.e., France, Germany, Italy, Russia, Turkey, and United Kingdom; mean population 82.5 million), four for countries with population > 25 million and < 50 million (i.e., Poland and Spain; mean population 42 million), and two for the remaining 16 ECMM countries with population < 25 million (mean population 9.4 million). Centres were recruited by ECMM council representatives of each participating country, and also via the EPICOVIDEHA[29] and FungiScope®[30] networks and among the ECMM Global Guidelines contributor and fellow groups.[27]

Each participating centre included the first ~10 culture proven adult candidaemia cases, defined according to European Society of Clinical Microbiology and Infectious Diseases (ESCMID) criteria,[31] occurring consecutively after July 1st, 2018. In total, 632 candidaemia patients were included from 60 centres in 20 European countries. Of these, 399 (63% of cases) bloodstream isolates were referred to the reference mycology laboratories at Statens Serum Institute (SSI), Copenhagen, Denmark (n = 329) or to the Department of Medical Microbiology, Hacettepe University (HU) Medical School, Ankara, Turkey (n = 70) (isolates from Turkish centres specifically) for confirmatory species identification and EUCAST susceptibility testing. The reasons for participating hospitals not referring their isolates were diverse and included workload challenges during Covid-19, isolates not being stored, lack of permission (Russia), and cost (some centres claimed > 1000€ for shipping, which was over the budget). The overall characteristics of the cases with referred

isolates were comparable to those cases for which isolates were not referred (e.g., origin being secondary or tertiary care centre, proportion with malignant disease and catheter related blood stream infection, and species distribution) with the exception that fewer referred isolates were from ICU patients (34% versus 43%, P = 0.0230), were *C. auris* (0.3% versus 6%, P < 0.0001) or were not identified to species level (0% versus 3%, P = 0.0012) (Supplementary Table 1).

Species identification was performed at the SSI using classical techniques (macro morphology on CHROMagar and thermotolerance at 45 °C for *C. albicans* versus *C. dubliniensis*), matrix assisted laser desorption/ionization - time-of-flight mass spectrometry (MALDI-TOF MS, Bruker, Bremen, Germany) with the online available spectrum database mass spectrometry imaging (MSI) when needed,[32,33] together with DNA sequencing as required.[34] For the Turkish isolates, species identification was performed using classical techniques and biochemical profiles (macro-morphology on CHROMagar, micro-morphology on cornmeal tween 80 agar, biochemical assimilation profiles using ID32C® - bioMérieux, Marcy-l'Étoile, France), and when required supplemented with MALDI-TOF (Bruker, Bremen, Germany) or DNA sequencing as previously described.[32,34]

Susceptibility testing and FKS gene sequence analysis

At the SSI, European Committee on Antimicrobial Susceptibility Testing (EUCAST) E.Def 7.3.2 susceptibility testing was performed prospectively during 2020–2022 using multiple batches of in house prepared trays.[35] Cell culture treated 96-well microplates (Nunc™ MicroWell™, ThermoFisher Scientific cat. no. 167008). Microtitre plates with two-fold dilutions were prepared using serial dilution and two pipette tip changes (well 4 and 7) and frozen at −80 °C prior to use.[36] Antifungal pure substances were stored in aliquots at −80 °C and 5000 mg/L stock solutions prepared in DMSO (Sigma-Aldrich, Brøndby, Denmark). The following compounds were investigated (source of compound and final concentration range in parentheses). Anidulafungin (Pfizer A/S, Ballerup, Denmark, 0.004–4 mg/L), micafungin (Astellas Pharma Inc., Tokyo, Japan, 0.004–4 mg/L), amphotericin B (Sigma- Aldrich, 0.004–4 mg/L), fluconazole (Sigma- Aldrich, either 0.03–16, 0.03–32 or 0.06–64 mg/L), and voriconazole (Pfizer A/S, Ballerup, Denmark, 0.004–4 mg/L).[37] The following quality control (QC) strains *C. albicans* CNM-CL F8555, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were included for quality control of prepared batches of plates and regular quality control, and results only accepted if MIC results for QC strains were within the target ranges. *FKS* sequencing was performed as previously described for *Candida* isolates with an elevated echinocandin MIC.[34] At the HU, EUCAST testing was performed similarly but with the following minor differences: 96-well microplates (TC-treated Corning Costar, catalogue no. 3595, Merck KGaA, Darmstadt, Germany) were used.

Data management

The number of centres were expressed as median, range, and the interquartile range (IQR=25th percentile–75th percentile) determined. Contingency analyses with Chi-square and Fisher's exact test was used to compare species proportions. Classification as wild-type (WT) and non-wild-type (NWT) and Susceptibility classification as Susceptible/ Susceptible, Increased exposure/ Resistant (S/I/R) were performed according to the available epidemiological cut off values (ECOFFs) and revised EUCAST Clinical breakpoints for fungi v. 10.0 valid from 4 February 2020.[38] For species where EUCAST has not set breakpoints, the recently proposed pragmatic breakpoints were applied.[39]

Results

A total of 399 blood culture isolates from 41 centres in 17 European countries were referred (Fig. 1). The median number of referred isolates per centre was 10 (range 1–18, IQR= 9–10; for cases with mixed *Candida* infections more than one isolate was sent). The most common species were *C. albicans* (47.1%), *C. glabrata* (22.3%), *C. parapsilosis* (15.0%), *C. tropicalis* (6.3%), and *C. dubliniensis* and *C. krusei* (2.3% each). Less common species represented by one to four isolates were *C. lusitaniae* (1%), *C. guilliermondii* and *C. kefyr* (0.8% each), *C. pelliculosa* and *C. inconspicua* (0.5% each), and *C. digboiensis, C. haemulonii, C. auris, C. rugosa* and *C. orthopsilosis* (0.3% each). The overall species distribution mirrored that of the entire patient population with the exception that fewer referred isolates were *C. auris* (1 (0.3%) versus 14 (6%), P < 0.0001). This difference reflected that Russian isolates (n = 35), which included 13 *C. auris*, were not referred (Supplementary Table 1).[28] However, the species distribution varied among centres. For 35/41 centres that included at least nine isolates, the proportion of *C. albicans* was ≥ 50% of the isolates in 15/35 (43%) centres but ≤ 30% in 7/35 (20%) centres (Turkey, *n* = 3; UK, *n* = 2; France, *n* = 1; and Belgium, *n* = 1) (105/169 isolates versus 16/69 isolates; P < 0.0001, Fisher's exact test) (Fig. 2). *C. glabrata* was the most common species in four centres (one each in UK, France, Slovenia and Belgium) and *C. parapsilosis* in two centres (UK and Turkey). When focusing on countries represented by more than one centre, a difference in species distribution across countries was also noted (Fig. 3). Austria had the highest *C. albicans* proportion (77%, P = 0.003 compared to other countries, Chi-square), France, Czech Republic and the UK the highest proportions of *C. glabrata* (25–33%, P = 0.2145) while the highest proportions of *C. parapsilosis* (24–26%, P = 0.0025 compared to other countries, Fisher's exact test) were found in Italy and Turkey.

