Original Article

Species Distribution and *In Vitro* Antifungal Susceptibility of Vulvovaginal *Candida* Isolates in China

Feng-Juan Wang, Dai Zhang, Zhao-Hui Liu, Wen-Xiang Wu, Hui-Hui Bai, Han-Yu Dong

Department of Obstetrics and Gynecology, Peking University First Hospital, Beijing 100034, China

Abstract

Background: Vulvovaginal candidiasis (VVC) was a common infection associated with lifelong harassment of woman's social and sexual life. The purpose of this study was to describe the species distribution and *in vitro* antifungal susceptibility of *Candida* species (*Candida spp.*) isolated from patients with VVC over 8 years.

Methods: Species which isolated from patients with VVC in Peking University First Hospital were identified using chromogenic culture media. Susceptibility to common antifungal agents was determined using agar diffusion method based on CLSI M44-A2 document. SPSS software (version 14.0, Inc., Chicago, IL, USA) was used for statistical analysis, involving statistical description and Chi-square test.

Results: The most common strains were *Candida (C.) albicans*, 80.5% (n = 1775) followed by *C. glabrata*, 18.1% (n = 400). Nystatin exhibited excellent activity against all species (<4% resistant [R]). Resistance to azole drugs varied among different species. *C. albicans*: clotrimazole (3.1% R) < fluconazole (16.6% R) < itraconazole (51.5% R) < miconazole (54.0% R); *C. glabrata*: miconazole (25.6% R) < clotrimazole (50.5% R) < itraconazole (61.9% R) < fluconazole (73.3% R); *Candida krusei*: clotrimazole (0 R) < fluconazole (57.7% R) < miconazole (73.1% R) < itraconazole (83.3% R). The susceptibility of fluconazole was noticeably decreasing among all species in the study period.

Conclusions: Nystatin was the optimal choice for the treatment of VVC at present. The species distribution and *in vitro* antifungal susceptibility of *Candida spp.* isolated from patients with VVC had changed over time.

Key words: Drug Resistance; Infection Diseases; Susceptibility Surveillance; Vulvovaginal Candidiasis; Women's Health

Introduction

Vaginitis is a common complaint for females and is the most frequent reason for gynecology consultation in primary health care services. Vulvovaginal candidiasis (VVC), which accounts for 40–50% of all cases of infectious vulvovaginitis, has been known as a common problem worldwide.[1] It is estimated that 75% of women experience at least one episode of VVC throughout her lifetime, and 5–8% of adult women have recurrent VVC (RVVC) which is defined as four or more culture-confirmed episodes in a 12-month period.[2] A multitude data show that non-*albicans Candida* species (*Candida spp.*) are associated with RVVC, increasing worldwide. An increasing prevalence of fungal resistance is also reported in global and local antifungal surveillance studies. The increased RVVC incidence and drugs resistance cause an important public health issue and give great challenge to clinician's treatment strategy.[3-7]

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Knowledge of local epidemiological patterns such as clinical characteristics of cases, distribution of strains, and antifungal susceptibilities of *Candida spp.* is important and essential in guiding appropriate clinical decisions since the species distribution and resistance to *Candida spp.* differ among geographies. The selection of initial antifungal therapy is also reliant on robust epidemiological data.[4,8-10] A few studies on the long-term monitoring of the species distribution and antifungal susceptibilities of vulvovaginitis *Candida spp.* have been reported by China. This study aimed to describe the trend of the species distribution and antifungal

Address for correspondence: Dr. Zhao-Hui Liu, Department of Obstetrics and Gynecology, Peking University First Hospital, Beijing 100034, China
E-Mail: 23662161@qq.com

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susceptibilities of vulvovaginitis *Candida spp.* in China from 2006 to 2013.

Methods

Candida strain collection

The study was conducted in Peking University First Hospital, a teaching facility of Peking University Health Science Center in Beijing, China. A total of 2204 isolates were collected from 2122 vulvovaginitis patients. We simply got these strains from the Repository Department of our hospital. All specimens were placed on CHROM-agar (Biocell Laboratory Ltd., Zhengzhou, China) to identify. Cultures were incubated for 24–48 h at 35°C in ambient air atmosphere before reading results.

Ethics

This study was an antifungal, susceptibility, surveillance study, and no human rights issues were involved. We obtained these strains in an anonymized and de-identified form.

