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Research Article

IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY OF *CANDIDA* SPECIES IN VULVOVAGINAL CANDIDIASIS FEMALE PATIENTS AT A VIETNAMESE HOSPITAL

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ABSTRACT

Objectives: This study aimed to investigate the prevalence rate of *Candida* species isolated from women clinically suspected of having vulvovaginal candidiasis and assess susceptibility patterns against commonly used antifungal agents.

Methods: This was a cross-sectional, hospital-based study conducted for candidiasis at the vaginal level from May 2023 to December 2023. A total of 146 women clinically suspected of infecting *Candida* spp. were subjected to the identification employing disc diffusion methods. Antifungal susceptibility assay was conducted on *Candida* isolates against four commonly used antifungal drugs of the azole group.

Results: Of the 146 *Candida* strain isolates from vaginal swabs, *Candida albicans* was the most prevalent occurrence accounting for 68.75%, followed by *Candida glabrata* (11.88%) and *Candida parapsilosis* (11.25%). Of the 110 *C. albicans* isolates, 108 cases were sensitive to clotrimazole, followed by miconazole (41 cases). Meanwhile, of the 50 non-*albicans* isolates, all cases were susceptible to clotrimazole, followed by miconazole (38 cases). Different rates of resistance against itraconazole and fluconazole (but not clotrimazole and miconazole) were documented in all isolated non-*albicans* strains. The 96 *C. albicans* cases exhibited resistance to fluconazole, followed by miconazole (5 cases) and clotrimazole (1 case).

Conclusion: These findings provided data for better disease management, aiming at reducing morbidity and mortality.

Keywords: Antifungal susceptibility, Azole, *Candida albicans*, *Candida* non-*albicans*, CHROM-agar, Identification.

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INTRODUCTION

Candidiasis is a prevalent fungal infection caused by yeasts belonging to the genus *Candida*, which includes more than 200 species of *Candida* to have been scientifically identified [1]. *Candida albicans* is well known as the most prevalent species causing Candidiasis globally. It might affect various parts of the body, typically oral thrush (oral candidiasis) and vaginal canal vulvovaginal candidiasis (VVC) [2]. However, recent epidemiological reports have revealed an increasing number of infections caused by non-*albicans* spp., typically *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis* [3].

VVC is the most frequent occurrence in reproductive-aged women. Typical clinical signs include swelling, itching, burning, and thick-white adherent vaginal discharge. VVC is attributed chiefly to a variety of factors, including abuse of broad-spectrum antibiotics, long-term use of contraceptive pills, immunosuppression, and inappropriate lifestyle habits such as poor hygiene practices, and wearing tight underwear [4,5]. There has been currently an increasing antifungal-resistant *Candida* spp. at an alarming rate over the last decade [6]. Resistance to antifungal agents is becoming a global concern to public health, particularly with the recent emergence of azole drug-resistant *Candida* spp. [7].

As a consequence, the sensitivity of *Candida* spp. to antifungal agents might be extremely variable depending on the virulence and the susceptibility of *Candida* strains. Antifungal susceptibility test thus plays a vital role in accurately providing the identity of *Candida* isolates, significantly contributing to proper effective therapy given to each patient [8]. In addition, the timely and accurate identification and antifungal susceptibility may help limit or prevent the emergence of antifungal resistance. In this regard, this study thus focused on the identification and their antifungal susceptibility patterns against four commonly used antifungal agents [9] (itraconazole, fluconazole, clotrimazole, and miconazole), aiming to facilitate valuable data for effective management of *Candida* species-infected patients as well as reducing morbidity and mortality [10].

METHODS

The various *Candida* strains were identified using various media employing disk diffusion methods including Sabouraud's Dextrose Agar and CHROM-agar. The antifungal susceptibility of the isolated clinical *Candida* spp. was assayed against four commonly used antifungal agents (itraconazole, fluconazole, clotrimazole, and miconazole). This was a cross-sectional, hospital-based study conducted for candidiasis at the vaginal level between May 2023 and December 2023 at the Department of Gynaecology and Obstetrics, Laboratory of Ho Chi Minh City-based Tan Binh district hospital (Vietnam). Approval from the Institutional Ethics Committee must be obtained before the commencement of the study.