All isolates were amphotericin B susceptible (MIC ≤ 1 mg/L) with the following species-specific modal MICs and (ranges): 0.125 mg/L (0.03–0.25 mg/L) for *C. dubliniensis*, 0.25 mg/L (0.06–1 mg/L) for *C. albicans*, 0.5 mg/L (0.03–1 mg/L) for *C. glabrata*, 0.5 (0.06–1 mg/L) for *C. parapsilosis,* 0.5 (0.125–1 mg/L) for *C. tropicalis*, and 0.5 mg/L (0.5–1 mg/L) for *C. krusei*, respectively. All *C. albicans* and *C. dubliniensis* isolates were susceptible to fluconazole and voriconazole (Table 1). However, fluconazole resistance was found in 4% of *C. tropicalis*, 12% *C. glabrata*, 17% *C. parapsilosis* and 20% other *Candida* spp. for which pragmatic breakpoints have been proposed. A correlation between fluconazole and voriconazole MICs was found both within each species and across species (Table 2). The 11 fluconazole resistant *C. glabrata* isolates derived from eight centres in six countries (Belgium, Czech Republic, Italy, Sweden, Turkey and the UK) and six of these were voriconazole non-wild-type (MIC > 1 mg/L) (Tables 2 and 3). In these six countries, the acquired fluconazole resistance rate in *C. glabrata* was 23% (11/48) versus 0/41 in the remaining countries (P = 0.0007, Fisher's exact test). The 10 fluconazole resistant *C. parapsilosis* isolates derived from seven centres in three countries (Greece, Italy and Turkey), and all were voriconazole non-wild-type and I or R (Tables 2 and 3). In these countries, the fluconazole resistance rate in *C. parapsilosis* was 37% (10/27) versus 0/33 (P = 0.0001, Fisher's exact test). The fluconazole resistant *C. tropicalis* derived from a centre in Germany and was also voriconazole resistant. The fluconazole resistant *C. guilliermondii* isolate derived from Slovenia and was voriconazole non-wild-type (MIC > 4 mg/L). Finally, the fluconazole resistant *C. auris* isolate derived from the UK and had a voriconazole MIC of 2 mg/L.

Four isolates were anidulafungin resistant, three of which were cross-resistant to micafungin (Table 1). These included a *C. albicans* (from Austria), which harboured an Fks1 alteration S645P, a *C. glabrata* (from Spain) that harboured an Fks2 alteration F659I and a *C. parapsilosis* (from Turkey), which harboured an Fks1 alteration L657W. In addition, one *C. inconspicua* (from Turkey) displayed a

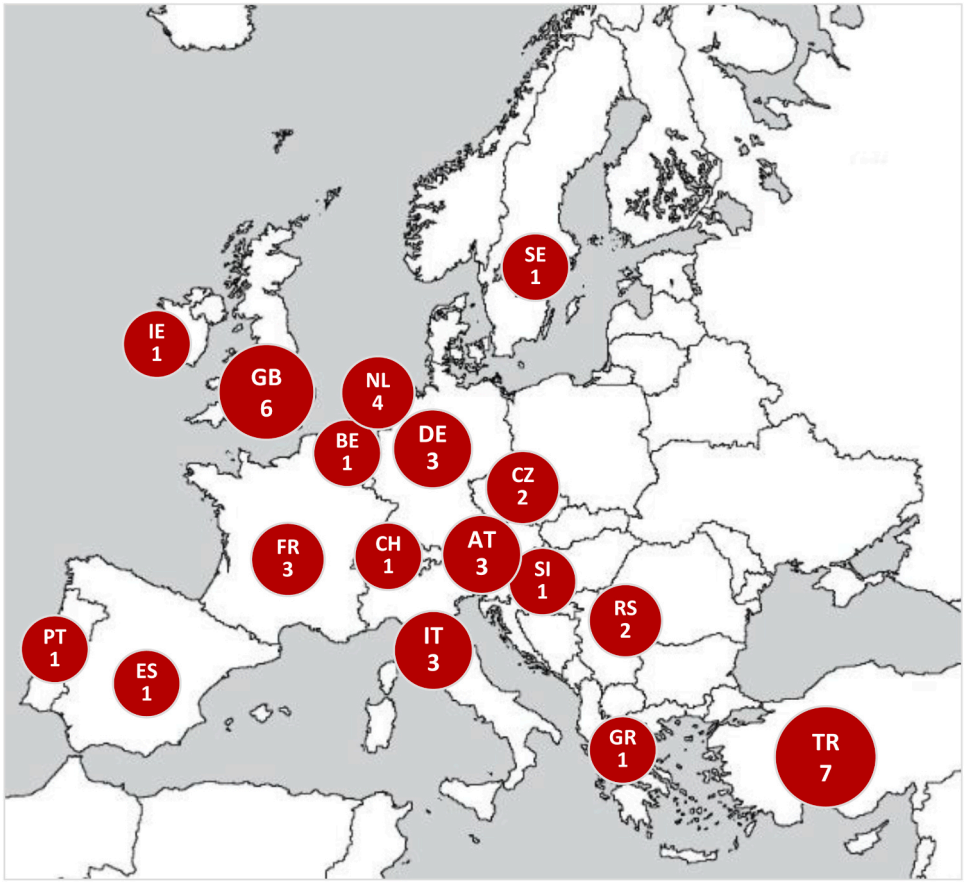

Fig. 1. Geographic location for the 41 participating centres from the 17 European countries. Two letter country codes and number of participating centres are indicated for each country. Circle diameter increase with increasing number of centres.

high anidulafungin MIC (0.125 mg/L) compared to the other *C. inconspicua* included (0.008 mg/L) and compared to the proposed pragmatic susceptibility breakpoint of 0.06 mg/L for this species.[39] This isolate did not have the *fks* genes sequenced. Five additional isolates were classified as micafungin resistant due to an MIC, which was one two-fold dilution above the breakpoint but remained anidulafungin susceptible. These included two *C. albicans* (from Sweden) that harboured an R647G Fks1 alteration and for which the anidulafungin MIC was 0.008 mg/L and micafungin MIC 0.06 mg/L, one *C. glabrata* (from UK) and two *C. parapsilosis* (from UK) with

