# The Impact of Antifungal Agents on the Morphology Dimorphism of Vaginal *Candida Albicans*

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**Abstract:** *Objective*: To compare the MICs of the five antifungal agents against *Candida albicans* isolated from patients with vulvovaginal candidiasis (VVC) in yeast form and in mycelial form. *Method*: 60 *Candida* organisms were cultured from samples obtained from patients with VVC of the Gynecology outpatient. Antifungal susceptibility testing was performed using the broth microdilution (BMD) method (CLSI, document M27-A2) of five agents. *Results*: The MIC values of miconazole and nystatin against *C. albicans* in mycelial form were significantly higher than those in yeast form, while the MIC of itraconazole against *C. albicans* in mycelial form was lower than those in yeast form ($P < 0.01$). MIC values of clotrimazole and fluconazole showed no difference between the two forms ($P > 0.05$). The susceptibility rate of the *C. albicans* in mycelia form (98.3%) to itraconazole was significantly higher than that in yeast form (51.7%). In yeast form, the susceptibility rate of *C. albicans* to itraconazole was significantly lower than that of fluconazole (51.7%, 100%). In mycelial form, the susceptibility rate of the two azoles were similar (98.3%, 100.0%). *Conclusion*: The mycelial form of *C. albicans* was more sensitive to itraconazole than the yeast form. All the azole agents had a good antifungal activity to the mycelium.

**Keyword:** *Candida albicans*, the yeast form, the mycelial form, antifungal susceptibility.

## INTRODUCTION

Vulvovaginal candidiasis (VVC) is a common gynecological infectious disease. The main pathogens were *Candida albicans* [1]. *C. albicans* is a dimorphic, opportunistic pathogen which exists ubiquitously in the human body; it does not grow remarkably as long as homeostatis is maintained; under conditions of decreased immunocompetence, it begins to grow and change from the yeast form to the mycelial form. In general, the mycelial form is associated with a higher degree of pathogenicity than the yeast form [2,3]. The *C. albicans* in mycelial form invading the tissues, causes the clinical symptoms. However, the traditional antifungal sensitivity researches of *C. albicans in vitro* used the yeast form as target cells to determine the MIC, and did not take the morphological characteristics and the pathogenesis of *C. albicans* into account. The MIC of mycelium maybe more effective to reflect the clinical efficacy than that of the yeast. In this study, the MICs of the five antifungal agents against vaginal *C. albicans* in the yeast form and in mycelial form were determined by the CLSI (Clinical and Laboratory Standards Institute, formerly NCCLS) document M27-A2 broth microdilution(BMD) method [4], to provide useful susceptibility information.

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## 1. MATERIALS AND METHODS

### 1.1. Study Design

The MICs of the five antifungal agents against 60 isolates of *C. albicans* in yeast form and mycelial form by BMD method (CLSI, document M27-A2) [4] were determined. The five antifungal agents were miconazole, itraconazole, clotrimazole, fluconazole and nystatin. Two quality controls were also included.

### 1.2. Clinical Isolates

Sixty clinical isolates of *C. albicans* were collected from the vaginal discharge of patients with VVC from the Gynecology outpatient at Peking University First Hospital. All the clinical strains were confirmed according to standard mycological methods by the Microbiological Research Laboratory. In addition, the two isolates *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were used as CLSI quality controls. All the isolates were preserved at -70°C and subcultured in antimicrobial-free medium to ensure viability and purity prior to testing.

### 1.3. Medium

Yeast medium used contained 10.4g RPMI 1640 (with L-glutamine but without sodium bicarbonate [GIBCO; Invitrogen, Breda Netherlands]) and 0.165 mol/L morpholine-propanesulfonic acid (MOPS;

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Amersco, Solon, OH, USA). The pH of the medium was adjusted with 1mol/L NaOH to 7.0 ± 0.1 at 25°C, sterilized by filtration and reserved at 4 ℃. Mycelial medium used was according to Wakabayashi H. [5]. 1L solution contained RPMI1640 10.4g, 20mmol / L HEPES, 2mmol / L L-glutamine, 2mmol/L glucose and 25% fetal calf serum, then adjusted the pH with NaHCO3 to 7.0 ±0.1 at 25°C, sterilized by filtration and reserved at 4°C. For colony counting, the medium used was Sabouraud dextrose agar (SDA; Jinzhang Biological Agent Co. Ltd., Tian Jin, China).