Specimen collection

Once a female patient (aged between 17 and 63 years) was admitted to the hospital, the attending gynecologists performed a comprehensive physical examination, noting clinical signs indicative of vaginal infection. As a result of clinically initial screening, a total of 374 women with clinically suspected fungal infections were subjected to this study. A sterile vaginal speculum was then utilized to visualize the cervix. Subsequently, two vaginal swabs were collected aseptically, labeled appropriately, and transferred to the laboratory for further analysis. The specimen was then divided into two parts; one was kept in a sterile tube and the other was spread onto a slide for Gram staining under the microscope [11].

Identification of *Candida* spp. of clinical isolates

Gram-staining

Once air-drying the smear, the specimen slide was placed on a staining tray and washed with crystal violet for 1 min, followed by rinsing with distilled water and applying Gram's iodine for another minute. The slide was then decolorized by washing under absolute alcohol, followed by rinsing with water and counterstaining with safranin for 1 min prior to optically microscopic observation under oil immersion at ×1,000 magnification [12].

Culture for isolation and CHROM-agar identification

The sterile swab-containing sample was spread evenly across the surface of a nutrient agar plate, followed by incubation at 35–37°C for 7 days. The result was recorded as "negative" in the case of non-fungal colonies on the plate. CHROM-agar identification - a common technique to identify different strains of *Candida* spp. based on the color of their growing colonies, was then applied for "positive" plates. 1–2 drops of 0.9% saline solution were added to a specimen swab and quadrant streaked onto a CHROM-agar plate in three different directions to ensure isolated colonies. The plates were promptly incubated at 35°C for 40–48 h, followed by the color observation of the colonies to identify the *Candida* spp. [13,14].

Antifungal susceptibility

A culture suspension was prepared using normal saline incubating overnight at 37°C in Sabouraud dextrose agar. The turbidity of the suspension was adjusted to 0.5 McFarland and subjected to the absorbance measurement at 530 nm using a spectrophotometer. The suspension was then spread evenly onto a Mueller–Hinton Agar plate. The disks impregnated with antifungals at specific concentrations (Table 1) were placed on the surface of the agar at equal distances. The antifungal agents used in susceptibility tests included clotrimazole 50 µg, fluconazole 25 µg, itraconazole 8 µg, and miconazole 10 µg. The plate was then incubated at 35°C, and the zone of inhibition was measured using a caliper as recommended by the Clinical and Laboratory Standards Institute M44-A document guidelines [15].

Statistical analysis

All experiments were performed in triplicate. The normally distributed data were expressed as the mean ± standard deviation (x ± s), and a t-test was used for comparisons between two groups of data. Chi-square test, Mann–Whitney U test, and Fisher's exact test were used for comparisons of the average values among multiple groups of data. Data analysis was performed using Statistical Package for the Social Sciences 25.0 software.

Ethical approvals

Ethical approval was obtained from the Biomedical Research Ethics Committee of Hong Bang International University through an official document (reference number: 86/PCT-HĐĐĐ). All patients were provided with information and voluntarily signed an informed consent form (ICF version 1, dated June 04, 2022) to participate in the study. In addition, the confidentiality of information was assured throughout the study.

RESULTS

Social-demographic characteristics of the study population

During the study period between May and December 2023, a total of 374 patients visited the Obstetrics Department at Tan Binh Hospital, corresponding to 374 clinical samples subjected to the investigation. The average age of the patients in this study was 37.24, ranging from 17 to 63 years old (Table 2), which is in line with the typical age range of patients visiting gynecology clinics. A few cases of postmenopausal patients (63 years old) were included in this study, provided they still met the inclusion criteria [16]. 360 participants live in city areas, accounting for 96.26% and an extremely high proportion of patients (97.33%) have at least a secondary education level. This study revealed that contraceptive use was common among participants (59.89%) and most patients were premenopausal (94.39%). There were low prevalence rates of long-term use of antibiotics (2.14%) and sexually transmitted diseases (2.41%) in the subjected population. External genital hygiene was the most vital genital care (78.34%) while douching and vaginal suppository insertion can disrupt the vaginal microbiota, creating opportunities for fungal or bacterial overgrowth.

