Year : 2018

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Kritikos Antonios, 2018, Accuracy of Sensititre YeastOne echinocandins epidemiological cut-off values for identification of FKS mutant Candida albicans and Candida glabrata: a ten year national survey of the Fungal Infection Network of Switzerland (FUNGINOS).

Originally published at : Thesis, University of Lausanne

Posted at the University of Lausanne Open Archive http://serval.unil.ch
Document URN : urn:nbn:ch:serval-BIB_625D354817D04

UNIL | Université de Lausanne

Ecole doctorale

UNIVERSITE DE LAUSANNE - FACULTE DE BIOLOGIE ET DE MEDECINE
Département de Médecine Interne

Service des Maladies infectieuses

"Accuracy of Sensititre YeastOne echinocandins epidemiological cut-off values for identification of FKS mutant Candida albicans and Candida glabrata: a ten year national survey of the Fungal Infection Network of Switzerland (FUNGINOS)."

THESE

préparée sous la direction du Docteur Frédéric Lamoth

et présentée à la Faculté de biologie et de médecine de
l'Université de Lausanne pour l'obtention du grade de

DOCTEUR EN MEDECINE

par

Antonios KRITIKOS

Médecin diplômé de l'Université de Héraklion (Grèce)
Originaire de Grèce

Lausanne
2018

UNIL | Université de Lausanne

Faculté de biologie et de médecine

Ecole Doctorale
Doctorat en médecine

Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

Directeur de thèse	*Monsieur le Docteur*	***Frédéric Lamoth***
Co-Directeur de thèse		
Expert	*Monsieur le Docteur*	***Guy Prod'Hom***
Vice-Directeur de l'Ecole doctorale	*Monsieur le Professeur*	***John Prior***

la Commission MD de l'Ecole doctorale autorise l'impression de la thèse de

Monsieur Antonios KRITIKOS

intitulée

Accuracy of Sensititre YeastOne echinocandins epidemiological cut-off values for identification of FKS mutant Candida albicans and Candida glabrata: a ten year national survey of the Fungal Infection Network of Switzerland (FUNGINOS)

Lausanne, le 20 septembre 2018

pour Le Doyen
de la Faculté de Biologie et de Médecine

Monsieur le Professeur John Prior
Vice-Directeur de l'Ecole doctorale

Clinical Microbiology and Infection 24 (2018) 1214.e1–1214.e4

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Research note

Accuracy of Sensititre YeastOne echinocandins epidemiological cut-off values for identification of *FKS* mutant *Candida albicans* and *Candida glabrata*: a ten year national survey of the Fungal Infection Network of Switzerland (FUNGINOS)

A. Kritikos [1], D. Neofytos [2], N. Khanna [3, 4], P.W. Schreiber [5], K. Boggian [6], J. Bille [1, 7], J. Schrenzel [2, 8], K. Mühlethaler [9], R. Zbinden [5], T. Bruderer [10], D. Goldenberger [11], G. Pfyffer [12], A. Conen [13], C. Van Delden [2], S. Zimmerli [8], D. Sanglard [7], D. Bachmann [7], O. Marchetti [1, 14], F. Lamoth [1, 7, *] on behalf of the Fungal Infection Network of Switzerland (FUNGINOS)[†]

[1] *Infectious Diseases Service, Department of Medicine, Lausanne University Hospital, Lausanne, Switzerland*
[2] *Service of Infectious Diseases, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland*
[3] *Division of Infectious Diseases and Hospital Epidemiology, Department of Biomedicine, University Hospital of Basel, Basel, Switzerland*
[4] *Division of Infectious Diseases and Hospital Epidemiology, Department of Clinical Research, University Hospital of Basel, Basel, Switzerland*
[5] *Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland*
[6] *Division of Infectious Diseases and Hospital Hygiene, Cantonal Hospital, Sankt Gallen, Switzerland*
[7] *Institute of Microbiology, Lausanne University Hospital, Lausanne, Switzerland*
[8] *Bacteriology Laboratory, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland*
[9] *Department of Infectious Diseases, Bern University Hospital, Bern, Switzerland*
[10] *Department of Bacteriology, Mycology and Parasitology, Centre of Laboratory Medicine, Cantonal Hospital of Sankt Gallen, Sankt Gallen, Switzerland*
[11] *Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland*
[12] *Department of Medical Microbiology, Canton Hospital of Luzern, Luzern, Switzerland*
[13] *Division of Infectious Diseases and Hospital Epidemiology, Cantonal Hospital of Aarau, Aarau, Switzerland*
[14] *Department of Medicine, Ensemble Hospitalier de la Côte, Morges, Switzerland*

