International Journal of Infectious Diseases 33 (2015) 1–4

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

Short Communication

Surveillance of antifungal susceptibilities in clinical isolates of *Candida* species at 36 hospitals in China from 2009 to 2013

Lei Zhang [a], Shusheng Zhou [a], Aijun Pan [a], Jiabin Li [b,c,*], Bao Liu [a,d,*]

[a] Department of Critical Care Medicine, Affiliated Anhui Provincial Hospital of Anhui Medical University, Hefei, China
[b] Department of Infectious Disease, the First Affiliated Hospital of Anhui Medical University, Hefei, China
[c] Anhui Centre for Surveillance of Bacterial Resistance, Hefei, China
[d] Department of Laboratory, Affiliated Anhui Provincial Hospital of Anhui Medical University, Hefei, China

ARTICLE INFO

Article history:
Received 18 August 2014
Received in revised form 20 November 2014
Accepted 17 December 2014

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Candida species
Azoles
Fluconazole
Antifungal susceptibility

SUMMARY

Background: The purpose of this study was to determine the species distribution and to monitor the antifungal susceptibility profiles of clinical Candida isolates collected in China from 2009 to 2013.
Methods: The antifungal susceptibilities of 952 Candida isolates were tested.
Results: *Candida albicans* was the most common species, accounting for 65.7% of the total isolates. The most frequently isolated non-albicans *Candida* species in this study was *Candida glabrata* (193, 20.3%). Nearly 7.6%, 3.2%, 1.8%, and 1.1% of the 952 isolates exhibited decreased susceptibility to fluconazole, voriconazole, itraconazole, and flucytosine, respectively. Moreover, seven *C. albicans* and one *Candida krusei* had an amphotericin B minimum inhibitory concentration (MIC) of 2 μg/ml.
Conclusions: The distribution of species and the prevalence of antifungal resistance in Candida isolates varied among different areas in China. Continuous monitoring of resistance patterns is necessary to control the spread of resistance in clinical isolates of *Candida* species.

1. Introduction

Candida species are commonly isolated fungal pathogens that are responsible for many types of fungal infection in immunocompromised patients and hospitalized patients with serious underlying diseases.[1,2] Azole drugs are the most prescribed antifungal agents for the treatment of such infections in clinical practice.[3] However, with long-term therapies, azole resistance often emerges in Candida isolates, especially *Candida albicans*, resulting in therapeutic failures.[3,4] In addition, the species distribution and antifungal resistance among Candida isolates vary from region to region.[5,6] Thus, it is important for clinicians to understand the local antifungal susceptibility profiles of *Candida spp* before treatment is initiated.

In order to provide effective guidelines for the treatment of candidiasis, we conducted this study to determine the distribution of *Candida* species and to monitor the antifungal susceptibility profiles of these isolates collected annually in September during the years 2009 to 2013 in Anhui, China.

2. Materials and methods

2.1. Isolates and identification

A total of 952 non-duplicate Candida isolates were collected from different sites from 952 patients who received treatment at their local hospital in different geographic regions of Anhui, China. All clinical specimens were incubated and isolated on Sabouraud dextrose agar (Oxoid) with chloramphenicol (50 μg/ml). Species identification was performed with CHROMagar *Candida* and the API 20C AUX system (bioMérieux, France) in accordance with the manufacturer's instructions, supplemented by conventional methods when needed.[7] All isolates were stored as suspensions in water until used in this study. To ensure optimal growth characteristics, each isolate was subcultured twice on Sabouraud dextrose agar at 35 °C before susceptibility testing.

2.2. Antifungal susceptibility testing

In vitro antifungal susceptibility testing of Candida isolates was performed by broth microdilution method according to the M27-A3 guidelines of the Clinical and Laboratory Standards Institute (CLSI).[8] The dilution of the yeasts was conducted in

* Corresponding authors. Tel.: +86 55162283823; fax: +86 55162283823.
E-mail addresses: biyoushenghua@sohu.com (J. Li), 98zl@sina.com (B. Liu).