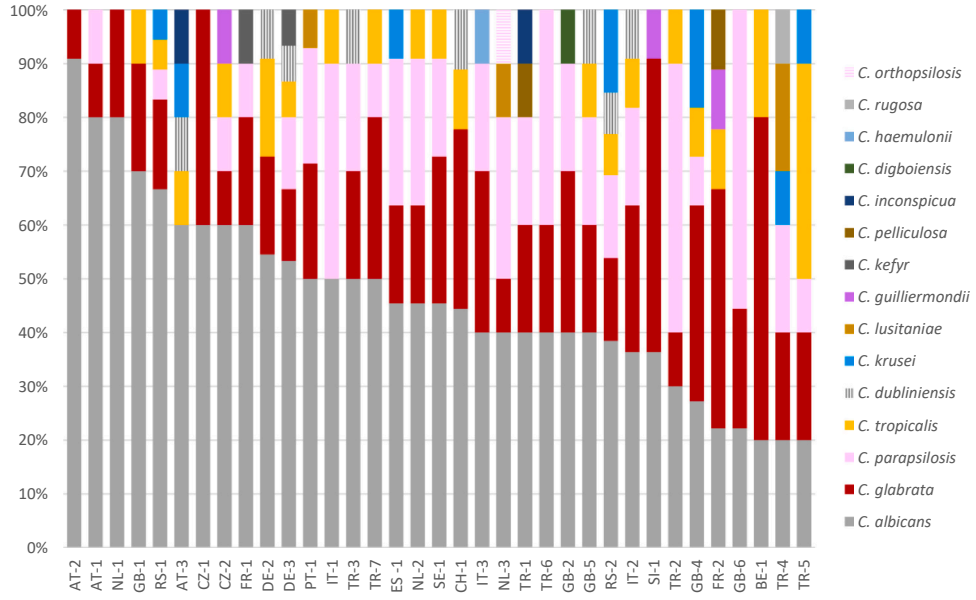

Fig. 2. Species distribution for 35/41 individual centres that included at least nine candidaemia isolates. Two letter country codes are used and a centre number. Centres are sorted according to descending proportion of *C. albicans*.

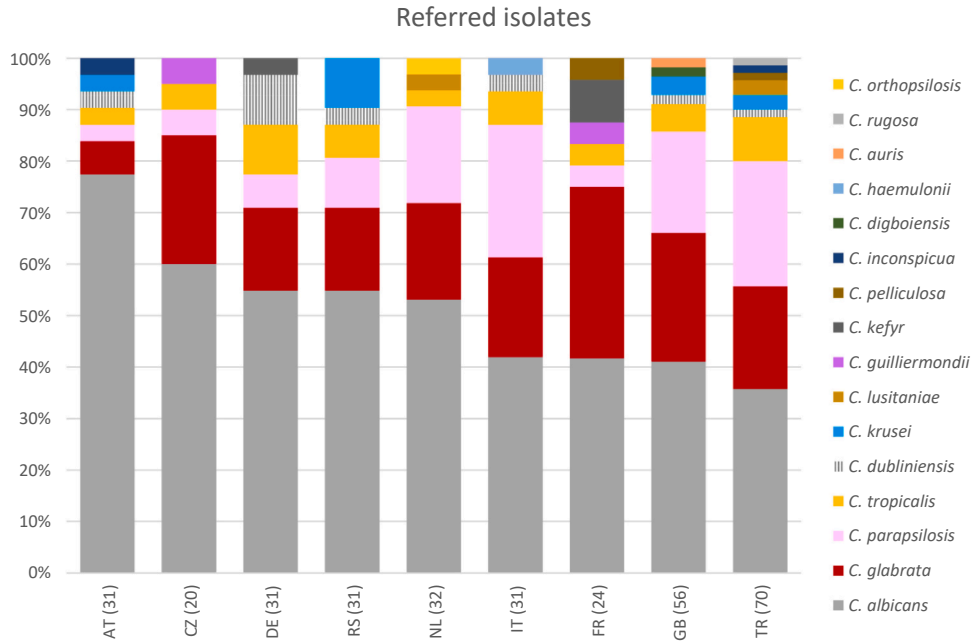

Fig. 3. Country specific species distribution for countries represented by at least 18 isolates (the number of isolates are indicated in parenthesis). Countries are sorted according to descending proportion of *C. albicans*.

Table 1
Susceptibility profile of the 399 European *Candida* blood-stream isolates for azoles and echinocandins.

	Fluconazole						Voriconazole						Anidulafungin				Micafungin			
Species (*N*)	S	% S	I	%I	R	%R	S/WT[a]	% S/WT	I	%I	R/NWT	% R/NWT	S	% S	R	%R	S/WT	% S/WT	R/NWT	%R/NWT
C. albicans (188)	188	100%	0	0%	0	0%	188	100%	0	0%	0	0%	187	99%	1	1%	185	98%	3	2%
C. glabrata (89)	0	0%	78	88%	11	12%	83	93%	na	na	6	7%	82	92%	1	1%	87	98%	2	2%
C. parapsilosis (60)	50	83%	0	0%	10	17%	50	83%	6	10%	4	7%	59	98%	1	2%	57	95%	3	5%
C. tropicalis (25)	24	96%	0	0%	1	4%	24	96%	0	0%	1	4%	25	100%	0	0%	25	100%	0	0%
C. dubliniensis (9)	9	100%	0	0%	0	0%	9	100%	0	0%	0	0%	na	na	na	na	na	na	na	na
C. krusei (9)	0	0%	0	0%	9	100%	9	100%	na	na	0	0%	9	100%	0	0%	9	100%	0	0%
Other *Candida* (19)[b]	10	53%	1	5%	8	42%	na	na	na	na	na	na	na	na	na	na	na	na	na	na

na. not applicable, neither breakpoints nor epidemiological cut-off value (ECOFF) have been set.
[a] Isolates are classified as **S**usceptible (S), wild-type (WT), susceptible, **I**ncreased exposure (I), **R**esistant (R) and non-wildtype (NWT) according to EUCAST breakpoints and ECOFFs (valid 4. Feb. 2020) [1] and the pragmatic breakpoints for rare yeast proposed by Astvad et al [2].
[b] Other species included *C. lusitaniae* (n=4), *C. guilliermondii* and *C. kefyr* (n=3 each), *C. pelliculosa* and *C. inconspicua* (n=2 each), and *C. digboiensis*, *C. haemulonii*, *C. auris*, *C. (Diutina) rugosa* and *C. orthopsilosis* (n=1 each).

wild-type *Fks* genes for which the micafungin MICs were only elevated one dilution above the breakpoint and modal MIC.

