Medical Mycology, 2014, 52, 549–553
doi: 10.1093/mmycol/myu016
Advance Access Publication Date: 12 June 2014
Short Communication

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Uncommon opportunistic yeast bloodstream infections from Qatar

Saad J. Taj-Aldeen[1,*], Atqah AbdulWahab[2,3], Anna Kolecka[4], Anand Deshmukh[1], Jacques F. Meis[5,6] and Teun Boekhout[4,7,8,9]

[1]Mycology Unit, Microbiology Division, Department of Laboratory Medicine and Pathology, Hamad Medical Corporation, Doha, Qatar, [2]Department of Pediatrics, Hamad Medical Corporation, Doha, Qatar, [3]Weill-Cornel Medical College, Qatar, [4]CBS Fungal Biodiversity Centre, Utrecht, The Netherlands, [5]Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands, [6]Department of Medical Microbiology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands, [7]Department of Internal Medicine and Infectious Diseases, University Medical Center, Utrecht, The Netherlands, [8]Department of Dermatology, Shanghai Key Laboratory of Molecular Medical Mycology, Institute of Dermatology and Medical Mycology, Changzheng Hospital, Second Military Medical University, Shanghai and [9]Institute of Microbiology, Chinese Academy of Science, Beijing, People's Republic of China

*To whom correspondence should be addressed. E-mail: stajaldeen@hmc.org.qa

Received 5 October 2013; Revised 10 February 2014; Accepted 3 March 2014

Abstract

Eleven uncommon yeast species that are associated with high mortality rates irrespective of antifungal therapy were isolated from 17/187 (201 episodes) pediatric and elderly patients with fungemia from Qatar. The samples were taken over a 6-year period (January 2004–December 2010). Isolated species included *Kluyveromyces marxianus*, *Lodderomyces elongisporus*, *Lindnera fabianii*, *Candida dubliniensis*, *Meyerozyma guilliermondii*, *Candida intermedia*, *Pichia kudriavzevii*, *Yarrowia lipolytica*, *Clavispora lusitaniae*, *Candida pararugosa*, and *Wickerhamomyces anomalus*. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry provided correct identifications compared with molecular analysis testing of the same isolates. Low minimal inhibitory concentrations were found when isavuconazole and voriconazole were used for all uncommon yeast species evaluated in this study. Resistance to antifungal drugs was low and remained restricted to a few species.

Key words: bloodstream infections, uncommon yeasts, antifungal susceptibility, risk factors, outcome, MALDI-TOF MS.

Introduction

The growing problem of fungemia reflects an increase in the number of patients at risk. Currently, bloodstream infections (BSIs) due to *Candida* spp. constitute the predominant group of hospital-based fungal infections [1,2]. The incidence of fungemia is growing and has dramatically increased during the past two decades [3–6]. Such infections have been attributed to prolonged hospitalizations

Table 1. Teleomorphic names of yeasts isolated in this study based on recent taxonomy with their GenBank accession numbers.

Anamorph	Teleomorph	Case isolate	Large subunit GenBank accession number
Candida kefyr (1970)	*Kluyveromyces marxianus* (1971)	1	KF959829
Unknown	*Lodderomyces elongisporus* (1971)	2	KF959832
C. fabianii (1964)	*Lindnera fabianii* (2008)	3	KF959834
C. guilliermondii (1938)	*Meyerozyma guilliermondii* (2010)	4	KF959844
		5	KF959836
C. intermedia(1938)	Unknown	6	KF959843
C. krusei (1923)	*Pichia kudriavzevii* (1965)	7	KF959838
		8	KF959839
C. lipolytica (1942)	*Yarrowia lipolytica* (1980)	9	KF959840
C. lusitaniae (1959)	*Clavispora lusitaniae* (1979)	10	KF959831
		11	KF959833
C. pararugosa(1978)	Unknown	12	KF959833
		13	KF959841
C. pelliculosa (1925)	*Wickerhamomyces anomalus* (2008)	14	KF959845
C. dubliniensis (1995)	Unknown	15	KF959842
		16	KF959835
		17	KF959837

of highly susceptible patients receiving advanced medical treatment.

The present trend of fungemia shows that a large proportion of BSIs are due to *Candida* species other than *C. albicans*, particularly among hematological, transplant, and intensive care unit patients [7–9]. The epidemiology of fungemia is changing, with an increase in the proportion of episodes caused by such opportunistic pathogenic yeasts.

Here we present data on clinically rare yeast species that we assessed in order to determine their antifungal susceptibility profiles, including susceptibility to new agents. Risk factors and clinical outcomes associated with fungemia caused by these yeasts were analyzed in patients admitted to a single tertiary care hospital, Hamad Medical Corporation (HMC) in Doha, Qatar.