### 1.4. Inoculum Preparation

Stock inoculum suspensions were obtained from 24-h cultures on SDA at 35°C. The turbidity of each yeast suspension was adjusted to match the turbidity of a 0.5 McFarland standard by 0.85% Saline and diluted to a concentration of $1.0 \times 10^3 \sim 5.0 \times 10^3$ colony-forming units/ml by the two kinds of mediums.

### 1.5. Drugs and Stock Solution Preparation

The five antifungal agents used in the study were miconazole, itraconazole, clotrimazole, fluconazole and nystatin, which were provided by the National Institute for the Control of Pharmaceutical and Biological Products, China. A 1600μg/ml stock solution was prepared for fluconazole by dissolving the powder in distilled water, while a 6400μg/ml stock solution for the other tested drugs was prepared by dissolving the powder in dimethyl sulfoxide .The stock solutions were stored at -70°C.

### 1.6. Drug Susceptibility Testing

Two kinds of mediums were used in drug susceptibility test. The test was carried out in 96-well flat-bottomed microtitration plates. A blank well (only medium) and growth control (drug-free well) were incubated each isolate testing. The plates were incubated in air at 35°C and MICs were determined visually at 48 h. The MICs were defined as the lowest drug concentration that showed 80% growth inhibition compared to the growth control. The final concentrations of the antifungal agents ranged from 0.015 to 128μg/ml. All experiments were performed twice. The MICs were read according to the existing breakpoints .The CLSI interpretive breakpoints for two agents were that: Itraconazole (S, ≤0.12μg/ml; S-DD, 0.2to 0.5μg/ml; R, ≥1μg/ml); fluconazole (S, ≤8μg/ml; S-DD, 16 to 32μg/ml; R, ≥64μg/ml)).

### 1.7. Statisitical Analysis

Statistical analysis was performed using the Wilcoxon signed-rank test and chi-squared test, $P<0.05$ was considered significant.

## 2. RESULTS

**2.1.** In this study, the growth control of all isolates grew well in the two mediums. Many long myceliums were observed in the mycelial medium (data not shown).

**2.2.** The MIC values (median and range) of 5 antifungal agents against 60 strains of *C. albicans* in yeast-phase and mycelium-phase were shown in Table **1**. The MIC values of miconazole and nystatin against *C. albicans* in mycelial form were significantly higher than those in yeast form. While the MIC values of itraconazole against *C. albicans* in mycelial form were significantly lower than those in yeast form ($P < 0.001$). Clotrimazole and fluconazole MIC values showed no difference between the two forms ($P > 0.05$).

**2.3.** Table **2** showed the susceptibility of the *C. albicans* to two antifungal agents in two forms. We determined the susceptibility of drugs according to the NCCLS breakpoints. The susceptibility rate of the *C. albicans* in mycelial form (98.3%) to itraconazole was significantly higher than that in yeast-phase (51.7%) ($P<0.001$). In yeast form, the susceptibility rate of *C. albicans* to itraconazole was 51.7 %, which was significantly lower than that of fluconazole. While in mycelial form, the susceptibility rate of the two azoles were similar (98.3%, 100.0%).

## 3. DISCUSSION

*C. albicans* has two forms. The yeast form was in favor of fungi to find a new host and spread in the environment, while the mycelial form can invade the tissues and cause tissue damage. The more myceliums the higher infection rate [6,7]. The pathogenic fungi form is mycelium in VVC, so we want to know the antifungal effect of the agents to mycelium.

There were many studies about the culture of the mycelial form of *C. albicans* [5,8]. In this study, we used the liquid medium which Wakabayashi H. [5] used to get the mycelial form of C. albicans. Using that mycelia medium, we can get many long myceliums.

There are many kinds of antifungal agents to treat VVC. The oral drugs are fluconazole, itraconazole and

nystatin. Vaginal suppositories are miconazole, clotrimazole, nystatin. The antifungal activity of miconazole, clotrimazole, fluconazole against *C. albicans* had no differences between the two forms. The mycelium was more sensitive to itraconazole than the yeast, while the yeast was more sensitive to nystatin than the mycelium ($P < 0.001$). In the yeast form, the susceptibility rate of *C. albicans* to itraconazole was significantly lower than that of the other drugs which may be related to the chemical nature of itraconazole. As its property of weak alkaline (pKa = 3.7), itraconazole ionized at the low- pH, and ions can play a better antifungal effect than compound [9], but the pH of the standard antifungal susceptibility test *in vitro* was 7.0, which affects the ionization.