Susceptibility testing

Susceptibility to clotrimazole (Rosco, Denmark), fluconazole (Rosco), miconazole (Rosco), itraconazole (Rosco), and nystatin (Rosco) was determined using an agar diffusion method (Neo-sensitabs, Rosco). The surface of improved Shadomy agar plate (Guangzhou Detgerm Microbiology Technology Co., Ltd.) was inoculated using a swab dipped in cell suspension which adjusted to a McFarland standard turbidity of 0.5. Disks (Rosco) of clotrimazole (10 μg), fluconazole (15 μg), miconazole (10 μg), itraconazole (8 μg), and nystatin (50 μg) were placed onto the surfaces of the inoculated plates. The plates were incubated in air at 35°C and read at 24 h. The zone diameters of all isolates were recorded accurately. Interpretation of all agents susceptibilities (susceptible [S], susceptible-dose dependent, and resistant [R]) was done according to the standard instructions. Quality control was performed using quality-control strains, ATCC 64548 and ATCC 64550.

Statistical analysis

Data processing and statistical analysis were performed with SPSS software (version 14.0, Inc., Chicago, IL, USA). Count data variables were expressed as frequencies and percentages. These enumeration data were performed with Chi-square test to compare the difference between groups. For all statistical analysis, statistical significance was accepted at $P < 0.05$ (two-sided).

Results

Distribution of *Candida*

During the study period from 2006 to 2013, a total of 2204 *Candida spp.* isolated from 2122 patients with VVC were collected. The distribution of *Candida species* was as follows: *Candida albicans* (*C. albicans*), 80.5% ($n = 1775$); *Candida glabrata* (*C. glabrata*), 18.1% ($n = 400$); *Candida krusei* (*C. krusei*) 1.2% ($n = 26$); *Candida tropicalis* (*C. tropicalis*), 0.1% ($n = 3$) [Table 1]. Although *C. albicans* maintained the most common species associated with vulvovaginitis (80.5%), the percentage reduced from about 90% in 2006 to 77% in 2013 meanwhile the prevalence of *C. glabrata* increased from about 10% in 2003 to 20% in 2013. The proportion of *C. krusei* and *C. tropicalis* was very low (1.2%, 0.1%, respectively) in our survey. Of all the patients with positive *Candida* cultures, 76 (3.6%) cases isolated more than one culture (158 isolates) in different time. The prevalence of non-*albicans Candida spp.* among patients with multiple positive cultures was higher than among patients with a single positive culture; however, there was no statistical significance between two groups ($P = 0.054$, $\chi^2 = 3.718$). Moreover, the predominant non-*albicans Candida spp.* recovered was also *C. glabrata*, which account for 24.7%.

Table 1: Species distributions of *Candida* isolates from patient with vulvovaginal candidiasis during 8-year period

Years	Isolates number	*Candida albicans* *n* (%)	*Candida glabrata* *n* (%)	*Candida krusei* *n* (%)	*Candida tropicalis* *n* (%)
2006	47	42 (89.4)	5 (10.6)	0 (0)	0 (0)
2007	138	126 (91.3)	8 (5.8)	4 (2.9)	0 (0)
2008	257	219 (85.2)	37 (14.4)	1 (0.4)	0 (0)
2009	284	223 (78.5)	53 (18.7)	7 (2.5)	1 (0.4)
2010	341	289 (84.8)	49 (14.4)	3 (0.9)	0 (0)
2011	334	257 (76.9)	74 (22.2)	2 (0.6)	1 (0.3)
2012	277	213 (76.9)	60 (21.7)	4 (1.4)	0 (0)
2013	526	406 (77.2)	114 (21.7)	5 (1.0)	1 (0.2)
Total	2204	1775 (80.5)	400 (18.1)	26 (1.2)	3 (0.1)

In vitro susceptibilities among *Candida albicans*

The susceptibility results for each species are presented in Tables 2 and 3. The results revealed that nystatin exhibited excellent activity against *C. albicans* and the whole resistance rates was only 0.2% [Table 2]. Notably, decreasing susceptibility to fluconazole, miconazole, and itraconazole was seen with *C. albicans* during 8-year period. Resistance to fluconazole increased from 2.4% in 2006 to 55.4% in 2012, but the rate dropped to 8.9% in 2013. With regards to miconazole and itraconazole, resistance rate increased from 2.4%, 7.1% in 2006 to 59.8%, 58.9% in 2013, respectively. There was only slightly fluctuant trend toward increased resistance to clotrimazole among *C. albicans* during the study period.