ARTICLE INFO

Article history:
Received 25 March 2018
Received in revised form
14 May 2018
Accepted 20 May 2018
Available online 15 June 2018

Editor: E. Roilides

Keywords:
Anidulafungin
Antifungal susceptibility testing
Candidaemia
Caspofungin
FKS hotspot
Micafungin
Minimal inhibitory concentration

ABSTRACT

Objectives: Echinocandins represent the first-line treatment of candidaemia. Acquired echinocandin resistance is mainly observed among *Candida albicans* and *Candida glabrata* and is associated with *FKS* hotspot mutations. The commercial Sensititre YeastOne™ (SYO) kit is widely used for antifungal susceptibility testing, but interpretive clinical breakpoints are not well defined. We determined echinocandins epidemiological cut-off values (ECV) for *C. albicans*/*glabrata* tested by SYO and assessed their ability to identify *FKS* mutants in a national survey of candidaemia.

Methods: Bloodstream isolates of *C. albicans* and *C. glabrata* were collected in 25 Swiss hospitals from 2004 to 2013 and tested by SYO. *FKS* hotspot sequencing was performed for isolates with an MIC≥ECV for any echinocandin.

Results: In all, 1277 *C. albicans* and 347 *C. glabrata* were included. ECV 97.5% of caspofungin, anidulafungin and micafungin were 0.12, 0.06 and 0.03 μg/mL for *C. albicans*, and 0.25, 0.12 and 0.03 μg/mL for *C. glabrata*, respectively. *FKS* hotspot sequencing was performed for 70 isolates. No mutation was found in the 52 'limit wild-type' isolates (MIC=ECV for at least one echinocandin). Among the 18 'non-wild-type' isolates (MIC>ECV for at least one echinocandin), *FKS* mutations were recovered in the only two isolates with MIC>ECV for all three echinocandins, but not in those exhibiting a 'non-wild-type' phenotype for only one or two echinocandins.

Conclusion: This 10-year nationwide survey showed that the rate of echinocandin resistance among *C. albicans* and *C. glabrata* remains low in Switzerland despite increased echinocandin use. SYO-ECV

* Corresponding author. F. Lamoth, Infectious Diseases Service and Institute of Microbiology, Lausanne University Hospital, Rue du Bugnon 48, 1011 Lausanne, Switzerland.
E-mail address: Frederic.Lamoth@chuv.ch (F. Lamoth).
[†] Membership of the FUNGINOS group is provided in the Appendix section.

https://doi.org/10.1016/j.cmi.2018.05.012

could discriminate *FKS* mutants from wild-type isolates tested by SYO in this population. **A. Kritikos, Clin Microbiol Infect 2018;24:1214.e1–1214.e4**

Introduction

Candida spp. are among the five major causes of bloodstream infections [1,2]. Echinocandins (caspofungin, anidulafungin, micafungin) are recommended as first-line treatment of candidaemia [3–5]. Echinocandin resistance in *Candida* spp. other than *Candida parapsilosis* is reported in <5% of isolates, being more frequent among *Candida glabrata* [6–10]. Hotspot mutations in the 1,3-β-D-glucan synthase genes *FKS1* and *FKS2*, encoding for the target of echinocandins, are the unique mechanism of resistance identified so far [11]. The Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have established antifungal testing methods and clinical breakpoints for interpretation of MICs of echinocandins [12,13]. Because CLSI and EUCAST antifungal susceptibility methods are laborious, many centres use a commercial broth microdilution kit (Sensititre Yeast One™; Trek Diagnostics Systems, Thermo Fisher Scientific, Cleveland, OH, USA). Although the manufacturer recommends use of CLSI breakpoints for interpreting echinocandin MICs, no specific breakpoints have been defined for this method. Epidemiological cut-off values (ECV) for Sensititre Yeast One™ (SYO) have been calculated in some studies [14,15]. In one of them, these ECV could correctly identify a collection of isolates with known *FKS* mutations [15].

