Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-016-2841-3

ORIGINAL ARTICLE

Epidemiology and reporting of candidaemia in Belgium: a multi-centre study

C. Trouvé[1] · S. Blot[2,3] · M.-P. Hayette[4] · S. Jonckheere[5] · S. Patteet[1,9] · H. Rodriguez-Villalobos[6] · F. Symoens[7] · E. Van Wijngaerden[8] · K. Lagrou[1,9]

Received: 18 September 2016 / Accepted: 3 November 2016

Abstract The primary aim of this study was to collect national epidemiological data on candidaemia and to determine the reporting time of species identification and antifungal susceptibility in clinical practice. During a 1-year period (March 2013 until February 2014), every first *Candida* isolate from each episode of candidaemia was included prospectively from 30 Belgian hospitals. Identification and susceptibility testing were performed according to local procedures and isolates were sent to the National Reference Center for Mycosis. Species identification was checked by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) and internal transcribed spacer (ITS) sequencing in case no reliable identification was obtained by MALDI-TOF MS. Antifungal susceptibility testing was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology. A total of 355 isolates were retrieved from 338 patients. The mean incidence rate of candidaemia was 0.44 (range: 0.07 to 1.43) per 1000 admissions or 0.65 (range: 0.11 to 2.00) per 10,000 patient days. *Candida albicans* was most frequently found (50.4 %), followed by *C. glabrata* (27.3 %) and *C. parapsilosis sensu lato* (9.8 %). The overall resistance to fluconazole was 7.6 %, ranging from 3.9 % in *C. albicans* to 20.0 % in *C. tropicalis*. Only one *C. glabrata* isolate was resistant to the echinocandins. Four days after blood culture positivity, 99.7 % of the identifications and 90.3 % of the antifungal profiles were reported to the treating clinician. Candidaemia incidence rates differed up to 20-fold among Belgian hospitals; no clear factors explaining this difference were identified. The overall antifungal resistance rates were low but high azole resistance rates were recorded in *C. tropicalis*.

✉ K. Lagrou
katrien.lagrou@uzleuven.be

1 Department of Laboratory Medicine, University Hospitals of Leuven, Herestraat 49, 3000 Leuven, Belgium

2 Department of Internal Medicine, Ghent University, Ghent, Belgium

3 Burns Trauma and Critical Care Research Center, The University of Queensland, Brisbane, Australia

4 Department of Clinical Microbiology and Reference Center for Mycosis, University Hospital of Liège, Liège, Belgium

5 Department of Clinical Microbiology, Jan Yperman Hospital, Ypres, Belgium

6 Department of Clinical Microbiology, University Hospitals of Saint-Luc, Brussels, Belgium

7 Department of Mycology & Aerobiology, Belgian Scientific Institute for Public Health, Brussels, Belgium

8 Department of Internal Medicine, University Hospitals of Leuven, Leuven, Belgium

9 National Reference Center for Mycosis, University Hospitals of Leuven, Leuven, Belgium

Introduction

Recent advances in modern healthcare have created a large population of patients at risk for fungal infections. Most European candidaemia incidence data derive from single- or multi-centre studies [1–9]. Studies from the USA report higher incidence rates compared to those from the European countries, except for Denmark, where the rate is close to that of the USA [1]. A similar high rate was reported from the only Belgian single-centre study focussing on both the incidence and antifungal susceptibility of fungi isolated from blood, dating back to 2001–2005 [10].

Species distribution varies also between geographical regions. For example, the prevalence of *Candida albicans* in

Published online: 17 November 2016

Norway and Switzerland is above 60 %, followed by *Candida glabrata* [5, 8], while in Mediterranean countries such as Italy and Spain, *Candida parapsilosis* is the most common non-*albicans Candida* species [3, 7].

Pfaller et al. reported that azole resistance was rare in *C. albicans* and *Candida dubliniensis* (1.4 % and 2.6 %, respectively) and limited in *C. parapsilosis* and *C. tropicalis* (3.6 % and 4.1 %, respectively) among a quarter of a million isolates from 41 countries worldwide [11]. This low-level resistance was confirmed in more recent multi-centre studies [12, 13]. Echinocandins have generally shown excellent activity against *Candida* species (resistance rates of <3 %), with the exception of *C. glabrata*, in which echinocandin resistance seems to be rising [14–18]. The finding of cross-resistance to azole antifungal agents in a considerable number of these isolates is particularly alarming [15].

