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Distribution and Drug Susceptibility of *Candida* spp. Associated With Female Genital Tract Infection, Chongqing, China

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Abstract

Background: Vulvovaginal candidiasis is defined as vulvovaginitis associated with vaginal carriage of *Candida* spp. and is a common problem with a high rate of morbidity.
Objectives: To investigate the distribution of *Candida* spp. and evaluate the corresponding antifungal susceptibility in women with genital tract infection in Chongqing, southwestern China.
Patients and Methods: Samples (n = 2.129) were obtained from female patients with symptoms of genital tract infection. *Candida* spp. were isolated from the specimens and were identified using a coloration medium and the VITEK 2 Compact automatic microbial identification system. Antifungal susceptibility testing was performed using the ATB FUNGUS drug susceptibility testing system.
Results: From 2,129 samples, 478 (22.45%) isolates of *Candida* were isolated, of which 395 (82.64%) were *Candida albicans*, 39 (8.16%) were *C. glabrata*, 21 (4.39%) were *C. tropicalis*, 9 (1.88%) were *C. parapsilosis*, and 14 (2.93%) were other *Candida* spp. The resistance of *C. albicans*, *C. glabrata*, and *C. tropicalis* to 5 antifungal drugs (amphotericin B, voriconazole, fluconazole, 5-fluorocytosine, and itraconazole) ranged from 0.5% to 6.4%, 0% to 7.7%, and 0% to 9.6%, respectively.
Conclusions: *Candida albicans* was the major pathogen associated with candidiasis of the female genital tract in patients in Chongqing. The results of the antifungal sensitivity of the isolates suggest that it is important for clinicians to administer appropriate antifungals for the treatment of *Candida* spp. infections.

Keywords: Genital Tract Infection, Antifungals, *Candida* spp

1. Background

Vulvovaginal candidiasis is defined as vulvovaginitis associated with vaginal carriage of *Candida* spp. and is a common problem with a high rate of morbidity. This infection is characterized by vulvar pruritus and increased vaginal discharge. At present, clinical treatment for vulvovaginal candidiasis consists of systemic and/or local use of imidazole-based antifungals (1).

Vaginal infection in pregnant women increases the incidence of fungal dermatitis and thrush in newborns, following vaginal delivery. Some fungal pathogens can move up the reproductive tract and penetrate the fetal membrane, leading to infection, which can result in abortion and/or premature delivery. The diagnosis and treatment of vulvovaginal candidiasis are subject to certain restrictions owing to the long period of time required for fungal culture and identification. In recent years, as a result of increasing sexual activity, the wide use of broad-spectrum antibiotics, anticancer drugs, immunosuppressants, corticosteroids, and oral contraceptives and an increase in the diabetes rate, the incidence of vaginitis associated with *Candida* spp. has increased significantly. An increase in the antifungal drug resistance in *Candida* spp. has also been observed, making treatment of this infection more difficult (2, 3). Some studies have identified alternative antifungal drugs that have been reported to be effective in the treatment of *Candida* infections. For example, Sadeghi Nejad et al. (4) showed that the extracts from fruits of *Heracleum persicum* had potential anti-*Candida* activity.

The emergence of antifungal resistance has resulted in an urgent need for laboratory support in the treatment of fungal infections, which includes the rapid identification of fungal pathogens and selection of appropriate methods for the in vitro susceptibility testing of clinical isolates. Shokohi et al. (5) determined the antifungal susceptibility of *Candida* spp. collected from oropharyngeal lesions in cancer patients.

2. Objectives

The aim of this retrospective study was to investigate recent *Candida* spp. infections in the genital tracts of female patients in Chongqing and to evaluate the resistance to commonly used antifungals by retrospectively analyzing the composition and drug susceptibility of *Candida* spp. isolated from the vaginal and cervical secretion specimens in both gynecological outpatients and inpatients between January 2010 and December 2011.