Discussion

We assessed *Candida* spp. distribution and antifungal resistance of candidaemia across Europe as part of the third ECMM *Candida* European multicentre observational study. Compared with the two earlier studies enrolling patients in 1997-'99 and 2006-'08, respectively, our study shows a decreasing proportion of *C. albicans* (56.4%, 54.0–47.1%, P = 0.0027, Chi-square), increasing *C. glabrata* (13.6%, 13.8–22.3%, P = < 0.0001, Chi-square) and the highest proportions of *C. parapsilosis* in Italy and Turkey.[40,41] This confirms the changing epidemiology of candidaemia reported in other parts of the world over the past decades.[1–11] Nevertheless, noticeable differences in species distribution were observed at the country level as exemplified by a *C. albicans* proportion ranging from 77% to 35.7%, and at the centre level with either *C. glabrata* or *C. parapsilosis* being the most common species at six of the 41 centres. While the underlying reasons for this difference are unknown, previous studies have shown that prior antifungal exposure and age impact the species distribution of subsequent candidaemia.[42,43]

Acquired fluconazole resistance was common in *C. glabrata* and *C. parapsilosis*. *C. glabrata* has long been recognised for the ability to acquire azole and echinocandin resistance, with fluconazole resistance rates of 8.1% (range 5.6–10.1%) during 2006–16 in the global SENTRY surveillance programme,[11] 10.7% in Belgium during 2004–15[21] and 13.8%, 9.1% and 10.6% in three nationwide studies covering 2011–18 in Denmark.[4,32,44] In this context, the overall rate of 12% fluconazole resistance in *C. glabrata*, with resistant *C. glabrata* detected in several countries throughout Europe, is concerning but not surprising. In contrast, the epidemiology of acquired fluconazole resistance in *C. parapsilosis* is changing with recent clonal outbreaks in southern Europe, India, the US (California, Indiana, New York and Texas) and South-Korea.[8,23,45–47] In agreement with this, we found fluconazole resistant *C. parapsilosis* in Greece, Italy and particularly Turkey, and a rate of 24% across these three countries. *C. parapsilosis* is a low virulent species that harbours an intrinsic mutation in the *fks* echinocandin target gene.[48] It is clinically susceptible to echinocandins despite elevated echinocandin MICs compared to *C. albicans*, although associated with a higher risk of persistence and relapse of infections in humans.[49–51] Of note, a highly pan-echinocandin resistant *C. parapsilosis* isolate harbouring an F652S Fks1 alteration was recently reported.[52] Consequently, the emerging azole resistance in this species suggest a potential of multidrug resistance,

Table 2
Correlation between fluconazole and voriconazole MICs for the 399 *Candida* isolates. The clinical breakpoint for susceptibility (S ≤ X) is indicated by a double line. The ECOFF (wild-type MIC ≤ Y) is indicated as a single line (when breakpoints are not established).

	Species (n)	Fluconazole MIC (mg/L)													Total
		0.06	≤0.125	0.125	0.25	0.5	1	2	4	8	16	32	64	> 64	
Voriconazole MIC (mg/L)	***C. albicans* (188)**														
	≤0.004	2		57	54										113
	≤0.016		2	3	52	14									71
	0.03				1	2									3
	0.06				1										1
	***C. glabrata* (89)**														
	0.03						2	5							7
	0.06							18	18						36
	0.125							2	23	1					26
	0.25						1			2	1				4
	0.5								2	1	2	1			6
	1											3		1	4
	2											1	2		3
	4													3	3
	***C. parapsilosis* (60)**														
	0.008					6									6
	≤0.016				1	12	16	1							30
	0.03						5	8							13
	0.06						1								1
	0.125														
	0.25									2	2	2			6
	0.5										1	2			3
	1												1		1
	***C. tropicalis* (25)**														
	≤0.016		1		5	3	1								10
	0.03				2	6	2								10
	0.06					1	3								4
	0.125														
	0.25														
	0.5									1					1
	***C. dubliniensis* (9)**														
	0.008				4										4
	≤0.016			1		4									5
	0.03														
	0.06														
	***C. krusei*[a] (9)**														
	0.25											5			5
	0.5											2	2		4
	Other *Candida* (19)														
	≤0.016		2		2	4	1								9
	0.03														0
	0.06							1	1						2
	0.125									2					2
	0.25									1					1
	0.5										1		1		2
	1														0
	2													1	1
	> 4													2	2

[a] For *C. krusei*, the ECOFFs are outside the illustrated MIC range (fluconazole: 128 mg/L and voriconazole 1 mg/L.

Table 3
Number of *C. glabrata* and *C. parapsilosis* isolates with acquired fluconazole resistance by country of isolation in the seven countries where fluconazole resistant *C. glabrata* or *C. parapsilosis* was detected. The countries are listed according to geographical location (North to South). Resistant isolates are highlighted in grey shading.

	C. glabrata		*C. parapsilosis*		*Candida*
	R	total	R	total	total
Sweden	1	3	0	2	11
UK	1	14	0	11	56
Belgium	2	6	0	0	10
Czech Republic	3	5	0	1	20
Italy	1	6	1	8	31
Greece	0	0	1	2	4
Turkey	3	14	8	17	70
Combined	11	48	10	41	202

which in light of its ability to cause outbreaks as emphasised during the COVID-19 pandemic is cause for concern.[24,26]

Acquired echinocandin resistance was infrequent in this pan-European study. Three isolates were anidulafungin and micafungin resistant, two of which harboured alterations at two of the most common "strong" hot spot codons in hot spot 1 of the target genes *fks1* (S645 in *C. albicans*) and *fks2* (F659 in *C. glabrata*) known to confer pan echinocandin resistance.[15] The third echinocandin resistant isolate was a *C. parapsilosis* that harboured an L657W alteration in Fks1 and was also fluconazole and voriconazole resistant. Acquired echinocandin resistance in *C. parapsilosis* is extremely rare, and to our knowledge, this specific alteration has not been described before. Moudgal et al. reported a case in 2004 of in vivo selection of an echinocandin and azole resistant *C. parapsilosis* isolate but without target gene analyses.[53] Subsequently in 2021, 2022 and 2023, Arastehfar et al., Siopi et al. and Ning et al. each reported