We determined echinocandin ECV among *Candida albicans* and *C. glabrata* bloodstream isolates tested by SYO from the Fungal Infection Network of Switzerland (FUNGINOS). The ability of ECV to discriminate *FKS* mutants from wild-type isolates was assessed.

Materials and methods

C. albicans and C. glabrata bloodstream isolates were prospectively collected in a cohort of 25 Swiss medical centres (5 university and 20 university-affiliated hospitals) of the FUNGINOS network over a 10-year period (2004–2013). Antifungal susceptibility testing was performed by SYO for all isolates. MICs were determined by visual inspection and according to the manufacturer's recommendations [16]. In all, 1277 *C. albicans* and 347 *C. glabrata* were tested. Caspofungin MICs were determined for all isolates (n = 1624), whereas susceptibility testing for micafungin and anidulafungin, included later in the SYO panel, was performed for 406 isolates. The ECVs were defined for each drug/species according to methods previously described, as the MIC value encompassing 97.5% of the theoretical distribution [15]. Sequencing of hotspots 1 and 2 of *FKS1* (*C. albicans* and *C. glabrata*) and *FKS2* (*C. glabrata* only) was performed using a Sanger chain termination method for all isolates with MIC≥ECV for at least one echinocandin (referred to as 'limit wild-type' and 'non-wild-type' populations, respectively). Primers and details are provided in the Supplementary material (Table S1). Antifungal susceptibility testing of anidulafungin and micafungin was performed by SYO for all those isolates that were not initially tested for these drugs (n = 28). This study was approved by the local ethics committee.

Results

Distributions of the MICs of the three echinocandins for *C. albicans* and *C. glabrata* are represented in Fig. 1. ECV for caspofungin, anidulafungin and micafungin were 0.12, 0.06 and 0.03 μg/mL for *C. albicans*, and 0.25, 0.12 and 0.03 μg/mL for *C. glabrata*, respectively.

FKS hotspots were sequenced in 70 isolates with MIC equal or above the ECV for at least one echinocandin. Although no mutation was found among the 52 'limit wild-type' isolates (MIC=ECV), 2/18 (11%) of the 'non-wild-type' isolates (MIC>ECV) harboured an *FKS* mutation: one *C. albicans* (R1361G in hotspot 2 of *FKS1*) and one *C. glabrata* (S663P in hotspot 1 of *FKS2*). Both amino acid substitutions are known to be associated with high levels of echinocandin resistance [11]. The two *FKS* mutants were the only isolates with MIC>ECV for all three echinocandins (MIC 1, 0.5 and 0.5 μg/mL and 8, 2 and 4 μg/mL for caspofungin, anidulafungin and micafungin, respectively), although no mutations were found among the 16 isolates classified as 'non-wild-type' for only one (n = 11) or two (n = 5) echinocandins.

The ECV breakpoints were a better predictor of *FKS* mutations for *C. glabrata* compared with *C. albicans* (Fig. 1). Overall, the MIC distribution curve of micafungin showed a clear distinction between the non-mutant population and the *FKS* mutant isolates, which were separated by at least three dilutions (Fig. 1). This distinction was less clear for anidulafungin and caspofungin with overlaps between the two populations.