http://dx.doi.org/10.1016/j.ijid.2014.12.033

RPMI 1640 medium containing a final inoculum concentration of 0.5–2.5 × 10^3 cells/ml. The drug concentrations of amphotericin B, flucytosine, and azoles (fluconazole, itraconazole, and voriconazole) obtained from Bristol-Myers Squibb, Sigma, and Pfizer, respectively, were prepared as described in CLSI document M27-A3.[8] Following incubation at 35 °C for 24 h, the minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug that caused more than 50% inhibition of growth for flucytosine and azoles, and completely inhibited cell growth for amphotericin B. The clinical breakpoints determined by 24-h CLSI broth microdilution method have been newly revised to provide species-specific interpretive criteria for the five most common *Candida* species (*C. albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, and *Candida parapsilosis*),[9,10] and they were adopted in this study. Given the absence of clinical breakpoints for some uncommon species, we applied the epidemiological cut-off values (ECVs) instead to detect the emergence of potential resistance to antifungal agents.[9]

Interpretive criteria for susceptibility to antifungal agents were as follows: for fluconazole, MICs ≤2 μg/ml were considered susceptible for *C. albicans*, *C. tropicalis*, and *C. parapsilosis* after 24 h of incubation, and MICs ≥8 μg/ml were resistant, 4 μg/ml susceptible-dose dependent (SDD). For *C. glabrata*, a fluconazole MIC ≤32 μg/ml was considered SDD, while ≥64 μg/ml was considered resistant. For voriconazole, MICs ≤0.125 μg/ml, 0.25–0.5 μg/ml, and ≥1 μg/ml were considered susceptible, intermediate, and resistant, respectively, for *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, whereas the clinical breakpoints for *C. krusei* were MICs ≤0.5 μg/ml susceptible, ≥2 μg/ml resistant, and 1 μg/ml intermediate. For itraconazole, the ECV determined after 24 h of incubation was 0.125 μg/ml for *C. albicans*, 0.25 μg/ml for *Candida dubliniensis*, and 0.5 μg/ml for other species, with the exception of *C. krusei* (1 μg/ml) and *C. glabrata* (2 μg/ml). The ECV of flucytosine was 0.5 to 1 μg/ml for all species, aside from *C. krusei* (ECV 32 μg/ml). The ECV of amphotericin B was 2 μg/ml for each species of *Candida*, as determined by the CLSI method.[8–10] Two reference strains, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019, were used as quality control strains in each susceptibility test.

3. Results

3.1. Distribution of Candida species

In this study, we collected 132, 216, 178, 194, and 232 clinical isolates of *Candida* species each year from 2009 to 2013, respectively. The distribution of these *Candida* species is presented in Table 1. Of the 952 isolates, *C. albicans* was the most common species, accounting for 65.7% of the total isolates in our study. *C. glabrata* (193, 20.3%) was the most frequently isolated non-albicans *Candida* species, followed by *C. tropicalis* (84, 8.8%), *C. krusei* (29, 3.0%), *C. parapsilosis* (13, 1.4%), and another three species (8, 0.8%), including four isolates of *Candida lusitaniae*, two isolates of *Candida guilliermondii*, and two isolates of *C. dubliniensis*.

When distributed according to the sources, the top three were as follows: 519 (54.5%) isolates from sputum, 242 (25.4%) from urine, and 65 (6.8%) from blood. There were also 29 (3.0%) Candida isolates from throat swabs, 26 (2.7%) from wounds, 12 (1.3%) from pus, 11 (1.2%) from ascites, nine (0.9%) from faeces, and 39 (4.1%) from another 15 various specimens. By comparison, *C. albicans* was also the most prevalent species of Candida isolated from urine (227, 93.8%), sputum (308, 59.3%), and blood (30, 46.2%).

In order to describe the geographical distribution of clinical isolates, we divided the regions in which the 36 hospitals were located into three groups: the south (10 hospitals), the middle (14 hospitals), and the north (12 hospitals). Among the 952 isolates, 35.7% were from the south region, 21.2% from the middle region, and 43.1% from the north region. *C. albicans* accounted for 73.5% of all Candida isolates from the south region, 44.1% from the middle region, and 69.8% from the north region. *C. glabrata* accounted for 19.4% of all Candida isolates from the south region, 9.4% from the middle region, and 26.3% from the north region.

The patients ranged in age from 8 months to 91 years (median age 53 years), and these patients were divided into four groups by age: <18 years, 18–44 years, 45–70 years, and >70 years. The majority of *C. albicans* isolates (341, 54.6%) were collected from patients aged 45–70 years. More *C. glabrata* were isolated from patients in the age group >70 years than from the other age groups (58.2% vs. 41.8%; $p < 0.05$).

