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In-Vitro Susceptibility of Fluconazole and Amphotericin B against *Candida* Isolates from Women with Vaginal Candidiasis in Taiwan

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ABSTRACT

Reported epidemiology references showed that vaginitis was caused either by bacteria (40-50%), *Trichomonas* (20-25%) or *Candida* (15-20%). The objective of this study was to investigate the prevalence and strains distribution of vaginal candidiasis in Taiwan. We evaluate the susceptibilities activity of fluconazole and amphotericin B against 101 *Candida* isolates from 97 patients suffering from vaginal candidasis. *Candida albicans* was the most common species (67.3%), followed by *C. glabrata* (24.7%), C. *tropicalis* (5.9%), *C. parapsilosis* (1%), and others (1%) among the identified strains. After evaluation by Etest, a total of 64 (63.4%) and 11 (10.9%) isolates were found to be susceptible, with dose dependent susceptibility, to fluconazole. All *Candida* isolates were susceptible to amphotericin B with one isolate *Saccharomyces cerevisiae* being resistant to this drug. Ten out of 68 isolates (14.7%) of *C. albicans* and 16 out of 25 isolates (64%) of *C. glabrata* showed fluconazole-resistant. *C. glabrata* commonly produced higher minimum inhibitory concentrations than all the other species of *Candida* to triazole agents.

Key words: susceptibility, fluconazole, amphotericin B, vaginal candidiasis

INTRODUCTION

Vaginal candidiasis, caused by opportunist yeast, *Candida*, is a common and increasing disease in women[(1)]. Approximately 75% of all women will have a vaginal infection episode during their life span[(2)]. Possible risk factors causing an increase in *Candida* infections include prior antibiotic therapy, pregnancy, diabetes mellitus (DM), oral contraceptives containing estrogen and progestin, and immunosuppressed patients (transplanted patients, cancer patients treated with chemotherapy, and HIV patients)[(2,3)]. *Candida albicans* is the dominant species that caused a prevalence of 70-90% of *Candida* vaginitis in various reports[(4)]. Only a minority of cases (< 10%) are caused by non-*albicans Candida* species, usually *C. glabrata* and *C. tropicalis*[(4)]. However, a significant increase in non- *C. albicans* species in some cases has been reported to be associated with recurrent vaginal candidiasis (RVC)[(5)]. These epidemiological phenomena are hypothetical due to incomplete eradications that either have increased virulence, or are drug resistant[(6)]. These changes suggest that further investigation of clinical *Candida* colonization and of its drug-resistant activity could bring discoveries that might lead to the complete eradication of these infections, or to the development of more effective antibiotic protocols.

Traditionally, oral amphotericin B has long been considered the "gold" standard for the treatment of vaginal candidiasis; however, toxicities, poor taste acceptance, and the need for multiple daily dosing limited its usefulness[(7)]. Today, a triazole antifungal agent, fluconazole, has been used for inhibiting fungal cytochrome P-450 sterol C-14 alpha-demethylation of cell membrane ergosterol synthesis pathways; it may be considered a reasonable alternative because it is significantly less toxic than amphotericin B and can be administered orally[(8)]. Unfortunately, the *in-vitro* antifungal susceptibility compariation of fluconazole and amphotericin B has not yet been identified clearly. The objective of this study was to evaluate the *in-vitro* pharmacological activity of fluconazole and amphotericin B against the clinical isolates of *Candida* according Etest, a highly studied and popular methodology for clinical conventional minimum inhibitory concentration (MIC) determination.

MATERIALS AND METHODS

I. *Strain Collection*

Vaginal swabs were collected from 182 patients who visited the Kuo General Hospital, a regional teaching hospital of Tainan, Taiwan. Vaginal secretions were cultured for 24 hr at 35˚C on Sabouraud's dextrose agar (Difco-BBL, USA), and each distinct colony was identified by germ tube test[(9)] and with commercial biochemical

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panels API 20C (Biomerieux-Vitek, France). Growth at 45˚C on Sabouraud's dextrose agar was used to distinguish between *C. albicans* and *C. dubliniensis*. Then the *Candida* species were identified on CHROMagar-*Candida* (Difco-BBL, USA), which is a chromgenic medium, allowing the initial identification of *C. albicans*, *C. tropicalis*, and *C. krusei* based on the colors and shapes of the colonies. *Candida* selected for testing include 5 American Type Culture Collection (ATCC) standard strains, *C. albicans* ATCC10231, *C. krusei* ATCC6258, *C. parasilosis* ATCC22019, *C. tropicalis* ATCC13803, and *C. glabrata* ATCC90030.