3. Patients and Methods

3.1. Subjects

From the outpatients and inpatients at the second affiliate hospital, Chongqing University of Medical Sciences, 2,129 women who presented with increased vaginal discharge or pus, vulvar itching, abdominal pain, and other symptoms of infection of the reproductive tract were selected. Patients from the Chongqing region were selected over a 2-year period from January 2010 to December 2011. This study was performed in accordance with the principles of the Helsinki declaration. ethical approval was obtained from the second affiliate hospital of the Chongqing University of Medical Sciences. The subjects were between 18 and 79 years of age (average age, 35.8 years), and of these subjects, 116 were $<$ 20 years old, 1,815 were between 20 and 49 years old, and 198 were $\geq$ 50 years old. Thirty subjects were unmarried. Patients who had undergone a general physical or gynecological examination for any reason and had used medication within 1 week before specimen collection were excluded from the study.

3.2. Specimen Collection

All specimens were collected by qualified gynecologists who obtained vaginal swabs from the dome and sidewalls of the vagina. Vaginal secretions were collected in sterile test tubes containing l mL of 0.9% saline for the culture of fungi. If the subject was unmarried, the sample was obtained from the vaginal orifice.

3.3. Culture and Identification of Candida spp

Vaginal secretions were cultured on Sabouraud dextrose agar (Oxoid, UK) at both 25°C and 37°C for 2 or 3 days, respectively. Putative positive colonies were white or cream in color with glossy and smooth surfaces. If no colonies were evident within 2 weeks, the sample was considered negative. Yeast-like colonies were Gram-stained to exclude any bacterial colonies that had been mistakenly identified as yeast. Colonies were then inoculated on CHROMagar medium (France) at 35°C for 24 - 48 hours. Green or emerald green colonies were identified as *C. albicans*, blue-gray colonies were identified as *C. tropicalis*, and purple-embossed colonies with smooth and glossy surfaces were identified as *C. glabrata*. Pink-to-purple large flat colonies with rough edges were identified as *C. krusei*, while colonies of the other phenotypes were identified as other *Candida spp*. These atypical isolates were further identified using the VITEK 2 compact automatic microbial identification system (bioMerieux, France).

3.4. Drug Susceptibility Test

Drug susceptibility testing was performed using the ATB FUNGUS drug susceptibility testing system (bioMerieux company, France). The following drugs and concentrations were tested: 5-flucytosine (0.125 - 64 μg/mL); amphotericin B, voriconazole, itraconazole (each between 0.03 and 16 μg/mL); and fluconazole (0.125 - 64 μg/mL). Testing was performed at 35°C for 24 hours. The minimum inhibitory concentrations of all drugs were obtained according to the CLSI M27-A3 guidelines (6, 7). In each test, the reference strain *C. albicans* ATCC 14053 was used for quality control.

According to the manufacturer instructions, a suspension of *C. albicans* with a turbidity of 2 McFarland was prepared, and 20 μL of this suspension was transferred to an ampule of ATB FUNGUS 3 medium. Then, 135 μL of the inoculated medium was transferred into each cupule. After incubation at 35°C for 24 hours, the strips were read using the ATB expression bacteriology analyzer automatic system (bioMerieux, France).

3.5. Statistical Analysis

Statistical analyses were performed using the SPSS software, version 17.0 (SPSS; Chicago, IL, USA). Data are presented as percentages. All data were compared and analyzed using the chi-square test. $P < 0.05$ were considered statistically significant.

4. Results

4.1. Detection and Distribution of Candida spp

From 2,129 samples of female genital tract secretions collected over a 2-year period, 478 isolates (positivity rate of 22.45%) representing 8 *Candida* spp. were detected. Of the isolates, 82.64% were *C. albicans*, 8.16% were *C. tropicalis*, 4.39% were *C. glabrata*, 1.88% were *C. parapsilosis*, 1.26% were *C. krusei*, 1.05% were *C. guilliermondii*, 0.42% were *C. lusitaniae*, and 0.21% were *C. dubliniensis* (Table 1).