Table 1: Antifungal agents used in susceptibility test

Antifungal agents	Concentration per disc	Zone of inhibition diameters (mm)		
		Sensitive	Intermediate	Resistant
Clotrimazole	50 µg	≥20	12–19	≤11
Fluconazole	25 µg	≥19	15–18	≤14
Itraconazole	8 µg	≥19	15–18	≤14
Miconazole	10 µg	≥20	12–19	≤11

Table 2: Sociodemographic characteristics of patients

N_{total}=374	Frequency (n)	Percentage
Ages		
17–41	117	80.14
41–60	29	19.86
Menopause		
Reproductive age	353	94.39
Menopause	21	5.61
Place of residence		
City areas	360	96.26
Rural areas	14	3.74
Educational background		
Under primary	2	0.53
Primary	8	2.14
Secondary	129	34.49
Graduated high school	140	37.43
Undergraduate-Postgraduate	95	25.40
Obstetric history		
Yes	274	73.26
No	100	26.74
Contraceptive methods		
Yes	224	59.89
No	150	40.11
Genital hygiene		
External washing	293	78.34
Douching	72	19.25
Vaginal suppository insertion	9	2.41
Long-term use of antibiotics		
Yes	8	2.14
No	366	97.86
History of sexually transmitted diseases		
Yes	9	2.41
No	365	97.59

Identification of *Candida* species

Table 3 details the relationship between *Candida* isolates distribution and social-epidemiological factors. There was a significant difference in the prevalence rates of *Candida* spp.-infected patients living in the city (37.88%) and rural areas (46.70%). Among different contraceptive methods, patients using condoms were recorded with the highest rate of *Candida* infection (45.63%), followed by patients with contraceptive devices (37.80%). There were generally no impacts on the prevalence rates of *Candida* infections with regard to other risk factors including menopause, genital hygiene, vaginal discharge, and other infectious diseases.

Identification of *C. albicans* and *Candida* non-*albicans* species

As illustrated by different colors in Fig. 1, this study revealed that *C. albicans* was predominant in frequency (n=110) accounting for 68.75% whereas all *C.* non-*albicans* spp. represented 31.25%, of which *C. glabrata* (n=19) was the second most frequently isolated species from vaginal specimens, accounting for 11.88%, followed by *C. parapsilosis*

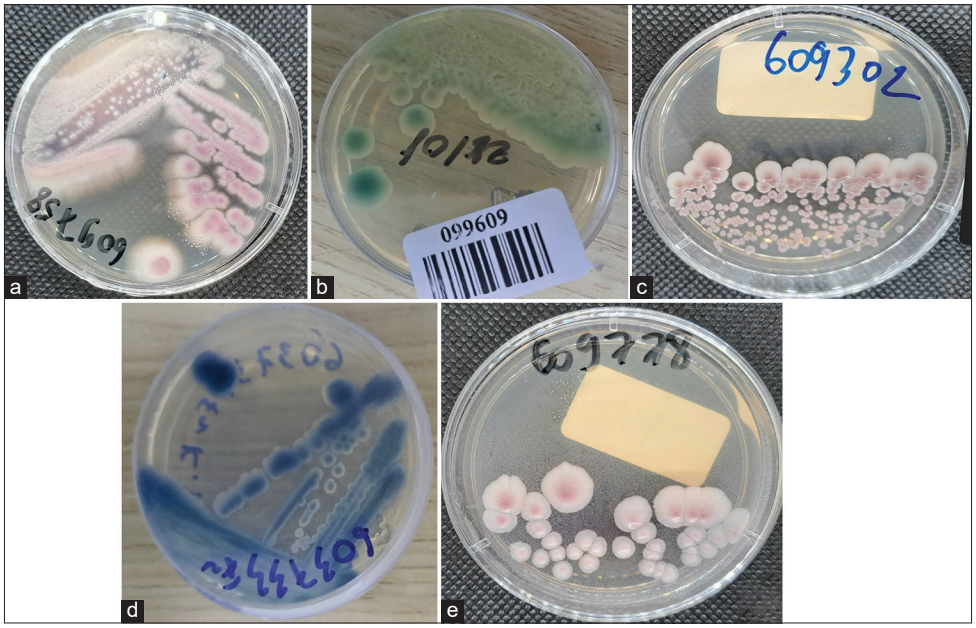

Fig. 1: CHROM-agar identification of *Candida* species including (a) *Candida krusei*; (b) *Candida albicans*; (c) *Candida glabrata*; (d) *Candida tropicalis*; (e) *Candida parapsilosis*

Table 3: The relationship between *Candida* isolates distribution and social-epidemiological factors