European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines recommend echinocandin drugs for the initial treatment of candidaemia in non-neutropaenic patients and a step-down to fluconazole after 10 days of intravenous treatment, if the species is susceptible and the patient is stable [19]. The feasibility and safety of an early step-down to fluconazole is an important research question [20]. In this respect, it is important to have good knowledge of the time necessary in routine practice to report species and susceptibility testing results to the treating clinician.

The first aim of this study was to determine the incidence and species distribution of candidaemia on a national level in Belgium and to determine antifungal resistance rates based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology. The second aim was to assess the reporting time of species identification and antifungal susceptibility in clinical practice.

Materials and methods

Study design

Thirty Belgian hospitals consented to participate in this study, including seven university hospitals. The amount of relevant hospitalisation beds per hospital was as follows: four with >1000 beds, 18 with 500–1000 beds and eight with <500 beds (total of 19,500 hospitals beds).

From March 1st, 2013 until February 28, 2014, every first *Candida* isolate from each episode of candidaemia was included. Episodes of candidaemia in a single patient were regarded as distinct if they were separated by at least a 30-day period during which no blood cultures became positive for *Candida*. Separate cultures with separate species from the same patient within a 30-day period were considered as polyfungal infections but not as different episodes. Identification and susceptibility testing were performed according to routine local procedures. In addition, all isolates were sent to the Belgian National Reference Center (NRC) for Mycosis (UZ Leuven) with a completed case report form (CRF). The following information had to be completed on the CRF: birth date, gender, hospitalisation ward, time of blood sampling, time to positivity (TTP) of the blood culture, time to reporting of identification and antifungal susceptibility to the treating clinician, next to the different blood culture incubation, species identification and antifungal susceptibility methods. Laboratory database systems were reviewed for missing cases to ensure completeness.

Identification

All isolates were subcultured upon arrival and stored at −70 °C. Species identification was checked at the NRC by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS; Microflex LT Biotyper, Bruker Daltonics, Bremen, Germany) and sequencing of the internal transcribed spacer (ITS) region if no reliable identification was obtained by MS [21]. In case of echinocandin resistance, the strain was sent to Rutgers, New Jersey Medical School for mutation analysis.

Antifungal susceptibility testing

The minimum inhibitory concentration (MIC) values were determined for fluconazole (FLC), posaconazole (POS), voriconazole (VRC), anidulafungin (AND), caspofungin (CAS), micafungin (MCF) and amphotericin B (AMB) by broth microdilution according to the EUCAST EDef 7.2 microdilution protocol [22]. The microdilution plates used were untreated MicroWell™ plates from Nunc™ (cat no. 732-2715). Reference powders for each antifungal agent were obtained from their respective manufacturers except FLC (Sigma) and AMB (Sigma). Antifungal resistance rates were calculated based on the EUCAST clinical breakpoints (version 7.0). The quality control (QC) strains *Candida krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019) were included each time a new batch of microtitre plates (VWR International) were used for the first time and once a week as a minimum.

Time to detection and reporting of identification and susceptibility results in daily clinical practice

Based on the requested information in the CRF, durations of the different diagnostic steps were calculated, including the time to positivity of the blood cultures (no missing data), the time to report the identification to the clinician (no missing data) and, finally, the time to report the antifungal susceptibility to the clinician. For 53 of the *Candida* isolates, antifungal reporting times were not available and, thus, not included in the data analysis.

Statistical analysis

Incidence data was expressed per 10,000 patient days or per 1000 admissions with 95 % confidence intervals (CIs). The data for categorical variables were expressed as a percentage and continuous variables were expressed as the mean, median and interquartile range (IQR). A Student's *t*-test (two-tailed, with equal or unequal variances, according to F-test for equality of two variances) was used to compare continuous variables. Categorical variables were analysed using χ^2 tests (two-tailed). Correlations between continuous variables were tested using Pearson analysis. Statistical analysis was performed using SPSS 23.0 (SPSS Inc., Chicago, IL. USA). A *p*-value of <0.05 was considered statistically significant.

Results

Patient characteristics and incidence data

Fifty-three percent of the patients with candidaemia were male. The median age was 67 years, with a range from 1 to 99 years.