3.2. Susceptibilities to azoles agents

The antifungal susceptibilities of all *Candida* species tested are shown in Table 2. The MIC_{50} and MIC_{90} were defined as the MICs at which 50% and 90% of the total isolates were inhibited in growth, respectively. Concerning the results of susceptibility testing to azole agents, 7.6%, 3.2%, and 1.8% of the 952 isolates exhibited decreased susceptibility to fluconazole, voriconazole, and itraconazole, respectively. Among these resistant isolates, four *C. albicans* isolates and two *C. tropicalis* isolates were simultaneously resistant to fluconazole (MICs ≥8 μg/ml) and voriconazole (MICs ≥1 μg/ml).

3.3. Susceptibilities to flucytosine

Overall, 1.1% of the total isolates were considered resistant to flucytosine. As shown in Table 2, these flucytosine-resistant isolates were detected in all different common *Candida* species, except for *C. parapsilosis*. There were five *C. albicans* isolates, two *C. glabrata* isolates, one *C. tropicalis* isolate, and two *C. krusei* isolates displaying reduced susceptibility to this agent on the basis of the broth microdilution MICs.

Table 1
Distribution of 952 clinical isolates of *Candida* species from different specimens

Specimens	*C. albicans*	*C. glabrata*	*C. tropicalis*	*C. krusei*	*C. parapsilosis*	Other species
Sputum (*n* = 519)	308 (49.3)[a]	148 (76.7)	53 (63.1)	7 (24.1)	2 (15.3)	1 (12.5)
Urine (*n* = 242)	227 (36.3)	2 (1.0)	5 (6.0)	4 (13.8)	3 (23.1)	1 (12.5)
Blood (*n* = 65)	30 (4.8)	14 (7.3)	6 (7.1)	7 (24.1)	5 (38.5)	3 (37.5)
Throat swab (*n* = 29)	11 (1.8)	10 (5.2)	5 (6.0)	3 (10.3)	0 (0)	0 (0)
Wound (*n* = 26)	19 (3.0)	3 (1.6)	2 (2.4)	1 (3.4)	0 (0)	1 (12.5)
Pus (*n* = 12)	4 (0.6)	4 (2.1)	1 (1.2)	2 (6.9)	1 (7.7)	0 (0)
Ascites (*n* = 11)	7 (1.1)	2 (1.0)	0 (0)	1 (3.4)	0 (0)	1 (12.5)
Faeces (*n* = 9)	4 (0.6)	1 (0.5)	4 (4.8)	0 (0)	0 (0)	0 (0)
Other sites (*n* = 39)	15 (2.4)	9 (4.7)	8 (9.5)	4 (13.8)	2 (15.4)	1 (12.5)
Total	625 (65.7)[b]	193 (20.3)	84 (8.8)	29 (3.0)	13 (1.4)	8 (0.8)

[a] The number of these isolates obtained from this specimen (the percentage of these isolates in the same species).
[b] The number of clinical isolates of this *Candida* species (the percentage of these isolates in the total isolates).

Table 2
In vitro antifungal susceptibilities of 952 clinical isolates of *Candida* species as determined by the Clinical and Laboratory Standards Institute (CLSI) method

Candida species (No. of isolates)	Antifungal agents	MIC (μg/ml)[a]			% R[b]
		Range	50%	90%	
C. albicans (625)	Fluconazole	0.125–64	0.25	2	4.3
	Voriconazole	0.0313–4	0.125	0.25	2.1
	Itraconazole	0.0078–0.5	0.0313	0.0625	1.3
	Flucytosine	0.0625–8	0.0625	0.125	0.8
	Amphotericin B	0.0313–2	0.25	0.25	1.1
C. glabrata (193)	Fluconazole	1–64	2	8	6.2
	Voriconazole	0.0313–2	0.0625	0.25	4.7
	Itraconazole	0.125–8	0.5	1	2.1
	Flucytosine	0.0313–4	0.25	0.25	1.0
	Amphotericin B	0.125–1	0.25	0.5	0
C. tropicalis (84)	Fluconazole	0.125–32	0.5	1	10.7
	Voriconazole	0.0313–4	0.0625	0.25	7.1
	Itraconazole	0.0313–2	0.0625	0.125	4.8
	Flucytosine	0.0313–8	0.25	0.25	1.2
	Amphotericin B	0.0625–1	0.25	0.5	0
C. krusei (29)	Fluconazole	16–>64	32	>64	75.9
	Voriconazole	0.125–8	0.25	1	6.9
	Itraconazole	0.125–2	0.25	0.5	3.4
	Flucytosine	2–32	4	16	6.9
	Amphotericin B	0.0625–2	0.5	0.5	3.4
C. parapsilosis (13)	Fluconazole	0.125–8	0.25	2	15.4
	Voriconazole	0.0313–0.5	0.125	0.25	0
	Itraconazole	0.0313–0.25	0.0625	0.0625	0
	Flucytosine	0.0625–0.25	0.125	0.125	0
	Amphotericin B	0.0625–1	0.125	0.25	0
Other species (8)	Fluconazole	0.125–4	0.25	1	0
	Voriconazole	0.0313–0.125	0.0313	0.0625	0
	Itraconazole	0.0078–0.25	0.0625	0.0625	0
	Flucytosine	0.0313–0.25	0.125	0.25	0
	Amphotericin B	0.0313–1	0.25	0.5	0