4.2. Detection Rate of Candidiasis in Patients in Different Age Groups

According to the world health organization (WHO) standards and the age demographics in China, patients were divided into three groups: (1) subjects $<$ 20 years of age, (2) subjects between 20 and 49 years of age, and (3) subjects $>$ 50 of age. The results showed that the detection rates of *Candida*-related vaginal disease in age groups 1 and 2 were significantly higher than those in age group 3 ($P < 0.05$). Differences between age groups 1 and 2 were not statistically significant ($P > 0.05$), as shown in Table 2.

Table 1. Distribution of the Detected *Candida* spp[a]

Species	Number of Strains
C. albicans	395 (82.64)
C. glabrata	39 (8.16)
C. tropicalis	21 (4.39)
C. parapsilosis	9 (1.88)
C. krusei	6 (1.26)
C. guilliermondii	5 (1.05)
C. lusitaniae	2 (0.42)
C. dubliniensis	1 (0.21)

[a]Values are expressed as No. (%).

4.3. Antifungal Sensitivity Test

Resistance of the 3 most commonly isolated *Candida* spp. to the 5 antifungals tested was low (Table 3). The resistance rate to amphotericin B was the lowest (0 - 0.5%) with sensitivity rates between 97.4% and 100%. The resistance rates to voriconazole were between 0% and 5.2% and the sensitivity rates were between 89.7% and 100%, while the resistance rates to itraconazole were between 0% and 7.7% and the sensitivity rates were between 82.1% and 90.4%.

5. Discussion

Vulvovaginal candidiasis frequently colonizes the female reproductive tract with typical symptoms of vaginal itching, redness, and tofu-like vaginal discharge (8). Owing to the inappropriate use of antifungal drugs (i.e., repeated administration or long-term use), the appearance of drug-resistant strains has been increasing in China, making antifungal treatment increasingly difficult (9).

In this study, 2.129 vaginal secretions from patients with reproductive tract infections in the Chongqing region were analyzed for fungal infection. The results showed that 478 subjects were infected with 8 *Candida* spp., of which *C. albicans* accounted for 395 cases, *C. glabrata* accounted for 39 cases, *C. tropicalis* accounted for 21 cases, and other *Candida* accounted for 23 cases. This indicates that *Candida* infection is high in patients with gynecological reproductive tract infections in the Chongqing region.

Colonization by *Candida* spp. is affected by the immune status of the host, lower body resistance, pH changes in the vagina, lifestyle, abuse of antibiotics and pregnancy (10, 11). In our study, the overall positivity rate of *Candida* infection was 22.45%, of which *C. albicans* was the main species identified. This is consistent with the results of the other studies (12-16). *Candida glabrata* and *C. tropicalis* were the next commonly detected species. This observation differs somewhat from the other studies, where *C. krusei* was more commonly isolated than *C. tropicalis,* but these variations may be owing to differences in the populations and geographical locations of the studies. Recent reports indicate that the association of *C. glabrata* with vaginal candidiasis has been gradually increasing (15), and this may be related to the wide use of clinical azole drugs or nonstandardized treatments.

Even if there is no direct evidence that vulvovaginal candidiasis is sexually transmitted, the incidence of vulvovaginal candidiasis is known to increase upon initiation of sexual activity (17). Indeed, sexual transmission between partners may be a factor in the increasing incidence of candidiasis. Studies have shown that the infection rates of spouses of *Candida* spp.-positive male partners were 4 times higher than those of male partners without *Candida* infection. In addition, *Candida* spp. infections were detected in the penis of 15% of the partners of female patients with candidiasis (18, 19). Our study has shown that the detection rates of *Candida* spp. in the under 20 and 20 - 49 years age groups were significantly higher than in the group of patients > 50 years. This indicates that vaginal candidiasis occurs more often in women of reproductive age in Chongqing. We believe that the increase in vaginal infections in this population may be related to their more active sexual behavior.