A total of 355 isolates were retrieved from 338 different patients. Thirteen patients had a mixed infection with two different *Candida* species. The mean incidence rate of candidaemia across all hospitals was 0.44 per 1000 admissions (95 % CI 0.40–0.49) or 0.65 per 10,000 patient days (95 % CI 0.58–0.72). The incidence rates ranged from 0.07 to 1.43 per 1000 admissions and from 0.11 to 2.00 per 10,000 patient days across the hospitals. The incidence of candidaemia in university hospitals (mean of 0.56/1000 admissions or 0.79/10,000 patient days) versus non-university hospitals (mean of 0.37/1000 admissions or 0.52/10,000 patient days) was not significantly different ($p = 0.23$ and $p = 0.26$, respectively). There was no correlation between the incidence rate per hospital and the mean duration of patient stay per hospital ($r = 0.01$, $p = 0.94$).

Species distribution

Eleven different *Candida* species were identified, with *C. albicans* being the most prevalent (50.4 %), followed by *C. glabrata* (27.3 %), *C. parapsilosis sensu lato* (9.8 %), *C. tropicalis* (5.6 %), *C. guilliermondii* (2.5 %), *C. dubliniensis* (1.2 %), *C. krusei* (1.2 %), *C. lusitaniae* (1.2 %), *Candida fermentati* (0.6 %) and *Candida palmioleophila* (0.3 %). The group of *C. parapsilosis sensu lato* contained *C. parapsilosis sensu stricto* (33/35) and *C. metapsilosis* (2/35). Table 1 shows the distribution of the top four recovered species according to the ward. The species distribution varied significantly between the wards ($p < 0.001$). In neonatal/paediatric services, the relative frequency of *C. parapsilosis* was higher than in other services. *Candida glabrata* was more frequent in internal medicine and surgery wards than in other wards, resulting in an almost equal prevalence as *C. albicans* in surgical patients. The proportion of *C. glabrata* was relatively low in haematology-oncology wards, this in favour of *C. tropicalis* and other *Candida* species. The proportion of *C. albicans* was the highest (64.2 %) in the intensive care unit (ICU).

There was also a difference in species distribution between male and female patients. The relative proportion of female versus male patients was higher for *C. glabrata* compared to *C. albicans* ($p = 0.15$) and *C. parapsilosis* ($p = 0.02$).

Antifungal susceptibility

The NRC MIC50, MIC90 and MIC ranges for the seven antifungal agents are shown in Table 2. The susceptibility of the isolates according to the EUCAST breakpoints is shown in Table 3. Five of the 20 *C. tropicalis* isolates showed decreased susceptibility to fluconazole, including four isolates with MIC values >4 mg/L, and were, thus, classified as resistant. Those fluconazole-resistant *C. tropicalis* isolates showed complete cross-resistance to voriconazole and posaconazole. There was a difference

Table 1 *Candida* species distribution for the top five hospitalisation wards of the patients with candidaemia

Ward	No. of isolates (%)					
	C. albicans	*C. glabrata*	*C. parapsilosis*	*C. tropicalis*	Other*	Overall
ICU	70 (64.2)	24 (22.0)	5 (4.6)	7 (6.4)	3 (2.8)	109 (100)
Internal medicine	48 (47.1)	36 (35.3)	12 (11.8)	1 (0.9)	5 (4.9)	102 (100)
Surgery	24 (42.1)	22 (38.6)	4 (7.0)	5 (8.7)	2 (3.5)	57 (100)
Haematology-oncology	18 (51.4)	5 (14.3)	2 (5.7)	5 (14.3)	5 (14.3)	35 (100)
Paediatrics	9 (50.0)	1 (5.6)	4 (22.2)	1 (5.6)	3 (16.6)	18 (100)

*Including *C. dubliniensis* (4/355), *C. guilliermondii* (9/355), *C. krusei* (4/355), *C. lusitaniae* (4/355), *C. fermentati* (2/355) and *C. palmioleophila* (1/355)

Table 2 MIC50, MIC90 values and MIC ranges (mg/L) of the top four most frequently isolated *Candida* species