II. *Susceptibility Testing*

Etest (AB BIODISK, Sweden) was applied for antifungal susceptibility test of *Candida*. Etest is an agar based quantitative susceptibility technique, which combines the concepts of both dilution and diffusion tests; comprised of a predefined gradient of antifungal agent for determining the minimum inhibitory concentration (MIC) in μg/mL of individual drugs. Each *Candida* isolate colony from a 24 hr SDA plate was picked and homogenized well in 0.85% saline to achieve 0.5 McFarland turbidity ($0.5\sim2.5 \times 10^3$ CFU/mL). This suspension was spread with a sterile swab on a PRMI 1640 plate (Sigma, USA) evenly onto the entire surface, in three directions, allowing excessive moisture to be absorbed for about 10-15 min, before applying the Etest plastic strips on the agar. The strips were in complete contact with the agar surface. Agar plates were incubated in a moist incubator at 35˚C until growth is seen (24-72 hr), before taking readings of the MIC values at the point of intersection between the clear zone edge and the Etest strip. For the fluconazole, the isolates were reported to be susceptible (S, MIC $\leqq$ 8 μg/mL), susceptible-dose dependent (S-DD, MIC16~32 μg/mL), or resistant (R, MIC $\geq$ 64 μg/mL) according to the National Committee for Clinical Laboratory Standards (NCCLS, 1997)[10] interpretive breakpoint guidelines. A MIC $\leqq$ 1 μg/mL was considered susceptible to amphotericin B.

RESULTS

I. *Species Distribution and Diversity*

Of the original 182 clinical diagnostic candidiasis patients enrolled in this study, 85 (47%) were either culture-negative or were cases of bacterial or *Trichomonas* vaginosis . A total of 102 clinical yeast isolates were found in 97 *Candida* culture-positive women with a recovery rate of 53%. Of those, 49.5% (45/97) patients had a history of recurrent vaginal candidiasis (RVC). An analysis by the test of association of each pair of the following variables: patient's status (sporadic vaginal candidiasis or RVC), clinical diagnostic (post-menopausal syndrome, oral antibiotics treatment, Diabetes mellitus, catamenia, pregnancy or other diseases), and fluconazole resistance (resistant, S-DD or susceptible) showed no significant correlation ($p > 0.05$, Pearson chi-square). All of the yeast isolates belonged to the *Candida* species, except one, which was *Saccharomyces cerevisiae*. The overall distribution of *Candida* spp. among the 101 *Candida* isolates obtained is summarized in Table 1: *C. albicans* was the predominant species (67%), followed by *C. glabrata* (25%), *C. tropicalis* (6%), *C. parapsilosis* (1%), and *Candida* sp. (1%). Five symptomatic women yield more than one species of *Candida*. In three of these patients, *C. albicans* and *C. glabrata* were isolated from the vaginal swabs. The other two were infected by *C. albicans* and *C. tropicalis*.

Table 1. Distribution of *Candida* species from visit patients

Candida species	No. (%) of isolates
Candida albicans	68 (67.3)
Non-albicans *Candida*	33 (32.6)
C. glabrata	25 (24.7)
C. tropicalis	6 (5.9)
C. parapsilosis	1 (1)
Candida sp.	1 (1)
Saccharomyces cerevisiae	1

II. *Fluconazole Susceptibility*

The Etest analysis showed that overall, 26 (25.7%) of *Candida* isolates were resistant to fluconazole, 11 (10.9%) were determined as S-DD, and 64 (63.4%) were considered fluconazole susceptible. Analysis of the 68 vaginal *C. albicans* isolates revealed 10 (14.7%) resistant, and 3 (4.4%) S-DD isolates. The remaining 55 (80.9%) *C. albicans* were susceptible. The other non-albicans 33 isolates showed 17 (51.5%) were fluconazole effective. Alarmingly, 48.5% non-albicans *Candida* isolates were fluconazole ineffective, and all of them were *C. glabrata* (Table 2).

III. *Amphotericin B Susceptibility*

The *in vitro* susceptibilities of *Candida* isolates with amphotericin B revealed that all strains were susceptible (MIC $\leqq$ 1 μg/mL). It was completely effective for all *C. albicans* and non-albicans *Candida* except for one *S. cerevisiae* isolate (Table 2).

DISCUSSION

The previous epidemiology reference showed that vaginitis was caused either by bacteria (40-50%), *Trichomonas* (20-25%) or *Candida* (15-20%)[11]. In this study, the incidence percentage of *Candida* vaginitis (53.3%) showing sharp increases may be due to a growing population of immunocompromised or immunosuppressed diseases[12], and the sampling population was screened from clinical candidal vaginitis women of signs and

symptoms. Among the clinical isolates, *C. albicans* was the most common species detected, followed by *C. glabrata*. Similarities were observed compared to the vaginal *Candida* distribution data from Malaysia[13].