The susceptibility of the yeast form of *C. albicans* to itraconazole was low, but previous studies showed that itraconazole had a good clinical efficacy, easy administration, short course, few and mild side effects. The treatments of acute VVC with fluconazole and itraconazole showed good clinical and mycological efficacy, especially for recurrent VVC, the clinical efficacy of itraconazole or even better than fluconazole [10], so that the routine susceptibility test of the yeast form at pH7.0 can't reflect the real antibacterial capacity of itraconazole.

In this study, the antifungal activity of nystatin to mycelium was weaker than yeast. Nystatin belongs to the polyenes antifungal agents, whose basic antifungal mechanism is that a lipophilic straight –chain of the drug molecule can combine with the two steroids of the cell membrane, then change the permeability of cell membranes, cause fungal cell contents leakage and lead the fungal cells to die. In a previous study, they found that the structure of the cell- wall was different between the yeast and the mycelium [12], which may affect the antifungal activity of the nystatin.

Previous study showed that the resistant rate of *C. albicans* to azole agents was 0 ~ 4.9% in yeast form [11]. In our study, the resistant rate of *C. albicans* to azole agents was 0 ~ 3.3% in both forms, which suggested that the azoles were the useful drugs to treat the VVC. All azoles had good antifungal activity against the mycelial form of *C. albicans in vitro*. Although the susceptibility rate of *C. albicans* to itraconazole was significantly lower than that of the other azole agents in the yeast form, it was reported as having a good clinical efficacy especially for recurrent VVC better than fluconazole. Futhermore, itraconazole has remarkable curative effect on moderate to severe VVC which means it has high antifungal activity against the mycelial form but still needs further study.

## CONDENSATION

The mycelial form of *C. albicans* is more sensitive to itraconazole than the yeast form and all the azole agents have a good antifungal activity to the mycelium.

## ACKNOWLEDGMENT

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## CONFLICT OF INTEREST

In this research, Jinan University and Peking University have made the same contribution. The first author currently works in Jiangxi Maternal and Child Health Hospital.

# Appendix

**Table 1: Antifungal Susceptibilities of Vagina Isolates in Two Forms according to the CLSI Microdilution Method after 48 h of Incubation**

| Dimorphism | Antifungal agents (median, range) | | | | |
|---|---|---|---|---|---|
| | Miconazole | Itraconazole | Clotrimazole | Fluconazole | Nystatin |
| Yeast form | 0.03(0.015 ~ 1) | 0.125 (0.03 ~ 1) | 0.03 (0.03 ~ 0.06) | 0.25 (0.125 ~ 8) | 2 (0.5 ~ 8) |
| Mycelial form | 0.05 (0.03 ~ 8) | 0.030 (0.015 ~ 2) | 0.03(0.03 ~ 0.125) | 0.25(0.125 ~ 16) | 4 (0.25 ~ 32) |
| *Z* | 2.968 | 5.576 | 0.905 | 0.402 | 5.281 |
| *P* | <0.001 | <0.001 | 0.366 | 0.687 | <0.001 |

**Table 2: *In Vitro* Susceptibility of Vaginal Isolates to Two Antifungal Agents in Two Forms**

| Antifungal agents | Itraconazole | | | Fluconazole | | |
|---|---|---|---|---|---|---|
| | S | SDD | R | S | SDD | R |
| Yeast-form | 31(51.7) | 27(45) | 3(3.3) | 60(100) | 0(0.0) | 0(0.0) |
| Mycelium-form | 59(98.3) | 0(0.0) | 1(1.7) | 60(100) | 1(1.7) | 0(0.0) |
| $\chi^2$ | 34.844 | | | - | | |
| *P* value | < 0.001 | | | 1.000 | | |

**Abbreviations:** R, resistant; S, sensitive; SDD, susceptible-dose dependent.
[a]Values are given as number (percentage) unless otherwise indicated.

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