MIC, minimum inhibitory concentration.
[a] 50% and 90% (MIC_{50} and MIC_{90}) represent the MICs at which 50% and 90% of the total isolates are inhibited in growth, respectively.
[b] % R is the percentage of resistance for each *Candida* species.

3.4. Susceptibilities to amphotericin B

The range of amphotericin B MICs of the 952 isolates was from 0.0313 to 2 μg/ml, and the values of MIC_{90} were 0.25 and 0.5 μg/ml for various *Candida* species (Table 2); this shows that amphotericin B exhibited good activity in vitro against the majority of the isolates (MICs <2 μg/ml). Nevertheless, some isolates, including seven *C. albicans* and one *C. krusei*, had an amphotericin B MIC of 2 μg/ml. None of eight unusual isolates tested were classified as resistant to amphotericin B, with the MICs ranging from 0.0313 to 1 μg/ml in this study.

4. Discussion

In this study, we investigated the distribution of species and determined in vitro susceptibilities to antifungal agents of 952 clinical Candida isolates collected from 36 different hospitals in Anhui, China from 2009 to 2013. Among the 952 clinical isolates, a large proportion (54.5%) were isolated from sputum, followed by urine (25.4%) and blood (6.8%). In contrast, the majority of Candida isolates were most frequently obtained from urine (45.2%), then blood (19.7%) and sputum (13%) in Taiwan, as documented in a previous survey.[11] *C. albicans* was the predominant species isolated in our study, which is consistent with some reports.[11,12] Moreover, *C. glabrata* (20.3%) was the most prevalent non-albicans *Candida* species in this region, which is in agreement with the results of the studies conducted by Hazen et al.[12] and Schmalreck et al.[13] However, the most prevalent non-albicans *Candida* species was *C. parapsilosis* in Latin America (25%), Canada (16%), and Europe (17%),[14] and *C. tropicalis* in Taiwan.[11] This demonstrates that the distribution of species differs from area to area.

Overall, resistance to fluconazole, voriconazole, and itraconazole was seen in all the common *Candida* species, except for *C. parapsilosis* with two fluconazole-resistant isolates. None of four *C. lusitaniae* isolates, two *C. guilliermondii* isolates, and two *C. dubliniensis* isolates was resistant to the antifungal agents in our study. Of the 952 isolates, 7.6% exhibited decreased susceptibility to fluconazole, and its ratio was higher than other antifungal agents. The high prevalence of fluconazole resistance among *Candida* species may be correlated with the increased use of fluconazole in this area, as well as in Korea.[15] Continuous exposure to azoles appears to have a major impact in selecting fluconazole-resistant *Candida* species, as described previously.[16] With regard to *C. krusei*, the reason for the high-level resistance to fluconazole (75.9%) is that *C. krusei* isolates are inherently resistant to fluconazole.[9] Of note, we found four isolates of *C. albicans* and two isolates of *C. tropicalis* with fluconazole MICs ≥8 μg/ml to be resistant to voriconazole. This phenomenon highlights the important issue of cross-resistance among azole agents. With regard to amphotericin B, its MIC_{90} values were 0.25 and 0.5 μg/ml for *Candida* species, and there were only seven *C. albicans* and one *C. krusei* (0.8%) with an amphotericin B MIC of 2 μg/ml. However, a German–Austrian multi-centre study showed nearly 11.2% of 1046 Candida isolates to be classified as amphotericin B-resistant.[13] This result reveals that the prevalence of antifungal resistance in Candida isolates varies among different areas.