Materials and methods

The names used for the yeasts isolated throughout the present work (Table 1) were based on the names most recently published for the teleomorphic forms [10]. Uncommon species recovered from 1 January 2004 to 31 December 2010 were retrospectively studied; information pertaining to yeast BSIs of patients hospitalized in all departments of HMC was included. The study subject population was composed of all adult and pediatric hospitalized patients of both genders who developed BSIs, the latter being defined as one or more blood samples from patients with relevant clinical signs and symptoms that yielded yeasts in culture [11]. This study was reviewed and approved by the HMC Medical Research Center ethics committee (11308/11); the requirement for written informed consent was waived because of the retrospective and observational nature of this study.

In order to identify the yeast species, genomic DNA was extracted as described by Bolano et al. [12], but the urea incubation step was omitted. Sequence analysis of the D1-D2 domains of the large subunit ribosomal DNA (rDNA) and the internal transcribed spacer 1 (ITS1) and ITS2 regions of the rDNA was performed according to the method of Okoli et al. [13]; see Table 1 for GenBank accession numbers. The sequences generated were compared with data in the National Center for Biotechnology Information database using the basic local alignment search tool (BLASTn; http://www.ncbi.nlm.nih.gov/). Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF MS)–based identification of all yeast isolates was performed according to the Bruker Daltonics GmbH ethanol/formic acid extraction protocol, as described previously (ethanol: J.T. Baker, Avantor Performance materials B.V., Deventer, the Netherlands; formic acid: Fuka, Zwijndrecht, the Netherlands) [14,15].

The susceptibility profiles of all strains were tested using the standard broth microdilution method, described in the Clinical and Laboratory Standards Institute (CLSI) document M27-A3 as described previously [16]. The susceptibilities were interpreted for some species by taking into account the new species-specific clinical breakpoints presented in M27-S4 [17]. Minimal inhibitory concentrations (MICs) $\leq$2 μg/ml for anidulafungin and caspofungin were categorized as susceptible for *Meyerozyma guilliermondii* strains for which a echinocandin MICs of $\geq$8 μg/ml was considered

Table 2. Fungemia cases caused by uncommon yeast species in Qatar.

Organism	Case number	Year of isolation	Age/sex	Risk factor	Treatment	Outcome
Kluyveromyces marxianus	1	2006	74 y/F	Chronic heart disease	NK	Survived
Lodderomyces elongisporus	2	2007	22 y/M	Trauma victim	Caspofungin, fluconazole	Died[a]
Lindnera fabianii	3	2007	19 y/M	55% burn	Fluconazole	Died[a]
Meyerozyma guilliermondii	4	2006	6 y/F	Malabsorption syndrome	L-AmB	Survived
	5	2008	2 m/M	Developmental delay	L-AmB	Survived
Candida intermedia	6	2006	14 m/F	Tetralogy of Fallot	L-AmB	Survived
Pichia kudriavzevii	7	2008	13 d/M	Congenital heart disease	L-AmB	Died[a]
	8	2008	84 y/M	Chronic disease	Caspofungin	Died[a]
Yarrowia lipolytica	9	2010	77 y/F	Diabetic mellitus, renal failure	Caspofungin	Died[b]
Clavispora lusitaniae	10	2007	7 m/F	Malabsorption	L-AmB, fluconazole	Survived
	11	2007	20 d/M	Motor development delay	L-AmB	Survived
Candida pararugosa	12	2006	6 m/M	Intrauterine growth restriction	L-AmB	Died[b]
	13	2010	5 y/M	NK	L-AmB	Survived
Wickerhamomyces anomalus	14	2006	18 m/F	Chronic heart disease, hepatomegaly	L-AmB	Died[b]
Candida dubliniensis	15	2005	85 y/F	Heart failure	NK	Died[a]
	16	2007	14 y/F	Road traffic accident	Caspofungin	Died[a]
	17	2008	67 y/M	30% burn	Fluconazole	Died[a]

L-AmB, liposomal amphotericin B; NK, not known.
[a]Mortality within 30 days.
[b]Patient died after 180 days.

to be resistant. MIC endpoints were determined for all other yeast species following the CLSI guideline M27-S4 [17]. A breakpoint of ≤1 μg/ml was selected to define the isolates as susceptible to amphotericin B [18]. The MICs for the quality control strains of *C. parapsilosis* American Type Culture Collection (ATCC) 22019 and *C. krusei* ATCC 6258 were all within the reference ranges (data not shown).

Results

Our data showed that MALDI-TOF MS correctly identified 100% of the uncommon yeast species examined when compared with molecular analysis, which is considered to be the "gold standard" (Kappa value = 1). Seventeen cases of fungemia due to uncommon yeast species detected in 187 patients (201 episodes) [19] are presented in Table 2. Of these, 10/17 patients were aged <15 years and 5/17 were aged >65 years. A high rate of fatal outcomes was noted at 30 days due to these uncommon yeast infections (7/17, or 41%) after the primary isolation of *Candida* in blood cultures irrespective of antifungal therapy.