	FLC		VRC		POS		AND		MCF		CAS		AMB	
	MIC50 (MIC range)	MIC90	MIC50 (MIC range)	MIC90	MIC50 (MIC range)	MIC90	MIC50 (MIC range)	MIC90	MIC50 (MIC range)	MIC90	MIC50 (MIC range)	MIC90	MIC50 (MIC range)	MIC90
C. albicans	0.25 (0.016–64)	2	0.016 (0.016–8)	0.06	0.016 (0.016–8)	0.06	<0.016 (<0.016 to <0.016)	<0.016	<0.016 (<0.016 to <0.016)	<0.016	0.03 (<0.016–0.25)	0.12	0.50 (0.25–1)	1
C. glabrata	8 (0.12–64)	64	0.25 (0.016–8)	1	1 (0.016–8)	2	<0.016 (<0.016–4)	<0.016	<0.016 (<0.016–4)	<0.016	0.06 (<0.016–4)	0.12	1 (0.25–1)	1
C. parapsilosis	0.50 (0.25–32)	1	<0.016 (<0.016–2)	0.03	<0.016 (<0.016–0.25)	<0.016	1 (0.06–2)	2	1 (0.12–1)	1	0.50 (0.12–8)	1	1 (0.50–1)	1
C. tropicalis	1 (0.50–64)	8	0.06 (<0.016–8)	4	0.03 (<0.016–8)	4	<0.016 (<0.016 to <0.016)	<0.016	<0.016 (<0.016 to <0.016)	<0.016	0.06 (<0.016–0.25)	0.06	1 (0.50–1)	1

MIC50/MIC90: minimum inhibitory concentration that inhibited 50 % and 90 % of the isolates, respectively

FLC: fluconazole; VRC: voriconazole; POS: posaconazole; AND: anidulafungin; MCF: micafungin; CAS: caspofungin; AMB: amphotericin B

in fluconazole resistance between the wards ($p = 0.007$), which disappeared when excluding the haematology-oncology ward. The mean rate of fluconazole resistance was 22.9 % for isolates from haematology-oncology patients, 10.5 % in surgical patients, 11.1 % in paediatrics, 6.0 % in internal medicine and 3.7 % in the ICU. All interpretable *Candida* isolates except one were fully susceptible to anidulafungin and micafungin. The MIC values of this *C. glabrata* for the three tested echinocandins (anidulafungin, caspofungin and micafungin) were all 4 mg/L. Mutation analysis of this strain revealed a deletion in the amino acid phenylalanine 658 (F658) just at the beginning of *HS1* in the *FKS2* gene. The highest MIC values for caspofungin were found for *C. parapsilosis*, *C. guilliermondii* and one *C. fermentati* isolate. Uniform susceptibility to amphotericin B was noted for all 355 isolates.

Duration of diagnostic steps

The mean/median TTP for blood cultures was 44 h 54 min/39 h 28 min (IQR 26 h 49 min to 57 h 28 min), with no statistical difference in the TTP of different automatic blood incubation systems (BACTEC versus BacT/ALERT, mean/median TTP of 45 h 44 min/41 h 00 min versus 44 h 36 min/38 h 50 min, respectively) ($p = 0.71$). For *C. glabrata*, the TTP (mean/median 58 h 58 min/57 h 23 min) was significantly longer than for *C. albicans* (mean/median 41 h 5 min/35 h 36 min, $p < 0.001$), *C. guilliermondii* (mean/median 36 h 16 min/41 h 50 min, $p = 0.02$), *C. parapsilosis sensu lato* (mean/median 42 h 47 min/39 h 9 min, $p = 0.001$) and *C. tropicalis* (mean/median 27 h 18 min/22 h 56 min, $p < 0.001$). The mean/median time between blood culture positivity and the moment of species identification reporting time (IDR) to the clinician was 33 h 39 min/29 h 33 min (IQR 23 h 20 min to 40 h 22 min). In 24 of 30 hospitals, MALDI-TOF MS was implemented for the identification of the *Candida* species. The IDRs were significantly shorter in hospitals using MALDI-TOF MS compared to hospitals with alternative methods (mean/median IDR 32 h 44 min/29 h 15 min versus 41 h 12 min/41 h 25 min, respectively, $p = 0.003$). The mean/median time between positive blood culture and the moment of reporting the antifungal susceptibility (antifungal susceptibility reporting, AFR) to the clinician was 65 h 47 min/58 h 57 min (IQR 48 h 28 min to 74 h 52 min) (in these 53 cases, susceptibility testing was not performed in routine practice; consequently, reporting times were not available). Twenty-five hospitals performed in-house antifungal susceptibility testing, while five hospitals sent their isolates to the NRC for susceptibility testing. The mean/median durations were