In vitro susceptibilities among non-*albicans Candida* species

The susceptibilities of the non-*albicans species* to five antifungal drugs are shown in Table 3. The results revealed that nystatin exhibited not only excellent antifungal activity against *C. albicans* but also one of the best choices for non-*albicans* species. Moreover, the resistance rates among *C. glabrata*, *C. tropicalis*, and *C. krusei* were 0.3%, 0, and 3.8%, respectively. However, resistant to different azole drugs was various among these species. The activity of all the four azole agents was low against *C. glabrata* (clotrimazole

Table 2: Trends *in vitro* susceptibilities to clotrimazole, fluconazole, miconazole, itraconazole, and nystatin among *Candida albicans* over an 8-year period

Years	Isolates number	Clotrimazole, *n* (%)				Fluconazole, *n* (%)				Miconazole, *n* (%)				Itraconazole, *n* (%)				Nystatin, *n* (%)			
		Tested number	*S*	SDD	*R*	Tested number	*S*	SDD	*R*	Tested number	*S*	SDD	*R*	Tested number	*S*	SDD	*R*	Tested number	*S*	SDD	*R*
2006	42	42	41 (97.6)	1 (2.4)	0 (0)	42	37 (88.1)	4 (9.5)	1 (2.4)	42	24 (57.1)	17 (40.5)	1 (2.4)	42	32 (76.2)	7 (16.7)	3 (7.1)	42	36 (85.7)	6 (14.3)	0 (0)
2007	126	126	125 (99.2)	1 (0.8)	0 (0)	126	119 (94.4)	6 (4.8)	1 (0.8)	110	56 (50.9)	6 (5.5)	48 (43.6)	92	58 (63.0)	3 (3.3)	31 (33.7)	126	126 (100.0)	0 (0)	0 (0)
2008	219	199	182 (91.5)	12 (6.0)	5 (2.5)	219	179 (81.7)	29 (13.2)	11 (5.0)	219	97 (44.3)	7 (3.2)	115 (52.5)	208	97 (46.6)	2 (1.0)	109 (52.4)	219	217 (99.1)	0 (0)	2 (0.9)
2009	223	221	197 (89.1)	22 (10.0)	2 (0.9)	221	185 (83.7)	27 (12.2)	9 (4.1)	221	97 (43.9)	6 (2.7)	118 (53.4)	221	106 (48.0)	0 (0)	115 (52.0)	218	216 (99.1)	2 (0.9)	0 (0)
2010	289	245	219 (89.4)	10 (4.1)	16 (6.5)	288	149 (51.7)	19 (6.6)	120 (41.7)	288	109 (37.8)	12 (4.2)	167 (58.0)	288	136 (47.2)	2 (0.7)	150 (52.1)	288	288 (100.0)	0 (0)	0 (0)
2011	257	256	240 (93.8)	13 (5.1)	3 (1.2)	256	200 (78.1)	36 (14.1)	20 (7.8)	256	97 (37.9)	24 (9.4)	135 (52.7)	256	120 (46.9)	4 (1.6)	132 (51.6)	256	256 (100.0)	0 (0)	0 (0)
2012	213	212	195 (92.0)	15 (7.1)	2 (0.9)	157	61 (38.9)	9 (5.7)	87 (55.4)	210	81 (38.6)	10 (4.8)	119 (56.7)	197	87 (44.2)	7 (3.6)	103 (52.3)	212	211 (99.5)	0 (0)	1 (0.5)
2013	406	405	358 (88.4)	22 (5.4)	25 (6.2)	405	284 (70.1)	85 (21.0)	36 (8.9)	405	143 (35.3)	20 (4.9)	242 (59.8)	387	151 (39.0)	8 (2.1)	228 (58.9)	405	405 (100.0)	0 (0)	0 (0)
Total	1775	1706	1557 (91.3)	96 (5.6)	53 (3.1)	1714	1214 (70.8)	215 (12.5)	285 (16.6)	1751	704 (40.2)	102 (5.8)	945 (54.0)	1691	787 (46.5)	33 (2.0)	871 (51.5)	1766	1755 (99.4)	8 (0.5)	3 (0.2)

R: Resistant; *S*: Susceptible; SDD: Susceptible-dose dependent.