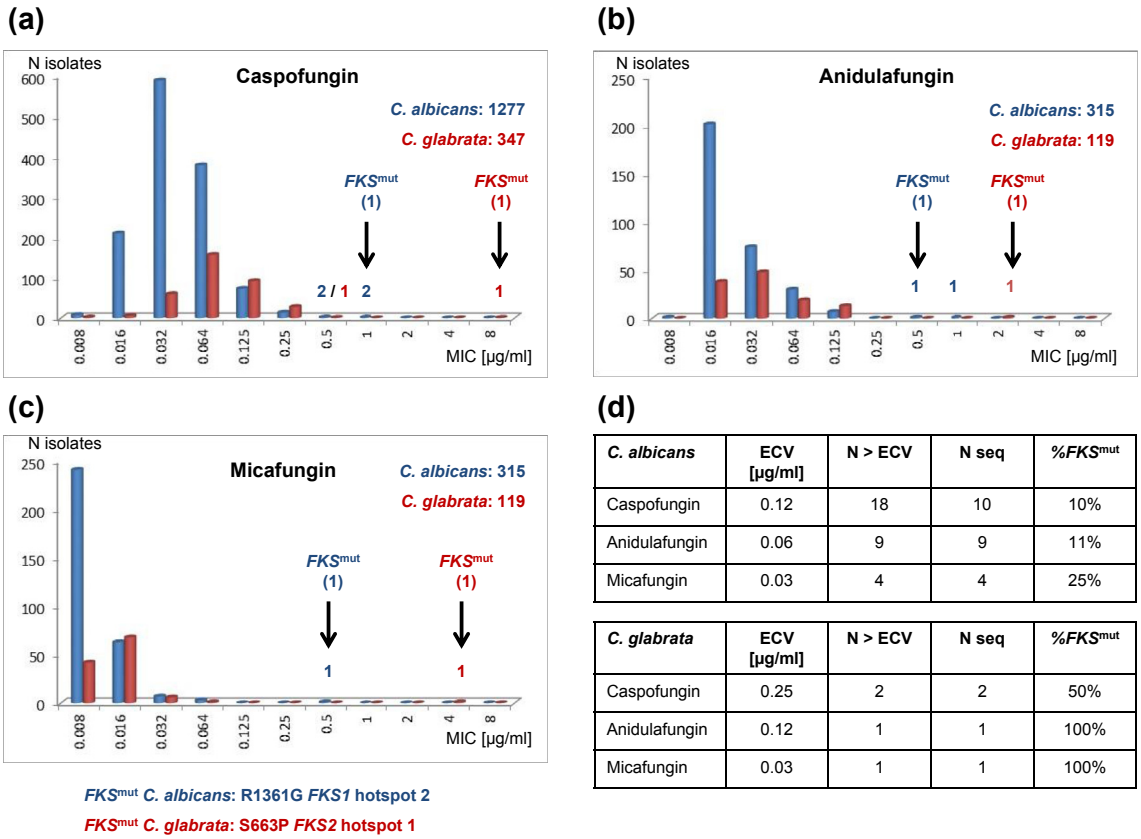
Data about echinocandin exposure before the candidaemic episode were obtained for 28 patients. Both patients infected with *FKS* mutant strains had received echinocandins within the last 3 months for 68 and 70 days, respectively. Only 2/26 (8%) of patients infected with non-*FKS* mutants have been exposed to echinocandins and duration was shorter (20 and 34 days).

Data for echinocandin consumption were available from three university hospitals, which represented 32% of isolates in this study. Caspofungin was the most widely used echinocandin: its consumption increased from 2.6 defined daily doses (DDD) per 1000 patient-days in 2004 to 5.4 in 2013 (Spearman's rank coefficient (r_s): 0.976, p <0.01). We did not observe a significant increase in the rate of *C. parapsilosis* or other *Candida* spp. with intrinsically higher echinocandin MICs over the study period.

Discussion

Although echinocandin resistance is an emerging concern, there are actually few epidemiological data about the rate of molecularly confirmed acquired resistance and the trends over years in relation to echinocandin consumption. This 10-year nationwide survey of *C. albicans* and *C. glabrata* bloodstream isolates in Switzerland showed that echinocandin resistance remained at a low level despite a significant increase in echinocandin use and was mainly associated with individual pre-echinocandin exposure of prolonged duration.