Table 3 Resistance percentages of the top four most frequently isolated *Candida* species according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints

	FLC		VRC		POS		AND		MCF		AMB	
	% S	% R	% S	% R	% S	% R	% S	% R	% S	% R	% S	% R
C. albicans	92.7	3.9	96.1	3.9	96.6	3.4	100	0	100	0	100	0
C. glabrata	0	11.3	/*	/*	/*	/*	99.0	1.0	99.0	1.0	100	0
C. parapsilosis	94.4	5.6	94.4	5.6	97.2	2.8	0**	0**	0**	0**	100	0
C. tropicalis	75.0	20.0	80.0	20.0	80.0	20.0	100	0	/*	/*	100	0
Total	65.5	7.6	/*	/*	/*	/*	99.7	0.3	99.7	0.3	100	0

FLC: fluconazole; VRC: voriconazole; POS: posaconazole; AND: anidulafungin; MCF: micafungin; AMB: amphotericin B

*No EUCAST breakpoints available

**All strains were classified as intermediate susceptible

longer when the susceptibility testing was outsourced ($n = 4$) compared to in-house testing ($n = 295$) (183 h 41 min/208 h versus 64 h 11 min/58 h 30 min) ($p = 0.05$). Figure 1 shows the cumulative percentages of the IDRs and AFRs against the time in days after blood culture positivity.

Discussion

The average incidence of candidaemia in this 1-year prospective study was 0.44 per 1000 admissions or 0.65 per 10,000 patient days. These rates are lower than the reported values in North and Latin America [23, 24], but similar to the rates from surveys conducted in European countries [4, 5, 9]. The highest incidence (1.43/1000 admissions or 2.00/10,000 patient days) was recorded in the tertiary care hospital with the largest number of transplantations. It is likely that incidence rates correlate with the size of the 'at-risk' population for these invasive infections, but an in-depth study of the patient populations in the different Belgian hospitals is needed in order to understand better the large differences in candidaemia incidences ascertained in this study.

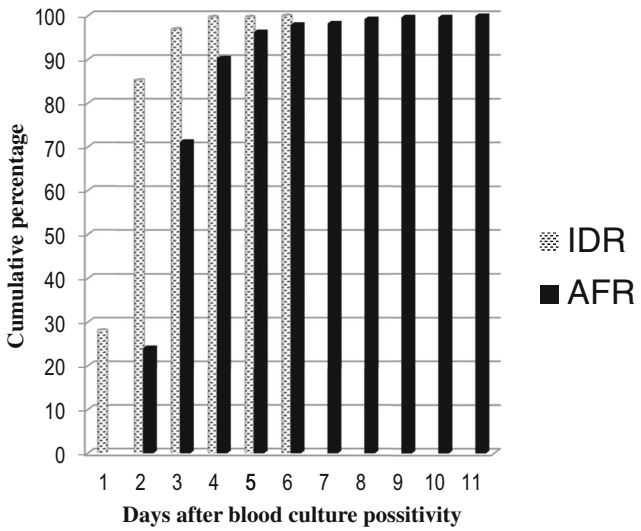

Fig. 1 Cumulative percentages of the identification reporting (IDR) and antifungal profile reporting (AFR) times against days after blood culture positivity. After 6 and 11 days, 100 % of the IDRs and AFRs, respectively, were reported

A surprisingly high number of azole-resistant *C. tropicalis* was noted (20 %) and confirmed by Clinical and Laboratory Standards Institute (CLSI) methodology. This azole resistance was not related to an outbreak as all four *C. tropicalis* strains were retrieved from different hospitals. This high percentage of azole resistance among *C. tropicalis* is not consistent with most other studies [11, 13, 25].

The echinocandins seem to retain excellent activity to all *Candida* species in this prospective study. This is consistent with other studies [12–14, 16, 26]. In *C. glabrata*, echinocandin resistance seems to be rising, mostly due to previous echinocandin use [14, 16]. The only echinocandin-resistant *C. glabrata* isolate retrieved in this study was isolated from a patient with proven *C. glabrata* endocarditis who received several treatment courses with anidulafungin.