Discussion

The reasons for the emergence of uncommon yeast species are not clear. However, some medical conditions may increase the risk of developing fungemia caused by these uncommon yeasts and those that are rarely recovered in the clinical setting. Also, these increases may be attributed to improvement in the sensitivity of diagnostic methodology, making it possible to identify uncommon species. As in other studies, we demonstrated that fungemia caused by *Pichia kudriavzevii* is rare in Qatar [20]. *Yarrowia lipolytica*, which was isolated from one patient in our study (case 9), was isolated from a variety of clinical specimens, including those from leukemia patients with fungemia, and described as a weakly pathogenic yeast [21]. *Lodderomyces elongisporus* was reported for the first time in 2008 as an etiologic agent of BSIs [22] and was isolated recently from a catheter tip in the Middle East [23]. The uncommon yeast, *Kluyveromyces marxianus*, isolated in the present study from an elderly patient (case 1), was reported as an emerging pathogen recovered from a fungemia case in Spain [24]. This isolate was erroneously referred to as *K. lactis* [19].

Resistance to antifungal drugs is low and remains restricted to only a few species. It is necessary to focus on drug resistance of uncommon species that cause fungemia, as some of them exhibit high, borderline MICs. For example, *M. guilliermondii* and *Y. lipolytica*, have MICs of 1–2 μg/ml against echinocandins (Table 3). When the new species-specific breakpoints (CLSI S4, 2012) were applied, *P. kudriavzevii* was resistant to fluconazole and

Table 3. Susceptibility of uncommon yeast species to systemic antifungal agents.

Isolate (*n*)	Minimal inhibitory concentration range (μg/ml)							
	Amphotericin B	Fluconazole	Itraconazole	Voriconazole	Posaconazole	Isavuconazole	Caspofungin	Anidulafungin
Kluyveromyces marxianus (1)	1	0.5	0.125	<0.016	0.25	<0.016	0.5	0.031
Lodderomyces elongisporus (1)	0.5	0.25	0.031	<0.016	0.063	<0.016	0.5	0.016
Lindnera fabianii (1)	0.5	2	0.25	0.031	0.25	0.031	0.5	0.063
Meyerozyma guillermondii (2)	0.5	4–8	0.5	0.125	0.25–0.5	0.125–0.25	0.5–1	1–2
Candida intermedia (1)	0.125	0.25	0.031	<0.016	<0.016	<0.016	1	0.031
Pichia kudriavzevii (2)	1	32–64	0.125–0.25	0.125–0.25	0.125–0.25	0.063–0.25	1	0.063
Yarrowia lipolytica (1)	1	4	0.5	0.063	0.5	0.063	2	1
Clavispora lusitaniae (2)	0.5	0.5	0.063–0.125	<0.016	<0.016–0.063	<0.016	1	0.125
Candida pararugosa (2)	0.5	8	0.25	0.125	0.125	0.031–0.063	0.25–1	0.063–0.125
Wickerhamomyces anomalus (1)	0.5	8	0.5	0.25	0.25	0.063	0.5	<0.016
Candida dubliniensis (3)	0.125–0.5	0.25	0.031	<0.016	0.031	<0.016	0.5	0.031

caspofungin (MIC = 1 μg/ml). Except for *M. guilliermondii* and *P. kudriavzevii*, there are no established breakpoints for other rare yeast species. Although isavuconazole and voriconazole exhibit low MICs for the uncommon yeast species reported in this study, it is known that isavuconazole has several pharmacokinetic properties that are more advantageous than those of currently used azoles [25,26]. Furthermore, oral and intravenous treatment regimens in a multiple-dose study in healthy volunteers were tested; excellent bioavailability with maximum drug concentration levels in serum of >1.85 μg/ml was found [26]. This serum level is much higher than the MICs obtained for the uncommon yeast species in the present study (MIC range, 0.016–0.25 μg/ml).

We conclude that rare fungal species caused fungemia associated with high mortality rates in hospitalized patients in patients aged <15 years and in patients aged >65 years. Our data suggest that isavuconazole has an equal or even better therapeutic efficacy than the currently used azoles and is a promising antifungal agent in the treatment of invasive uncommon yeast infections.

Acknowledgments

This work was made possible by grant NPRP 5–298–3–086 from the Qatar National Research Fund (a member of Qatar Foundation) to S. J. T.-A., A. A-W., A. D., J. M., and T. B. The statements herein are solely the responsibility of the authors.

Declaration of interest

J. F. M received grants from Astellas, Basilea, and Merck. He has been a consultant to Astellas, Basilea, and Merck and received speaker's fees from Merck and Gilead. The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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