echinocandin resistant *C. parapsilosis* harbouring R658G (four isolates, three of which were isogenic and azole resistant), F652S (one isolate, azole susceptible) and S656P (one isolate, and also azole resistant), respectively.[52,54,55] These findings suggest that the increased use of echinocandins selects emerging echinocandin and multidrug resistant *C. parapsilosis* which pose a significant concern for clinical management. Five isolates were anidulafungin susceptible but micafungin resistant. Differential susceptibility to the echinocandins may either be due to occasional misclassification of wild-type isolates with an outlier MIC, typically one dilution above the breakpoint. This occurs when the breakpoint is set at the epidemiological cut-off value (ECOFF). ECOFFs are set visually, where the MIC distribution ends, and statistically, including 95–99% of a modelled distribution. Due to inherent variation associated with phenotypic susceptibility testing, a minority of test results for wild-type isolates will fall one dilution above the ECOFF. For anidulafungin and micafungin the breakpoints are set at the ECOFFs. It is therefore plausible that the three isolates in our study without Fks alterations and with differential anidulafungin versus micafungin susceptibility classification represent misclassification of clinically susceptible wild-type isolates. However, true differential susceptibility to the three echinocandins has been documented for *C. albicans* and *C. glabrata*. Arendrup et al. demonstrated that an Fks alteration Fks2p-S663F in *C. glabrata* conferred anidulafungin and caspofungin resistance but retained susceptibility to micafungin in vitro and in vivo in an animal model.[56] Lackner et al. reported a case of clinical failure during caspofungin treatment but recovery on anidulafungin therapy for a patient treated for chronic mucocutaneous candidiasis.[57] This caspofungin non-responding *C. albicans* isolate harboured a double mutation in *fks1* resulting in R647R/G and P649P/L alterations and was by EUCAST susceptibility testing classified as anidulafungin susceptible but micafungin resistant and by Etest also caspofungin resistant. A laboratory-generated mutant only carrying a heterozygous R647G alteration retained the differential susceptibility with resistance to micafungin and susceptibility to anidulafungin. The two *C. albicans* isolates in our study, which shared this differential susceptibility phenotype (micafungin resistant but anidulafungin susceptible), harboured the same R647G alteration on both alleles. These findings support that some alterations confer clinically relevant differential susceptibility to the echinocandins, although the activity of the three agents is generally similar. The findings also support the need for testing both anidulafungin and micafungin as markers for echinocandin susceptibility and guidance of therapy as advised by EUCAST.[58]

This study is associated with strengths and limitations. Strengths are that many European countries across the breadth of the continent were represented and the number of isolates per centre and country were comparable according to population, thereby avoiding an imbalanced representation of Europe. Consistent susceptibility testing was demonstrated, using the EUCAST standard reference method at experienced reference laboratories. Limitations included not all isolates from the ECMM *Candida* III study being referred for confirmatory identification and susceptibility testing. Yet the species distribution was similar in the overall case collection and the referred isolates. Given that the number of isolates per centre was limited to ~10 consecutive isolates, data should be interpreted with caution, particularly in countries with few centres. The case mix (i.e., patient cohort) at the participating centres may not reflect the nationwide epidemiology of the countries, as it is likely that the recruited centres will be biased towards tertiary centres with mycology-interested colleagues. This is especially true since the current isolate collection excluded neonates and paediatric patients (< 18 years) where *C. parapsilosis* is more frequent.[28] Nevertheless, as the epidemiology aligns with current published trends and neonates/paediatric candidaemia only constituted 3–3.7% of candidaemia in nationwide studies,[4,8] we believe these limitations do not detract from the conclusions of this study. From a technical aspect, confirmatory species identification and susceptibility testing were conducted at two different sites, with all isolates from Turkey tested at the HU as a tertiary care centre Mycology laboratory in Turkey with advanced experience in fungal diagnostics and antifungal susceptibility testing using reference microdilution methods and the remaining isolates tested at the EUCAST development laboratory hosted at the reference mycology laboratory in Denmark. However, both centres used MALDI-TOF with updated databases for identification and EUCAST E.Def 7.3 for susceptibility testing. Of note, the drug and species-specific modal MICs generated at the two centres were either identical or fell at two neighbouring MICs for the six most common species represented by at least nine isolates (data not shown), confirming consistent MIC testing performance across the two laboratories.

In conclusion, this study demonstrates a continued change in species distribution from *C. albicans* towards *C. glabrata* and *C. parapsilosis*. It confirms a high but stable fluconazole resistance rate in *C. glabrata* and a concerning emerging fluconazole resistance in *C. parapsilosis* in southern Europe, which in one isolate was combined with acquired echinocandin resistance due to a novel L657W *fks*-alteration. However, *C. auris* and other potential MDR species (e.g. *C. haemulonii* and *C. digboiensis*) were infrequently detected and acquired echinocandin resistance was overall rare.

Funding

The confirmatory species identification, susceptibility testing and target gene sequencing conducted at the Statens Serum Institut and at Hacettepe University did not receive any external funding. Otherwise, the study, including case-based reimbursements for participating centres for study participation and reimbursements for shipment of isolates from each centre to the SSI, Copenhagen, Denmark, was partly funded by an Investigator Initiated Research Grant from Scynexis. The funder had no influence on the study design or on the analysis of the results.

Declaration of Competing Interest

Outside this work the authors have the following potential conflicts to declare: MaCA has received research grants/contract work (paid to the SSI) from Amplyx, Basilea, Cidara, F2G, Gilead, Novabiotics and Scynexis, and speaker honoraria (personal fee) from Astellas, Chiesi, Gilead, MSD, and SEGES over the past 5 years. She is the current chairman of the EUCAST-AFST. SAA reports research grant from Cidara, lecture honoraria from Gilead, and travel grant from Astellas. MH reports research funding from Astellas, Gilead, MSD, Pfizer, Euroimmun, and Scynexis. PK reports grants or contracts from German Federal Ministry of Research and Education (BMBF) B-FAST (Bundesweites Forschungsnetz Angewandte Surveillance und Testung) and NAPKON (Nationales Pandemie Kohorten Netz, German National Pandemic Cohort Network) of the Network University Medicine (NUM) and the State of North Rhine-Westphalia; Consulting fees Ambu GmbH, Gilead Sciences, Mundipharma Resarch Limited, Noxxon N.V. and Pfizer Pharma; Honoraria for lectures from Akademie für Infektionsmedizin e.V., Ambu GmbH, Astellas Pharma, BioRad Laboratories Inc., European Confederation of Medical Mycology, Gilead Sciences, GPR Academy Ruesselsheim, HELIOS Kliniken GmbH, Lahn-Dill-Kliniken GmbH, medupdate GmbH, MedMedia, MSD Sharp & Dohme GmbH, Pfizer Pharma GmbH, Scilink Comunicación Científica SC and University Hospital and LMU Munich; Participation on an Advisory Board from Ambu GmbH, Gilead Sciences, Mundipharma Resarch Limited and Pfizer Pharma; A pending patent currently reviewed at the German Patent and Trade Mark Office (DE 10 2021 113 007.7); Other non-