Compared with our study, Espinel-Ingroff *et al.* reported ECV values that were similar for *C. glabrata* and one dilution higher for *C. albicans* [15]. In their analysis, these ECVs correctly classified as non-wild-type about 90% of 81 isolates with known *FKS* hotspot mutations. This study provided crucial information about the sensitivity of these cut-offs for identifying hotspot mutations, but their positive and negative predictive values in routine clinical application were not determined. Our approach was different because we tested 70 isolates phenotypically classified as 'non-



(d)

C. albicans	ECV [µg/ml]	N > ECV	N seq	%*FKS*^{mut}
Caspofungin	0.12	18	10	10%
Anidulafungin	0.06	9	9	11%
Micafungin	0.03	4	4	25%

C. glabrata	ECV [µg/ml]	N > ECV	N seq	%*FKS*^{mut}
Caspofungin	0.25	2	2	50%
Anidulafungin	0.12	1	1	100%
Micafungin	0.03	1	1	100%

Fig. 1. Distribution of MICs of *Candida albicans* and *Candida glabrata* isolates for the three echinocandins; caspofungin (a), anidulafungin (b) and micafungin (c). FKS^{mut}, isolates with documented *FKS* hotspot mutations. (d) ECV, epidemiological cut-off values derived from the MIC distribution curves, N>ECV: number of isolates with MIC>ECV ('non-wild-type' isolates), N seq: number of 'non-wild-type' isolates for which *FKS* hotspot sequencing was performed (note: some isolates could not be recovered), %*FKS*^{mut}, rate (percentage) of *FKS* hotspot mutant isolates among 'non-wild-type' isolates.

wild-type' or 'limit wild-type' according to the ECVs. The absence of *FKS* mutations among the 52 'limit wild-type' isolates suggests an excellent negative predictive value of these cut-offs. Although ECV were able to correctly identify the only two *FKS* mutants, only 11% of 'non-wild-type' isolates actually harboured a mutation. Using the same ECVs as Espinel-Ingroff *et al.* (i.e. one dilution higher for *C. albicans*) would increase the positive predictive value of *FKS* mutation to overall 33% [15]. Application of CLSI breakpoints, which is recommended by the SYO manufacturer, classified 26 (1.6%) isolates of this collection as 'non-susceptible' to at least one echinocandin with *FKS* mutations recovered in only 8% of them. Of note, CLSI criteria for resistance would have missed one of the mutants classified as 'intermediate' for anidulafungin and micafungin.

Importantly, micafungin ECV demonstrated a better ability to discriminate *FKS* mutants from wild-type population compared with other echinocandins and *FKS* mutations were always associated with MIC above the ECV cut-offs for all three echinocandins. However, these two mutations are known to be associated with a high level of echinocandin resistance and we cannot exclude that other mutations, which have been associated with low levels of resistance and were not found in this study, could be more difficult to detect on the basis of MIC values [11].

Our analysis was performed in a predominantly wild-type population with low prevalence of *FKS* mutations, which actually corresponds to epidemiological data reported from other European countries [7,8]. This may explain the lower ECV found in this analysis compared with the multicentre study of Espinel-Ingroff *et al.* [15]. These results should be validated in settings with higher rates of echinocandin resistance. Despite this limitation, our analysis further supports the appropriateness of these ECV to exclude or predict *FKS* mutation-related echinocandin resistance among *C. albicans* and *C. glabrata* tested by SYO.

Transparency declaration

The Fungal Infection Network of Switzerland Foundation (FUNGINOS) received unrestricted grant support from (in alphabetical order): Essex Schering-Plough, Gilead, Merck, Novartis, Pfizer, and Sharp and Dohme-Chibret. None of the above funding sources has been involved in study design and conduct, patient recruitment, data collection, analysis, and interpretation; writing of the article; or decision to submit the article for publication.