Currently, Etest has been distributed as a time-saving methodology for clinical antifungal susceptibility testing. Fluconazole and amphotericin B Etest and NCCL microdilution methods have been compared extensively and shown to produce a good agreement[14]. In this study, Etest analysis results showed that 25.7% of Candida isolates were resistant to fluconazole and more than one-half of these strains belong to *C. glabrata*. Fluconazole susceptible percentage of *C. albicans* was 80.9% (at MIC ≦ 8 μg/mL), close to the data of Argentina (87%)[15] and Belgium (79%)[16], but with statistical significant difference from those of Spain (100%)[17] and America (96%)[1] (Table 3). The percentage of S-DD *C. albicans* was 10.9%; often falling into the S-DD category. Identification of this yeast often results in treatment with amphotericin B or other non-azole antifungal or higher dosage of azoles drugs. For example, an antifungal susceptibility testing suggested that a 400~800 mg daily dosage of fluconazole is likely to be effective against fluconazole S-DD (MIC16~32 μg/mL) *C. albicans* in the treatment of candidiasis[14].

A striking observation in this study is that 64% of the *C. glabrata* isolates were fluconazole resistance. This percentage was far higher than the reported data of Spain (4.3%)[17] and America (4.5%)[18]. The present study is the only drug susceptibility result of *Candida* isolates from women with vaginal candidiasis in Taiwan, although one previous drug resistance research indicated 47.3% of *C. albicans* and 70% of *C. glabrata* collection from patients with bloodstream infections in National Taiwan University Hospital were fluconazole ineffective[12]. *C. glabrata* is of particular importance as an emerging fungal pathogen because it appears to be innately resistant to moderate levels of fluconazole[19]. Some observations of certain cases indicated that this drug resistance has been correlated with either over-expression of membrane ergosterol synthesis enzyme or the strain's genetic modification[20]. Spinillo *et al.* have also shown that *C. glabrata* causes approximately 20-30% of all recurrent candidiasis cases and it's possible that long-term or inappropriate maintenance treatment could encourage this selection of fluconazole resistance species[21].

S. cerevisiae has never been found to cause candidal vaginitis[22]. In this case, the only one *S. cerevisiae* isolate showed resistance to both fluconazole (MIC = 48 μg/mL) and amphotericin B (MIC ≥ 32 μg/mL). Its drug resistant performance may be due to the lack of sterol D desaturase, a drug target enzyme of cell membrane synthesis pathway[23].

Knowledge of local antifungal susceptibility pattern, in conjunction with knowledge of local prevalence or identification of more resistant species, can greatly aid in the selection of effective antifungal agents for empirical use.

Table 2. Comparative susceptibilities of *Candida* vaginal isolates against fluconazole and amphotericin B

Candida species	No. of isolates	No. (%) of isolates				
		Fluconazole*			Amphotericin B**	
		S[a]	S-DD[b]	R[c]	S[d]	R[e]
C. albicans	68	55 (80.9)	3 (4.4)	10 (14.7)	68 (100)	0
Non-albicans *Candida*	33	9 (27.3)	8 (24.2)	16 (48.5)	33 (100)	0
C. glabrata	25	1 (4)	8 (32)	16 (64)	25 (100)	0
C. tropicalis	6	6 (100)	0	0	6 (100)	0
C. parapsilosis	1	1 (100)	0	0	1 (100)	0
Candida sp.	1	1 (100)	0	0	1 (100)	0
S. cerevisiae	1	0	0	1 (100)	0	1 (100)

[a]Susceptible (MIC ≦ 8 μg/mL).
[b]Susceptible-dose dependent (MIC16~32 μg/mL).
[c]Resistant (MIC ≥ 64 μg/mL).
[d]Susceptible (MIC ≦ 1 μg/mL).
[e]Resistant (MIC > 1 μg/mL).
*p < 0.001: Chi-squares, JMP (SAS Institute, USA).
**p = 0.08: Chi-squares, JMP (SAS Institute, USA).

Table 3. Comparative susceptibilities of *Candida albicans* vaginal isolates against fluconazole of different countries

Country	No. (%) of isolates				
	This study[a]	Spain[b,(17)]	America[c,(18)]	Argentina[d,(15)]	Belgium[e,(16)]
S*	55 (80.9)	108 (100)	373 (96)	79 (87)	68 (79)
S-DD**	3 (4.4)	0	2 (0.5)	0	0
R***	10 (14.7)	0	14 (3.5)	12 (13)	18 (21)

[ab,ac]p < 0.005: Chi-squares, JMP (SAS Institute, USA).
[ad,ae]p = 0.08: Chi-squares, JMP (SAS Institute, USA).
*Susceptible (MIC ≦ 8 μg/mL).
**Susceptible-dose dependent (MIC16~32 μg/mL).
***Resistant (MIC ≥ 64 μg/mL).

However, the role of antifungal susceptibility testing is usually dependent on local availability and often cost issues. On the basis of these results, we do recommend *in-vitro* susceptibility testing for patients with recurrent vaginitis. In this setting, species identification and susceptibility testing may enhance antifungal selection and patient response to treatment.

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