Table 3: Antifungal activities of clotrimazole, fluconazole, miconazole, itraconazole and nystatin against non-*albicans* species

Species	Isolates number	Clotrimazole, *n* (%)				Fluconazole, *n* (%)				Miconazole, *n* (%)				Itraconazole, *n* (%)				Nystatin, *n* (%)			
		Tested number	*S*	SDD	*R*	Tested number	*S*	SDD	*R*	Tested number	*S*	SDD	*R*	Tested number	*S*	SDD	*R*	Tested number	*S*	SDD	*R*
Candida glabrata	400	374	62 (16.6)	123 (32.9)	189 (50.5)	382	35 (9.2)	67 (17.5)	280 (73.3)	391	221 (56.5)	70 (17.9)	100 (25.6)	375	132 (35.2)	11 (2.9)	232 (61.9)	393	392 (99.7)	0 (0)	1 (0.3)
Candida krusei	26	26	25 (96.2)	1 (3.8)	0 (0)	26	3 (11.5)	8 (30.8)	15 (57.7)	26	3 (11.5)	4 (15.4)	19 (73.1)	24	4 (16.7)	0 (0)	20 (83.3)	26	25 (96.2)	0 (0)	1 (3.8)
Candida tropicalis	3	2	0 (0)	1 (50.0)	1 (50.0)	2	0 (0)	1 (50.0)	1 (50.0)	2	0 (0)	0 (0)	2 (100.0)	2	0 (0)	0 (0)	2 (100.0)	2	2 (100)	0 (0)	0

R: Resistant; *S*: Susceptible; SDD: Susceptible-dose dependent.

16.6% S, fluconazole 9.2% S, itraconazole 35.2% S), and only miconazole was active against 56.5% of the isolates. Clotrimazole-resistance *C. krusei* strains were not found in this study. The activity of clotrimazole against to *C. krusei* was 96.2%, similar to nystatin. However, resistance to fluconazole (57.7%), miconazole (73.1%), and itraconazole (83.3%) was frequent among *C. krusei* isolates.

Discussion

Antifungal susceptibility surveillance study was playing an increasingly important role in tracking the development of antifungal resistance and starting initial antifungal treatment.[4,8,9] With the increasing use of antimicrobial agents, the distribution and susceptibilities of pathomycete may be changing from time to time.[4] In our study, we found a descending prevalence of *C. albicans* (90–77%) and a rising prevalence of *C. glabrata* (10–20%) from 2006 to 2013, similar as described in other surveys.[3,11-13] Factors such as antimicrobial agents, tumor chemotherapy agents, human organs transplantation, and human immunodeficiency diseases were deemed to contribute to the changing of species distribution. Regimens should be adjusted according to local surveillance results.[3,14]

As we all know, treatment choice based on local susceptibility tests was indispensable and credible. In our study, we found that nystatin exhibited excellent activity against all *Candida* species (<4% R), which was in accordance with other reports.[15,16] Besides the unique mechanism of changing cell membrane permeability, the excellent antifungal activity of nystatin was likely associated with relatively low-frequency use in clinical setting. In accordance with previous reports, increasing resistant to fluconazole was obviously among all species.[3,17,18] Although the susceptibility of fluconazole was descending, it was still active against *C. albicans* (70% S) in our study. However, when implemented on *C. glabrata* and *C. krusei,* fluconazole had little effect to these species due to intrinsic resistance which prompted reconsideration of fluconazole as the first-line therapy to these species. With regard to clotrimazole, it maintained activity against *C. albicans* (>90% S) and *C. krusei* (>90% S) but shows less effect to *C. glabrata* (<20% S). The activity of miconazole and itraconazole against all species, especially for *C. krusei* was not sufficient. In a study by Fan *et al.*, the resistant rate of *C. albicans* to azole agents was 0–4.9%.[13] Richter *et al.* showed that resistance to fluconazole was observed infrequently (3.7%).[11] Pfaller *et al*. reported that 90.2% of the *Candida* isolates tested were susceptible to fluconazole.[19] The data shown in our study were relatively higher compared to other reports, requiring the attention of clinicians in this situation. However, the possibility of some system bias cannot be excluded due to the potential reasons of the different specimen, test method, and regional disparity.[5,13,14,17,19,20]

There were several limitations in this study. First, some uncommon species may be absent using chromogenic culture media as a prequalification test limits the variety of species that can grow. Second, susceptibility tests were conducted using commercially available products, agar diffusion method (Neo-sensitabs, Rosco, Denmark) based in CLSI M44-A2 document. The high resistance rate in this study may be related to it. Third, expanding the scope of monitoring was necessary and more drug agents should be involved.

In conclusion, this study found that non-*albicans Candida,* especially *C. glabrata,* which was associated with RVVC was increasing. The drugs *in vitro* susceptibility were different among different vulvovaginal *Candida* species and it had changed over time. Nystatin was the optimal choice for the treatment of VVC at present. Regimens should be adjusted according to local surveillance results.

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Conflicts of interest

There are no conflicts of interest.

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