Acknowledgements

The FUNGINOS Group thank Mrs Isabel Cobos, Mrs Aurélie Guillet, Mrs Corine Guyaz, Mrs Monika Ochsner and Mrs Annie Savoie (Infectious Diseases Service, Department of Medicine, Lausanne University Hospital) for assistance in collecting and managing clinical data, as well as Mr Christian Durussel, Mrs Dominique Pilloud, Dr Philippe Hauser and colleagues (FUNGINOS Reference Mycology Laboratory, Institute of Microbiology, Lausanne University Hospital) for technical support for identification and antifungal susceptibility testing of *Candida* isolates.

We acknowledge the Santos-Suarez foundation for financial support to F. Lamoth laboratory.

Preliminary results of this work have been presented as a poster (P1747) at the 27th ECCMID at Vienna (Austria), 22–25 April 2017.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2018.05.012.

Appendix. Fungal Infection Network of Switzerland (FUNGINOS): Investigators of the Candidaemia Study

Clinical Investigators (Institutions and persons in alphabetical order):

Thomas Bregenzer, Anna Conen, Kantonsspital, Aarau.

Anna Conen, Ursula Flückiger, Nina Khanna, Christina Orasch, University Hospital, Basel.

Ulrich Heininger, Universitätskinderspital, Basel, Mario Franciolli, Ospedale San Giovanni, Ente Ospedaliero Cantonale, Bellinzona.

Lauro Damonti, Stefan Zimmerli, University Hospital, Bern.

Madeleine Rothen, Claudine Zellweger, Spitalzentrum, Biel.

Madeleine Rothen, Philipp Tarr, Kantonsspital, Bruderholz.

Felix Fleisch, Kantonsspital, Chur.

Christian Chuard, Véronique Erard, Hôpital Cantonal, Fribourg.

Stéphane Emonet, Jorge Garbino, Christian van Delden, University Hospital, Geneva.

Daniel Genne, Hôpital Communal, La-Chaux-de-Fonds.

Pierre-Yves Bochud, Thierry Calandra, Véronique Erard, Frédéric Lamoth, Oscar Marchetti, Christina Orasch, University Hospital, Lausanne.

Jean-Philippe Chave, Clinique Bois-Cerf, Clinique Cécil, and Clinique La Source, Lausanne.

Peter Graber, Kantonsspital, Liestal.

Rita Monotti, Ospedale Regionale, Ente Ospedaliero Cantonale, Locarno.

Enos Bernasconi, Ospedale Civico, Ente Ospedaliero Cantonale, Lugano.

Marco Rossi, Kantonsspital, Luzern.

Martin Krause, Kantonsspital, Münsterlingen.

Rein-Jan Piso, Kantonsspital, Olten.

Frank Bally, Nicolas Troillet, Institut Central des Hôpitaux Valaisans, Sion.

Katia Boggian, Kantonsspital, Sankt Gallen.

Gerhard Eich, Jacques Gubler, Kantonsspital, Winterthur.

Jan Fehr, Alexander Imhof, Christian Ruef, University Hospital, Zürich.

Gerhard Eich, Jacques Gubler, Stadtspital Triemli, Zürich.

Christoph Berger, Universitätskinderspital, Zürich.

Microbiology Laboratory Investigators (Institutions and persons in alphabetical order):

Hans Fankhauser, Ivo Heinzer, Kantonsspital, Aarau.

Reno Frei, University Hospital, Basel.

Roland Hertel, Universitätskinderspital, Basel.

Marisa Dolina, Orlando Petrini, Istituto Cantonale di Microbiologia, Bellinzona.

Olivier Dubuis, Viollier Microbiology Laboratories, Bienne.

Konrad Mühlethaler, University Hospital, Bern.

Suzanne Graf, Kantonsspital, Bruderholz and Kantospital, Liestal.

Martin Risch, Eva Ritzler, Kantonsspital, Chur.

Dominique Fracheboud, Hôpital Cantonal, Fribourg.

Peter Rohner, Jacques Schrenzel, University Hospital, Geneva.

Reto Lienhardt, Hôpital Communal, La-Chaux-de-Fonds.

Jacques Bille, Frédéric Lamoth, University Hospital, Lausanne.

Corinne Andreutti-Zaugg, Alberto Gallusser, Clinique La Source, Lausanne.