In patients with candidaemia, the TTP has been reported to be significantly longer for *C. glabrata* than for other *Candida* species in the BacT/Alert and BACTEC 9240 systems [27]. This is confirmed in our study. Also, consistent with previous studies, we could not find a significant difference between TTP for *Candida* isolates in blood cultures incubated in the BACTEC versus the BacT/ALERT system [28].

The median time between positive blood culture and the reporting of the yeast identification to the clinician was 29 h 33 min, with a shorter time for laboratories that used MALDI-TOF MS as the identification method,

which is in line with expectations and was recently underlined in a systematic review [29]. However, the clinical impact of this faster reporting remains unknown.

The median time to report the antifungal susceptibility profile to the clinician was 58 h 57 min. These times represent a realistic estimate of what is currently achieved in routine clinical practice.

In conclusion, this TANSIR trial is the first multi-centre study focussing on both the incidence and antifungal susceptibility of candidaemia in the Belgian patient population. Azole resistance among *Candida* isolates remains low, but is emerging among *C. tropicalis* strains. Echinocandin resistance remains rare in Belgian *Candida* isolates.

Acknowledgements We are very grateful to prof. dr. David Perlin (Public Health Research Institute, New Jersey Medical School—Rutgers Biomedical and Health Sciences, Newark) for the mutation analysis of the echinocandin-resistant isolate.

We thank the following members of the participating Belgian hospitals: Wim Achtergael, ASZ Aalst; Anne Piette, AZ Alma Eeklo Sijsele; Gudrun Alliët, AZ Damiaan Oostende; Eric Nulens, AZ Sint Jan Brugge; Wouter Vandewal, AZ Sint Lucas Brugge; Anne-Marie Van Den Abeele, AZ Sint Lucas Gent; Truus Goegebuer, AZ Sint Maarten Mechelen; Tom Spiritus, AZ St.-Elisabeth Herentals; Danielle Van Der beek, AZ Turnhout; Inge Thoelen, AZ Vesalius Tongeren; Salah Eddine LALI, CHU de Charleroi; Te-Din Huang, CHU Mont-Godinne; Jean-Sébastien Goffinet, Clinique Saint Joseph CSL Arlon; Julie Cadrobbi, Clinique Sainte Elisabeth Namur; Valérie Verbelen, Clinique Saint-Pierre Ottignies; Carlota Montesinos, Erasme Brussel; Jef Vanschaeren, GZA Antwerpen; Emmanuel De Laere, AZ Delta; Johan Frans, Imelda Ziekenhuis Bonheiden; Louis Ide, Jan Palfijn Gent; Patricia Vandecandelaere, Jan Yperman Ziekenhuis Ieper; An Boel, OLV Ziekenhuis Aalst; Frederik Van Hoecke, Sint-Andries Ziekenhuis Tielt; Hector Rodriguez-Villalobos, UCL; Annelies De Bel, UZ Brussel; Jerina Boelens, UZ Gent; Katrien Lagrou, UZ Leuven; Koen Magerman, Virga Jessa Hasselt; Els Oris, ZOL Genk; Marie Pierre Hayette, CHU Liège.

Compliance with ethical standards

Transparency declaration The results of this study were partially presented at the 25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID, Copenhagen, Denmark, 25–28 April 2015), in the form of an orally presented E-poster.

Katrien Lagrou reports a grant from MSD during the conduct of the study, personal fees and travel support from Gilead and Pfizer and personal fees from MSD outside the submitted work.

Marie-Pierre Hayette reports personal fees and travel support from Gilead and Pfizer and grants and travel support from MSD outside the submitted work.

Stijn Blot reports personal fees and travel support from Gilead, grants from MSD and grants, personal fees and travel support from Pfizer outside the submitted work.

Hector Rodriguez-Villalobos reports travel support from Gilead, MSD and Pfizer outside the submitted work.

Eric Van Wijngaerden reports personal fees from Gilead, personal fees and travel support from Pfizer and personal fees and travel support from MSD outside the submitted work.

Charlotte Trouvé reports travel support from Pfizer outside the submitted work.

Stijn Jonckheere, Sofie Patteet and Françoise Symoens declare no conflicts of interest.

Funding This work was supported by a research grant from MSD.