In conclusion, continuous surveillance of antifungal susceptibilities in clinical isolates of *Candida* species at the national and international levels is required in order to control the spread of resistance and provide effective strategies for the prophylaxis and treatment of humans with fungal infections. However, the reason for the resistance trend of antifungal agents in our study is unclear. This problem may be resolved by further studies on multiple

resistance mechanisms in combination with continuous surveillance and extensive clinical evaluations.

Acknowledgements

We thank all of the contributing hospitals that provided isolates for this study.

Funding: This study was supported by the National Natural Science Foundation of China (No. 81172737).

Ethical approval: Not required.

Conflict of interest: None declared.

References

1. Chen S, Slavin M, Nguyen Q, Marriott D, Playford EG, Ellis D, et al. Active surveillance for candidemia, Australia. *Emerg Infect Dis* 2006;**12**:1508–16.
2. Büchner T, Fegeler W, Bernhardt H, Brockmeyer N, Duswald KH, Herrmann M, et al. Treatment of severe Candida infections in high-risk patients in Germany: consensus formed by a panel of interdisciplinary investigators. *Eur J Clin Microbiol Infect Dis* 2002;**21**:337–52.
3. Zhang L, Yang HF, Liu YY, Xu XH, Ye Y, Li JB. Reduced susceptibility of *Candida albicans* clinical isolates to azoles and detection of mutations in the ERG11 gene. *Diagn Microbiol Infect Dis* 2013;**77**:327–9.
4. Xu Y, Chen L, Li C. Susceptibility of clinical isolates of *Candida* species to fluconazole and detection of *Candida albicans* ERG11 mutations. *J Antimicrob Chemother* 2008;**61**:798–804.
5. Jang MJ, Shin JH, Lee WG, Kim MN, Lee K, Lee HS, et al. In vitro fluconazole and voriconazole susceptibilities of Candida bloodstream isolates in Korea: use of the CLSI and EUCAST epidemiological cutoff values. *Ann Lab Med* 2013;**33**:167–73.
6. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Barton R, Bijie H, et al. Geographic variation in the frequency of isolation and fluconazole and voriconazole susceptibilities of *Candida glabrata*: an assessment from the ARTEMIS DISK Global Antifungal Surveillance Program. *Diagn Microbiol Infect Dis* 2010;**67**:162–71.
7. Warren NG, Hazen KC. Candida, Cryptococcus, and other yeasts of medical importance. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. *Manual of clinical microbiology*. 7th ed., Washington DC: ASM Press; 1999. p. 1184–99.
8. Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard—third edition. CLSI document M27-A3. Wayne, PA: CLSI; 2008.
9. Pfaller MA, Diekema DJ. Progress in antifungal susceptibility testing of Candida spp by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. *J Clin Microbiol* 2012;**50**:2846–56.
10. Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts. Fourth informational supplement. CLSI document M27-S4. Wayne, PA: CLSI; 2012.
11. Yang YL, Chen HT, Lin CC, Chu WL, Lo HJ. TSARY Hospitals. Species distribution and drug susceptibilities of Candida isolates in TSARY 2010. *Diagn Microbiol Infect Dis* 2013;**76**:182–6.
12. Hazen KC, Baron EJ, Colombo AL, Girmenia C, Sanchez-Sousa A, del Palacio A, et al. Comparison of the susceptibilities of *Candida spp* to fluconazole and voriconazole in a 4-year global evaluation using disk diffusion. *J Clin Microbiol* 2003;**41**:5623–32.
13. Schmalreck AF, Willinger B, Haase G, Blum G, Lass-Flörl C, Fegeler W, et al. Species and susceptibility distribution of 1062 clinical yeast isolates to azoles, echinocandins, flucytosine and amphotericin B from a multi-centre study. *Mycoses* 2012;**55**:e124–37.
14. Cuenca-Estrella M, Rodero L, García-Effrón G, Rodriguez-Tudela JL. Antifungal susceptibilities of *Candida spp* isolated from blood in Spain and Argentina, 1996–1999. *J Antimicrob Chemother* 2002;**49**:981–7.
15. Kim SH, Shin JH, Kim EC, Lee K, Kim MN, Lee WG, et al. The relationship between antifungal usage and antifungal susceptibility in clinical isolates of Candida: a multicenter Korean study. *Med Mycol* 2009;**47**:296–304.
16. Kontoyiannis DP. Why prior fluconazole use is associated with an increased risk of invasive mold infections in immunosuppressed hosts: an alternative hypothesis. *Clin Infect Dis* 2002;**34**:1281–3.