N_{Total}=374	Frequency (n)–percentage		p-value OR (CI=95%)
	$n_{Positive}$ (%)	$n_{Negative}$ (%)	
Place of residence			
City (359)	136 (37.88)	223 (62.12)	0.493*
Rural (15)	7 (46.70)	8 (53.30)	0.697 (0.25–1.97)
Contraceptive methods			
None (151)	57 (37.75)	94 (62.25)	0.067*
Condom (103)	47 (45.63)	56 (54.37)	
Contraceptive pills (38)	8 (21.10)	30 (78.90)	
Contraceptive devices (82)	31 (37.80)	51 (62.20)	
Menopause			
Premenopausal (352)	136 (38.64)	216 (61.36)	0.523*
Menopause (22)	7 (31.82)	15 (68.18)	0.741 (0.30–1.86)
Genital hygiene			
Appropriate (94)	35 (37.23)	59 (62.77)	0.817*
Inappropriate (280)	108 (38.57)	172 (61.43)	1.06 (0.65–1.72)
Vaginal discharge			
Diluted (156)	63 (40.38)	93 (59.62)	0.469*
Concentrated (218)	80 (36.70)	138 (63.30)	0.86 (0.56–1.31)
Infection diseases			
Underlying (214)	90 (42.06)	12 (57.94)	0.079*
None (160)	53 (33.13)	107 (66.87)	1.47 (0.96–2.25)

*Chi-square test. OR: Odds ratio, CI: Confidence interval

(n=18), *C.tropicalis* (n=7) and *C. krusei* (n=6) accounting for 11.25%, 4.38%, and 3.75%, respectively.

There were extremely significant differences in the distribution of clinical isolates of *C. albicans* in terms of age and places of residence (Table 4). Accordingly, *C. albicans* was detected in 88 patients of reproductive age accounting for 81.48% versus 20 cases of menopause. On the other hand, patients living in city areas were apparently vulnerable to *C. albicans* infection representing 97.22%. On the contrary, *C. glabrata* infection was attributed to all considered factors with significant differences. However, there was no significant difference in the distribution of clinical isolates of *C. parapsilosis* in terms of age and contraceptive methods. Notably, significant differences in the distribution of clinical isolates of *C. krusei* were noted in all factors excluding contraceptive methods.

Antifungal susceptibility of *Candida* species

All antifungal agents exhibited zones of inhibition against five isolated *Candida* spp. at different levels (Fig. 2 and Table 5). There was a significant difference ($p<0.001$) in the antifungal zone diameters between *C. albicans* and *C.* non-*albicans*. *C. albicans* and *C. krusei* were found to be strongly resistant to itraconazole and fluconazole whereas *C. glabrata* and *C. tropicalis* were recorded to be insensitive to fluconazole only. Meanwhile, miconazole exerted moderate antifungal activity against the isolated *Candida* spp. with the inhibitory zones ranging from 17.17±3.66 to 26.67±3.61 mm in diameter while clotrimazole was highly effective against all clinically isolated *Candida* spp. (30.17±6.24–41.44±6.99 mm).

Significant differences in antifungal susceptibility profiles were observed between *C. albicans* and *C.* non-*albicans* for itraconazole, fluconazole, and miconazole ($p<0.05$) (Table 6). However, no significant differences in antifungal susceptibility were found between the two groups for clotrimazole ($p>0.05$). *C. albicans* was found to be extremely sensitive to clotrimazole (108/110 cases), followed by miconazole (41/110 cases) whereas resistance to fluconazole represented 96 cases on 110 *C. albicans*-infected patients. The same pattern was also noted in *C.* non-*albicans* against clotrimazole and miconazole with frequencies of 50 and 38 cases over 50 non-*albicans* cases, respectively.

As also indicated in Table 6, all four non-*albicans* spp. were found to be extremely sensitive to clotrimazole among four tested antifungal agents, reflecting the efficacy of this antifungal agent in the treatment of *C.* non-*albicans* spp. In contrast to clotrimazole, fluconazole was the least effective against *C.* non-*albicans* spp. as the resistance to fluconazole was noted ranging from 50% to 83.33%. Miconazole was recorded to be effective against *C. glabrata* and *C. parapsilosis* (but not against *C. krusei* and *C. tropicalis*) with 83.33% and 28.57% intermediate, respectively. Itraconazole, on the other hand, was documented to be resistant to all non-*albicans* spp., particularly *C. krusei* and *C. tropicalis* with a high percentage of 66.47 and 42.86%, respectively.