Informed consent Informed consent was not obtained from all individual participants in the study, since no additional sample-taking was necessary and the patient's medical files were not needed to be consulted.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Vallabhaneni S, Mody RK, Walker T, Chiller T (2016) The global burden of fungal diseases. Infect Dis Clin North Am 30:1–11
2. Tortorano AM, Peman J, Bernhardt H, Klingspor L, Kibbler CC, Faure O, Biraghi E, Canton E, Zimmermann K, Seaton S, Grillot R; ECMM Working Group on Candidaemia (2004) 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 23:317–322
3. Caggiano G, Coretti C, Bartolomeo N, Lovero G, De Giglio O, Montagna MT (2015) *Candida* bloodstream infections in Italy: changing epidemiology during 16 years of surveillance. Biomed Res Int. doi:10.1155/2015/256580
4. Ericsson J, Chryssanthou E, Klingspor L, Johansson AG, Ljungman P, Svensson E, Sjölin J (2013) Candidaemia in Sweden: a nationwide prospective observational survey. Clin Microbiol Infect 19(4):E218–E221
5. Hesstvedt L, Gaustad P, Andersen CT, Haarr E, Hannula R, Haukland HH, Hermansen NO, Larssen KW, Mylvaganam H, Ranheim TE, Sandven P, Nordøy I; Norwegian Yeast Study Group, Kanestrøm A, Grub C, Onken A, Thielsen C, Skaare D, Tofteland S, Sønsteby LJ, Hjetland R, Hide R, Vik E, Kümmel A, Åsheim S (2015) Twenty-two years of candidaemia surveillance: results from a Norwegian national study. Clin Microbiol Infect 10:938–945
6. Nieto MC, Tellería O, Cisterna R (2015) Sentinel surveillance of invasive candidiasis in Spain: epidemiology and antifungal susceptibility. Diagn Microbiol Infect Dis 81:34–40
7. Guinea J, Zaragoza Ó, Escribano P, Martín-Mazuelos E, Pemán J, Sánchez-Reus F, Cuenca-Estrella M; CANDIPOP Project, GEIH-GEMICOMED (SEIMC), and REIPI (2014) Molecular identification and antifungal susceptibility of yeast isolates causing fungemia collected in a population-based study in Spain in 2010 and 2011. Antimicrob Agents Chemother 58:1529–1537
8. Orasch C, Marchetti O, Garbino J, Schrenzel J, Zimmerli S, Mühlethaler K, Pfyffer G, Ruef C, Fehr J, Zbinden R, Calandra T, Bille J (2014) *Candida* species distribution and antifungal susceptibility testing according to European Committee on Antimicrobial Susceptibility Testing and new vs. old Clinical and Laboratory Standards Institute clinical breakpoints: a 6-year prospective candidaemia survey from the fungal infection network of Switzerland. Clin Microbiol Infect 7:698–705
9. Bitar D, Lortholary O, Le Strat Y, Nicolau J, Coignard B, Tattevin P, Che D, Dromer F (2014) Population-based analysis of invasive fungal infections, France, 2001–2010. Emerg Infect Dis 20:1149–1155
10. Lagrou K, Verhaegen J, Peetermans WE, De Rijdt T, Maertens J, Van Wijngaerden E (2007) Fungemia at a tertiary care hospital: incidence, therapy, and distribution and antifungal susceptibility of causative species. Eur J Clin Microbiol Infect Dis 26:541–547

11. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, Rodloff A, Fu W, Ling TA; Global Antifungal Surveillance Group (2010) Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of *Candida* species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. J Clin Microbiol 48: 1366–1377
12. Arendrup MC (2014) Update on antifungal resistance in *Aspergillus* and *Candida*. Clin Microbiol Infect 20:42–48
13. Pfaller MA, Messer SA, Woosley LN, Jones RN, Castanheira M (2013) Echinocandin and triazole antifungal susceptibility profiles for clinical opportunistic yeast and mold isolates collected from 2010 to 2011: application of new CLSI clinical breakpoints and epidemiological cutoff values for characterization of geographic and temporal trends of antifungal resistance. J Clin Microbiol 51: 2571–2581
14. Arendrup MC, Perlin DS (2014) Echinocandin resistance: an emerging clinical problem? Curr Opin Infect Dis 27:484–492
15. Farmakiotis D, Tarrand JJ, Kontoyiannis DP (2014) Drug-resistant *Candida glabrata* infection in cancer patients. Emerg Infect Dis 20: 1833–1840
16. Alexander BD, Johnson MD, Pfeiffer CD, Jiménez-Ortigosa C, Catania J, Booker R, Castanheira M, Messer SA, Perlin DS, Pfaller MA (2013) Increasing echinocandin resistance in *Candida glabrata*: clinical failure correlates with presence of *FKS* mutations and elevated minimum inhibitory concentrations. Clin Infect Dis 56:1724–1732
17. Vallabhaneni S, Cleveland AA, Farley MM, Harrison LH, Schaffner W, Beldavs ZG, Derado G, Pham CD, Lockhart SR, Smith RM (2015) Epidemiology and risk factors for echinocandin nonsusceptible *Candida glabrata* bloodstream infections: data from a large multisite population-based candidemia surveillance program, 2008–2014. Open Forum Infect Dis 2(4):ofv163
18. Pfaller MA, Castanheira M, Lockhart SR, Ahlquist AM, Messer SA, Jones RN (2012) Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of *Candida glabrata*. J Clin Microbiol 50:1199–1203
19. Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, Meersseman W, Akova M, Arendrup MC, Arikan-Akdagli S, Bille J, Castagnola E, Cuenca-Estrella M, Donnelly JP, Groll AH, Herbrecht R, Hope WW, Jensen HE, Lass-Flörl C, Petrikkos G, Richardson MD, Roilides E, Verweij PE, Viscoli C, Ullmann AJ; ESCMID Fungal Infection Study Group (2012) ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect 18:19–37
20. van der Geest PJ, Rijnders BJ, Vonk AG, Groeneveld AB (2016) Echinocandin to fluconazole step-down therapy in critically ill patients with invasive, susceptible *Candida albicans* infections. Mycoses 59:179–185
21. Pryce TM, Palladino S, Kay ID, Coombs GW (2003) Rapid identification of fungi by sequencing the ITS1 and ITS2 regions using an automated capillary electrophoresis system. Med Mycol 41:369–381
22. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Antifungal Agents. Breakpoint tables for interpretation of MICs. Version 7.0, valid from 2014-08-12
23. Cleveland AA, Harrison LH, Farley MM, Hollick R, Stein B, Chiller TM, Lockhart SR, Park BJ (2015) Declining incidence of candidemia and the shifting epidemiology of *Candida* resistance in two US metropolitan areas, 2008–2013: results from population-based surveillance. PLoS One 10(3):e0120452
24. Lockhart SR, Iqbal N, Cleveland AA, Farley MM, Harrison LH, Bolden CB, Baughman W, Stein B, Hollick R, Park BJ, Chiller T (2012) Species identification and antifungal susceptibility testing of *Candida* bloodstream isolates from population-based surveillance studies in two U.S. cities from 2008 to 2011. J Clin Microbiol 50: 3435–3442
25. Pfaller MA, Messer SA, Jones RN, Castanheira M (2015) Antifungal susceptibilities of *Candida*, *Cryptococcus neoformans* and *Aspergillus fumigatus* from the Asia and Western Pacific region: data from the SENTRY antifungal surveillance program (2010–2012). J Antibiot (Tokyo) 68:556–561
26. Marcos-Zambrano LJ, Escribano P, Sánchez C, Muñoz P, Bouza E, Guinea J (2014) Antifungal resistance to fluconazole and echinocandins is not emerging in yeast isolates causing fungemia in a Spanish tertiary care center. Antimicrob Agents Chemother 58: 4565–4572
27. Huang L, Zhang YY, Sun LY (2013) Time to positivity of blood culture can predict different *Candida* species instead of pathogen concentration in candidemia. Eur J Clin Microbiol Infect Dis 32: 917–922
28. Horvath LL, George BJ, Murray CK, Harrison LS, Hospenthal DR (2004) Direct comparison of the BACTEC 9240 and BacT/ALERT 3D automated blood culture systems for *Candida* growth detection. J Clin Microbiol 42:115–118
29. Dixon P, Davies P, Hollingworth W, Stoddart M, MacGowan A (2015) A systematic review of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry compared to routine microbiological methods for the time taken to identify microbial organisms from positive blood cultures. Eur J Clin Microbiol Infect Dis 34:863–876