financial interests from Elsevier, Wiley and Taylor & Francis online outside the submitted work. JSG reports speaker honoraria from Gilead and Pfizer outside of the submitted work. OAC reports grants or contracts from Amplyx, Basilea, Cidara, F2G, Gilead, Matinas, MedPace, MSD, Mundipharma, Octapharma, Pfizer, Scynexis; Consulting fees from Abbvie, Amplyx, Biocon, Biosys, Cidara, Gilead, IQVIA, Janssen, Matinas, MedPace, Menarini, Molecular Partners, MSG-ERC, Noxxon, Octapharma, Pardes, Pfizer, PSI, Scynexis, Seres; Honoraria for lectures from Abbott, Abbvie, Al-Jazeera Pharmaceuticals/Hikma, Astellas, Gilead, Grupo Biotoscana/United Medical/Knight, MedScape, MedUpdate, MSD, Mylan, Noscendo, Pfizer, Shionogi; Payment for expert testimony from Cidara; Participation on a Data Safety Monitoring Board or Advisory Board of Actelion, Allecra, Cidara, Entasis, IQVIA, Janssen, MedPace, Paratek, PSI, Pulmocide, Shionogi, The Prime Meridian Group; A patent at the German Patent and Trade Mark Office (DE 10 2021 113 007.7). CLF has received research grants from Pfizer, Basilea, F2G, Gilead, and Scynexis, and speaker honoraria from Gilead, MSD, and Pfizer over the past 5 years. RK received research grants from Merck and Pfizer and served on the speakers' bureau of Pfizer, Gilead, Astellas, Basilea, Merck, Angelini, and Shionogi. GD has received lecture honoraria from Gilead and Pfizer, outside of the submitted work. He was also invited to symposia and congresses by the two aforementioned companies. AFT has received grants and speaker honoraria from Pfizer and Gilead outside of this work. PLW performed diagnostic evaluations and received meeting sponsorship from Bruker, Dynamiker, and Launch Diagnostics; Speakers fees, expert advice fees and meeting sponsorship from Gilead; Speaker and expert advice fees from F2G and speaker fees MSD and Pfizer: Speakers fees and performed diagnostic evaluations for Associates of Cape Cod and IMMY. JPG has received lecture honoraria from Gilead, MundiPharma and Pfizer. NK is a member of the Gilead, Merck Sharp & Dohme AG (MSD) and Pfizer advisory boards for invasive fungal infections, chair of the DSMB of Pulmicide, and reports grants from The Swiss National Science Foundation (grant number 32003B_204944 and the National Centre of Competence in Research AntiResist Grant 51NF40_180541), outside the submitted work. ER reports grants to his institutions from Astellas, MSD, Scynexis, Shionogi, GSK, Pfizer, Gilead and Allergan. He has served as consultant to Amplyx, Astellas, Gilead, MSD, Pfizer, Scynexis, GSK and Shionogi. CL reports conference sponsorship from Gilead Sciences. BD reports consulting fees from Gilead, Pfizer and travel grant from Gilead. KL received consultancy fees from MRM Health, MSD and Gilead, speaker fees from FUJIFILM WAKO, Pfizer and Gilead and a service fee from Thermo fisher Scientific and TECOmedical. MB reports research grants and/or personal fees for advisor/consultant and/or speaker/chairman from Bayer, BioMérieux, Cidara, Cipla, Gilead, Menarini, MSD, Pfizer, and Shionogi. VÖ reports research funding and/or collaborations with Abbott, Accelerate diagnostics, Astrego AB, Becton, Dickinson and Company (BD), BioFire Diagnostics, bioMérieux, Bruker, Nittobo Medical Co, QuantaMatrix, Quidel, SD Biosensor, SSI Diagnostica, and T2 Biosystems. BW reports research grant from Pfizer and Schülke & Mayr, advisory board for Merck Sharp & Dohme and Pfizer and speaker honoraria from Gilead, Pfizer, bioMerieux, Euro Immun, CapeCod, Immy and Diasorin. VAA reports research funding from Pfizer. JS has received lecture honoraria from Gilead and Pfizer, outside of the submitted work. JBB has received research grants from F2G and Gilead outside of the submitted work. The following authors had no potential conflicts to declare: Tadeja Matos, Eelco F.J. Meijer, Beyza Ener, Ebru EVREN, Laura Loughlin, Riina Rautemaa-Richardson, Suleyha Hilmioglu-Polat, Deborah E. A. Lockhart, Benedict R.S. Rogers, Ulrike Scharmann, Filip Růžička, Axel Hamprecht, Karin Van Dijk, Anna Grancini, Petr Hamal, Tuğçe Ünalan-Altıntop, Yasemin OZ, Aleksandra Barac, Assunta SARTOR, Faruk Aydin, Özlem Koyuncu Özyurt.

Acknowledgements

The authors wish to thank research technician Birgit Brandt and Désiré Mageme Nahimana at the SSI, Copenhagen, Denmark and Dilek Çakmak at HU, Ankara, Turkey for excellent technical assistance.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2023.08.001.