Suzanne Graf, Kantonsspital, Liestal.

Gaby Pfyffer, Kantonsspital, Luzern.

Karin Herzog, Kantonsspital, Münsterlingen.

Urs Schibli, Kantonsspital, Olten.

Lysiane Tissière, Institut Central des Hôpitaux Valaisans, Sion.

Thomas Bruderer, Detlev Schultze, Kantonsspital, Sankt Gallen.

Reinhard Zbinden, University Hospital, Zürich.

References

[1] Marchetti O, Bille J, Fluckiger U, Eggimann P, Ruef C, Garbino J, et al. Epidemiology of candidemia in Swiss tertiary care hospitals: secular trends, 1991–2000. Clin Infect Dis 2004;38:311–20.

[2] Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24 179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004;39:309–17.

[3] Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, et al. ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect 2012;18:19–37.

[4] Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 2016;62:e1–50.

[5] Ullmann AJ, Akova M, Herbrecht R, Viscoli C, Arendrup MC, Arikan-Akdagli S, et al. ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: adults with haematological malignancies and after haematopoietic stem cell transplantation (HCT). Clin Microbiol Infect 2012;18:53–67.

[6] Arendrup MC, Dzajic E, Jensen RH, Johansen HK, Kjaeldgaard P, Knudsen JD, 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.

[7] Marcos-Zambrano LJ, Escribano P, Sanchez C, Munoz P, Bouza E, Guinea J. Antifungal resistance to fluconazole and echinocandins is not emerging in yeast isolates causing fungemia in a Spanish tertiary care center. Antimicrob Agents Chemother 2014;58:4565–72.

[8] Pfaller MA, Messer SA, Moet GJ, Jones RN, Castanheira M. Candida bloodstream infections: comparison of species distribution and resistance to echinocandin and azole antifungal agents in Intensive Care Unit (ICU) and non-ICU settings in the SENTRY Antimicrobial Surveillance Program (2008–2009). Int J Antimicrob Agents 2011;38:65–9.

[9] Pham CD, Iqbal N, Bolden CB, Kuykendall RJ, Harrison LH, Farley MM, et al. Role of FKS mutations in *Candida glabrata*: MIC values, echinocandin resistance, and multidrug resistance. Antimicrob Agents Chemother 2014;58:4690–6.

[10] Shields RK, Nguyen MH, Press EG, Cumbie R, Driscoll E, Pasculle AW, et al. Rate of FKS mutations among consecutive *Candida* isolates causing bloodstream infection. Antimicrob Agents Chemother 2015;59:7465–70.

[11] Arendrup MC, Cuenca-Estrella M, Lass-Florl C, Hope WW. Breakpoints for antifungal agents: an update from EUCAST focussing on echinocandins against *Candida* spp. and triazoles against *Aspergillus* spp. Drug Resist Updat 2013;16:81–95.

[12] Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts. 4th ed. Wayne, PA: CLSI; 2017 (M27-Ed4).

[13] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Antifungal clinical breakpoint table V8.1. Copenhagen: EUCAST; 2017.

[14] Canton E, Peman J, Hervas D, Iniguez C, Navarro D, Echeverria J, et al. Comparison of three statistical methods for establishing tentative wild-type population and epidemiological cutoff values for echinocandins, amphotericin B, flucytosine, and six *Candida* species as determined by the colorimetric Sensititre YeastOne method. J Clin Microbiol 2012;50:3921–6.

[15] Espinel-Ingroff A, Alvarez-Fernandez M, Canton E, Carver PL, Chen SC, Eschenauer G, et al. Multicenter study of epidemiological cutoff values and detection of resistance in *Candida* spp. to anidulafungin, caspofungin, and micafungin using the Sensititre YeastOne colorimetric method. Antimicrob Agents Chemother 2015;59:6725–32.

[16] MCS Diagnostics. Sensititre Yeastone for in vitro diagnostic use. 2012. Available at: http://www.mcsdiagnostics.com/site/upload/file/pdf/yo_8_yo10_v1.4_e.pdf.