In recent years, drug resistance in *Candida* spp. to common antifungal drugs has been increasing (20, 21). In our study, drug susceptibility tests were performed on 478 strains of *Candida*. Our results showed that 3 main species, including *C. albicans*, had low resistance to the 5 antifungal drugs. Resistance rates were < 8%, while the sensitivity rates were > 80%. In particular, resistance rates to amphotericin B were the lowest, ranging from 0% to 0.5%, with sensitivity rates ranging from 97.4% to 100%; resistance rates to voriconazole ranged from 0% to 5.2% and sensitivity rates ranged from 89.7% to 100%. However, owing to the side effects of amphotericin B and voriconazole, their use in candidiasis treatment is limited. The sensitivity rates of *C. glabrata* to fluconazole and itraconazole were 84.6% and 82.1%, respectively, which were lower than those of *C. tropicalis* and *C. albicans*.

Buitron Garcia-Figueroa et al. (22) reported that in vaginal candidiasis, the infection rates of *C. glabrata* is rising commensurate with the drug resistance rates to fluconazole (68.2%). Studies by Peman et al. (23) also showed that the overall susceptibility rates for itraconazole and fluconazole were 77.6% and 91.9%, respectively. Resistance rates were only observed in *C. glabrata* for itraconazole (24.1%) and posaconazole (14.5%), and in *C. krusei* for itraconazole (81.5%). In contrast Gualco et al. (24) reported that

Table 2. Detection Rates of Vaginitis in Women of Different Ages[a]

Age Group, y	Number of Cases	*Candida*	*C. albicans*	Other *Candida* spp
<20	116	26 (22.4)	20 (76.9)	6 (23.1)
20 - 49	1815	429 (23.6)	357 (83.2)	72 (16.8)
> 49	198	23 (11.6)	18 (78.3)	5 (21.7)
Total	2129	478	395	83

[a]Values are expressed as No. (%).

Table 3. Drug Susceptibility in the Most Prevalent *Candida* spp[a]

Antifungals	*C. albicans* (395 Isolates)			*C. glabrata* (39 Isolates)			*C. tropicalis* (21 Isolates)		
	R	I	S	R	I	S	R	I	S
Amphotericin	0.5	0.5	99.0	0.0	2.6	97.4	0.0	0.0	100.0
Voriconazole	1.7	5.1	93.2	5.2	5.1	89.7.5	0.0	0.0	100.0
Fluconazole	5.3	4.6	90.1	7.7	7.7	84.6	0.0	4.8	95.2
5-Florocytosineosinosine	3.1	6.3	90.6	5.1	2.6	92.3	4.8	0.0	95.2
Itraconazole	6.4	7.8	85.8	7.7	10.2	82.1	4.8	4.8	90.4

[a]Values are expressed as percentages.

the resistance rates of *C. albicans* to fluconazole and itraconazole were 0.7% and 2.7%, respectively. The different resistance rates observed in these studies may be owing to the differences in the study populations and geographical locations or differences in antifungal use, which is not regulated in China.

On the basis of our results, we suggest that the identification of yeast infections by smear microscopy alone is not sufficient and that clinicians should attempt to cultivate causative *Candida* spp. from vaginal secretions and design antifungal treatments based on drug susceptibility testing (25). This approach should help to reduce the recurrence of *Candida* infections and to mitigate against increasing antifungal resistance.

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Footnote

Authors' Contribution: Xiaodong Luo conducted the specimen studies, participated in the susceptibility testing, and drafted the manuscript; Xiaojing Dong contributed to the study design and helped to draft the manuscript; Zhi Pen conceived of the study, contributed to the study design and coordination, and performed the statistical analysis. All authors read and approved the final manuscript.

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