References

1. Nielsen H, Stenderup J, Bruun B. *Fungemia in a university hospital 1984–1988. Scand J Infect Dis* 1991;**23**:275–82. https://doi.org/10.3109/00365549109024310
2. Wenzel RP. *Nosocomial candidemia: risk factors and attributable mortality. Clin Infect Dis* 1995;**20**:1531–4.
3. Bretagne S, Sitbon K, Desnos-Ollivier M, et al. *Active surveillance program to increase awareness on invasive fungal diseases: the French RESSIF Network (2012 to 2018). mBio* 2022;**13**(3):e0092022. https://doi.org/10.1128/mbio.00920-22
4. Risum M, Astvad K, Johansen HK, et al. *Update 2016–2018 of the nationwide danish fungaemia surveillance study: epidemiologic changes in a 15-year perspective. J Fungi* 2021;**7**:491.
5. Public Health England PHE. *Laboratory surveillance of candidaemia in England: 2020*. Health Protection Report; 2021. p. 15. ⟨https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1017262/hpr1521_cnddm20_final4.pdf⟩.
6. Tsay SV, Mu Y, Williams S, et al. *Burden of Candidemia in the United States, 2017. Clin Infect Dis* 2020;**71**:449–53.
7. Chapman B, Slavin M, Marriott D, et al. *Changing epidemiology of candidaemia in Australia. J Antimicrob Chemother* 2017;**72**:1103–8.
8. Mamali V, Siopi M, Charpantidis S, Samonis G, Tsakris A, Vrioni G. *Increasing incidence and shifting epidemiology of candidemia in Greece: results from the first nationwide 10-year survey. J Fungi* 2022;**8**:1–20.
9. Díaz-García J, Gómez A, Machado M, et al. *Blood and intra-abdominal Candida spp. from a multicentre study conducted in Madrid using EUCAST: emergence of fluconazole resistance in Candida parapsilosis, low echinocandin resistance and absence of Candida auris. J Antimicrob Chemother* 2022;**77**:3102–9.
10. Bassetti M, Merelli M, Righi E, et al. *Epidemiology, species distribution, antifungal susceptibility, and outcome of candidemia across five sites in Italy and Spain. J Clin Microbiol* 2013;**51**:4167–72.
11. Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. *Twenty years of the SENTRY antifungal surveillance program: results for Candida species from 1997-2016. Open Forum Infect Dis* 2019;**6**:S79–94.
12. Plachouras D, Lötsch F, Kohlenberg A, et al. *Candida auris: epidemiological situation, laboratory capacity and preparedness in the European Union and European Economic Area, january 2018 to may 2019. Eurosurveillance* 2020;**25**:1–6.
13. Rhodes J, Fisher MC. *Global epidemiology of emerging Candida auris. Curr Opin Microbiol* 2019;**52**:84–9.
14. Kohlenberg A, Monnet DL, Plachouras D. *Increasing number of cases and outbreaks caused by Candida auris in the EU/EEA, 2020 to 2021. Eurosurveillance* 2022;**27**(46):2200846. https://doi.org/10.2807/1560-7917.ES.2022.27.46.2200846
15. Rogers TR, Verweij PE, Castanheira M, et al. *Molecular mechanisms of acquired antifungal drug resistance in principal fungal pathogens and EUCAST guidance for their laboratory detection and clinical implications. J Antimicrob Chemother* 2022;**77**:2053–73.
16. Díaz-García J, Mesquida A, Gómez A, et al. *Antifungal susceptibility testing identifies the abdominal cavity as a source of candida glabrata-resistant isolates. Antimicrob Agents Chemother* 2021;**65**:e0124921.
17. Jensen RH, Johansen HK, Søes LM, et al. *Posttreatment antifungal resistance among colonizing Candida isolates in candidemia patients: results from a systematic multicenter study. Antimicrob Agents Chemother* 2016;**60**:1500–8.
18. Zhao Y, Prideaux B, Nagasaki Y, et al. *Unraveling drug penetration of echinocandin antifungals at the site of infection in an intra-abdominal abscess model. Antimicrob Agents Chemother* 2017;**61**:e01009-17.
19. Jensen RH, Astvad KMT, Silva LV, et al. *Stepwise emergence of azole, echinocandin and amphotericin B multidrug resistance in vivo in Candida albicans orchestrated by multiple genetic alterations. J Antimicrob Chemother* 2015;**70**:2551–5.
20. Morogovsky A, Handelman M, Abou Kandil A, Shadkchan Y, Osherov N. *Horizontal gene transfer of triazole resistance in Aspergillus fumigatus. Microbiol Spectr* 2022;**10**(3):e0111222. https://doi.org/10.1128/spectrum.01112-22
21. Goemaere B, Lagrou K, Spriet I, Hendrickx M, Becker P. *Clonal spread in Candida glabrata bloodstream isolates and fluconazole resistance affected by prolonged exposure: a 12-year single-center study in Belgium. Antimicrob Agents Chemother* 2018;**62**(8):e0059118.
22. Sarvikivi E, Lyytikäinen O, Soll DR, et al. *Emergence of fluconazole resistance in a Candida parapsilosis strain that caused infections in a neonatal intensive care unit. J Clin Microbiol* 2005;**43**:2729–35.
23. Alcoceba E, Gómez A, Lara-Esbrí P, et al. *Fluconazole-resistant Candida parapsilosis clonally related genotypes: first report proving the presence of endemic isolates*

harbouring the Y132F ERG11 gene substitution in Spain. Clin Microbiol Infect 2022;**28**:1113–9.
24. Trevijano-Contador N, Torres-Cano A, Carballo-González C, et al. *Global emergence of resistance to fluconazole and voriconazole in Candida parapsilosis in tertiary hospitals in Spain during the COVID-19 pandemic. Open Forum Infect Dis* 2022:1–6.
25. Ramos-Martínez A, Pintos-Pascual I, Guinea J, et al. *Impact of the COVID-19 pandemic on the clinical profile of candidemia and the incidence of fungemia due to fluconazole-resistant Candida parapsilosis. J Fungi* 2022;**8**:451.
26. Daneshnia F, de Almeida Júnior JN, Arastehfar A, et al. *Determinants of fluconazole resistance and echinocandin tolerance in C. parapsilosis isolates causing a large clonal candidemia outbreak among COVID-19 patients in a Brazilian ICU. Emerg Microbes Infect* 2022;**11**:2264–74.
27. Koehler P, Arendrup MC, Arikan-Akdagli S, et al. *ECMM CandiReg—a ready to use platform for outbreaks and epidemiological studies. Mycoses* 2019;**62**:920–7.
28. Hoenigl M, Salmanton-García J, Egger M, et al. *Guideline adherence and survival of patients with candidaemia in Europe: results from the ECMM Candida III multinational European observational cohort study. Lancet Infect Dis* 2023;**23**:751–61.
29. Salmanton-García J, Busca A, Cornely OA, et al. *EPICOVIDEHA: a ready to use platform for epidemiological studies in hematological patients with COVID-19. HemaSphere* 2021;**5**:e612.
30. Hoenigl M, Gangneux J-P, Segal E, et al. *Global guidelines and initiatives from the European Confederation of Medical Mycology to improve patient care and research worldwide: new leadership is about working together. Mycoses* 2018;**61**:885–94.
31. Cuenca-Estrella M, Verweij PE, Arendrup MC, et al. *ESCMID* guideline for the diagnosis and management of Candida diseases 2012: diagnostic procedures. Clin Microbiol Infect* 2012;**18 Suppl 7**:9–18.
32. Arendrup MC, Dzajic E, Jensen RH, et al. *Epidemiological changes with potential implication for antifungal prescription recommendations for fungaemia: data from a nationwide fungaemia surveillance programme. Clin Microbiol Infect* 2013;**19**:E343–53.
33. Normand AC, Becker P, Gabriel F, et al. *Validation of a new web application for identification of fungi by use of matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol* 2017;**55**:2661–70.
34. Astvad KMT, Johansen HK, Røder BL, et al. *Update from a 12-year nationwide fungemia surveillance: increasing intrinsic and acquired resistance causes concern. J Clin Microbiol* 2018;**56**:1–15.
35. Arendrup MC, Meletiadis J, Mouton JW, et al. *EUCAST technical note on isavuconazole breakpoints for Aspergillus, itraconazole breakpoints for Candida and updates for the antifungal susceptibility testing method documents. Clin Microbiol Infect* 2016;**22**(6):571.e14.
36. Arendrup MC, Jørgensen KM, Hanemaaijer N, Verweij PE. *ISO standard 20776-1 or serial 2-fold dilution for antifungal susceptibility plate preparation: that is the question!. J Antimicrob Chemother* 2021;**76**:1793–9.
37. Arendrup MC, Prakash A, Meletiadis J, Sharma C, Chowdhary A. *Comparison of EUCAST and CLSI reference microdilution MICs of eight antifungal compounds for candida auris and associated tentative epidemiological cutoff values. Antimicrob Agents Chemother* 2017;**61**(6):e0048517.
38. Arendrup MC, Friberg N, Mares M, et al. *How to interpret MICs of antifungal compounds according to the revised clinical breakpoints v. 10.0 European Committee on Antimicrobial Susceptibility Testing (EUCAST). Clin Microbiol Infect* 2020;**26**:1464–72.
39. Astvad KMT, Arikan-Akdagli S, Arendrup MC. *A pragmatic approach to susceptibility classification of yeasts without EUCAST clinical breakpoints. J Fungi* 2022;**8**:141.
40. Klingspor L, Tortorano AM, Peman J, et al. *Invasive Candida infections in surgical patients in intensive care units: a prospective, multicentre survey initiated by the European Confederation of Medical Mycology (ECMM) (2006-2008). Clin Microbiol Infect* 2015;**21**:87.e1–87.e10.
41. Tortorano AM, Peman J, Bernhardt H, et al. *Epidemiology of candidaemia in Europe: results of 28-month European Confederation of Medical Mycology (ECMM) hospital-based surveillance study. Eur J Clin Microbiol Infect Dis* 2004;**23**:317–22.
42. Lortholary O, Desnos-Ollivier M, Sitbon K, Fontanet A, Bretagne S, Dromer F. *Recent exposure to caspofungin or fluconazole influences the epidemiology of candidemia: a prospective multicenter study involving 2,441 patients. Antimicrob Agents Chemother* 2011;**55**:532–8.
43. Kullberg BJ, Arendrup MC. *Invasive candidiasis. N Engl J Med* 2015;**373**:1445–56.
44. Astvad KMTT, Johansen HK, Røder BL, et al. *Update from a 12-year nationwide fungemia surveillance: increasing intrinsic and acquired resistance causes concern. J Clin Microbiol* 2018;**56**:1–15.
45. Arastehfar A, Hilmioğlu-Polat S, Daneshnia F, et al. *Clonal candidemia outbreak by Candida parapsilosis carrying Y132F in Turkey: evolution of a persisting challenge. Front Cell Infect Microbiol* 2021;**11**:1–10.
46. Martini C, Torelli R, de Groot T, et al. *Prevalence and clonal distribution of azole-resistant Candida parapsilosis isolates causing bloodstream infections in a large Italian hospital. Front Cell Infect Microbiol* 2020;**10**:e232. https://doi.org/10.3389/fcimb.2020.00232
47. Singh A, Singh PK, De Groot T, et al. *Emergence of clonal fluconazole-resistant Candida parapsilosis clinical isolates in a multicentre laboratory-based surveillance study in India. J Antimicrob Chemother* 2019;**74**:1260–8.
48. Garcia-Effron G, Katiyar SK, Park S, Edlind TD, Perlin DS. *A naturally occurring proline-to-alanine amino acid change in Fks1p in Candida parapsilosis, Candida orthopsilosis, and Candida metapsilosis accounts for reduced echinocandin susceptibility. Antimicrob Agents Chemother* 2008;**52**:2305–12.
49. Chiotos K, Vendetti N, Zaoutis TE, et al. *Comparative effectiveness of echinocandins versus fluconazole therapy for the treatment of adult candidaemia due to Candida parapsilosis: a retrospective observational cohort study of the Mycoses Study Group (MSG-12): Table 1. J Antimicrob Chemother* 2016;**71**:3536–9.
50. Mora-Duarte J, Betts R, Rotstein C, et al. *Comparison of caspofungin and amphotericin B for invasive candidiasis. N Engl J Med* 2002;**347**:2020–9.
51. Reboli AC, Rotstein C, Pappas PG, et al. *Anidulafungin versus fluconazole for invasive candidiasis. N Engl J Med* 2007;**356**:2472–82.
52. Siopi M, Papadopoulos A, Spiliopoulou A, et al. *Pan-Echinocandin Resistant C. parapsilosis Harboring an F652S Fks1 alteration in a patient with prolonged echinocandin therapy. J Fungi* 2022;**8**:931.
53. Moudgal V, Little T, Boikov D, Vazquez JA. *Multiechinocandin- and multiazole-resistant Candida parapsilosis isolates serially obtained during therapy for prosthetic valve endocarditis. Antimicrob Agents Chemother* 2005;**49**:767–9.
54. Arastehfar A, Daneshnia F, Hilmioglu-Polat S, et al. *Genetically related micafungin-resistant Candida parapsilosis blood isolates harbouring novel mutation R658G in hotspot 1 of Fks1p: a new challenge? J Antimicrob Chemother* 2021;**76**:418–22.
55. Ning Y, Xiao M, Perlin DS, et al. *Decreased echinocandin susceptibility in Candida parapsilosis causing candidemia and emergence of a pan-echinocandin resistant case in China. Emerg Microbes Infect* 2023;**12**(1):e2153086.
56. Arendrup MC, Perlin DS, Jensen RH, Howard SJ, Goodwin J, Hope W. *Differential in vivo activities of anidulafungin, caspofungin, and micafungin against Candida glabrata isolates with and without FKS resistance mutations. Antimicrob Agents Chemother* 2012;**56**:2435–42.
57. Lackner M, Tscherner M, Schaller M, et al. *Positions and numbers of FKS mutations in Candida albicans selectively influence in vitro and in vivo susceptibilities to echinocandin treatment. Antimicrob Agents Chemother* 2014;**58**:3626–35.
58. Arendrup MC, Kahlmeter G, Guinea J, Meletiadis J. Subcommittee on Antifungal SusceptibilityTesting (AFST) of the ESCMID European Committee for AntimicrobialSusceptibility Testing (EUCAST). *How to: perform antifungal susceptibility testing of microconidia-forming dermatophytes following the new reference EUCAST method E.Def 11.0, exemplified by Trichophyton. Clin Microbiol Infect* 2021;**27**:55–60.