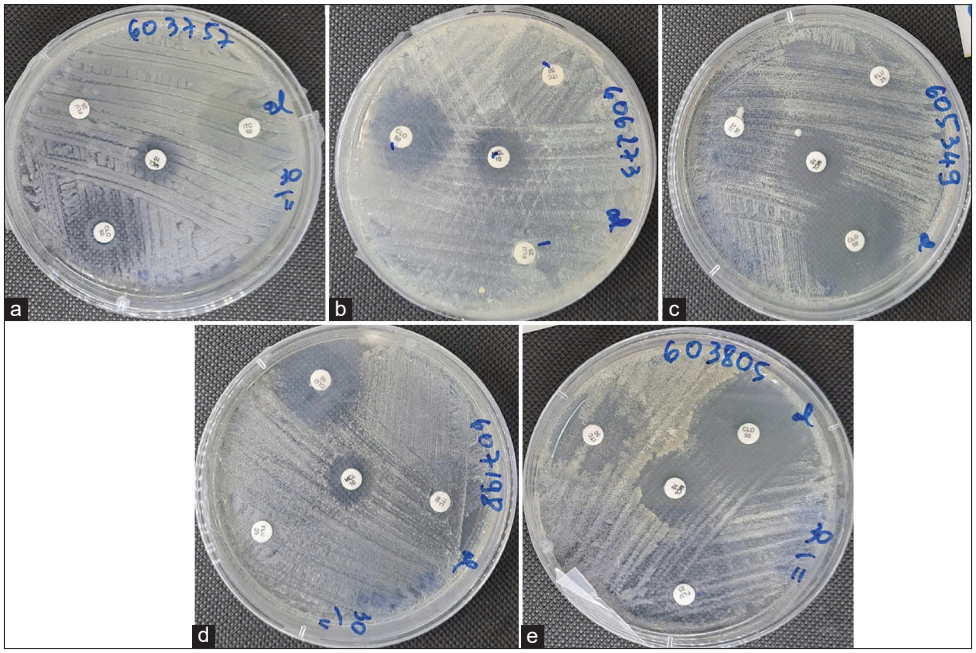

Fig. 2: Antifungal susceptibility of *Candida* species: (a) *Candida krusei*; (b) *Candida albicans*; (c) *Candida glabrata*; (d) *Candida tropicalis*; (e) *Candida parapsilosis*, against antifungal agents: (CLO)-Clotrimazole; (FLU)-Fluconazole; (ITC)-Itraconazole

Table 4: Distribution of clinically isolated *Candida* species

$N_{Candida}$=146	*Candida albicans* (n=108) (%)	*Candida* non-albicans				p-value**
Parameters		***Candida glabrata* (n=18) (%)**	***Candida parapsilosis* (n=12) (%)**	***Candida krusei* (n=6) (%)**	***Candida tropicalis* (n=2) (%)**	
Age						
Reproductive age	88 (81.48)	17 (94.45)	6 (50.00)	5 (83.33)	1 (50.00)	0.025
Menopause	20 (18.52)	1 (5.55)	6 (50.00)	1 (16.67)	1 (50.00)	
Place						
City areas	105 (97.22)	17 (94.45)	9 (75.00)	6 (100)	2 (100)	0.045
Rural areas	3 (2.78)	1 (5.55)	3 (25.00)	-	-	
Obstetric history						
Yes	68 (62.96)	15 (83.33)	10 (83.33)	5 (83.33)	2 (100)	0.238
No	40 (37.04)	3 (16.67)	2 (16.67)	1 (16.67)	-	
Contraceptive methods						
Yes	63 (58.33)	14 (77.78)	6 (50.00)	3 (50.00)	2 (100)	0.360
No	45 (41.67)	4 (22.22)	6 (50.00)	3 (50.00)	-	

**Fisher's exact test

"-": No cases recorded within the species indicate 0% prevalence

Table 5: Antifungal susceptibility of *Candida* species

***Candida* species**	**Zone of inhibition in diameters (mm)**			
	Itraconazole	**Fluconazole**	**Clotrimazole**	**Miconazole**
Candida albicans (n=110)	6 (6–24)	6	31.62±6.37	18.41±6.12
Non-*albicans*				
Candida parapsilosis (n=18)	32	17	41.44±6.99	26.67±3.61
Candida glabrata (n=19)	32	6	39.79±5.53	26.11±4.19
Candida krusei (n=6)	6 (6–27)	6 (6–10)	30.17±6.24	17.17±3.66
Candida tropicalis (n=7)	26	6	36.14±7.63	23.86±8.59
p-value	p<0.001*	p<0.001*	p<0.001**	p<0.001**

*Mann–Whitney U test; **Student's t-test

DISCUSSION

Between May and December 2023, 374 patients with different social-demographic characteristics (Table 1) presented to the Obstetrics Department at Tan Binh Hospital, and these 374 corresponding clinical samples were included in this study. After the specimens were collected and identified, there were generally no impacts on the prevalence rates of *Candida* infections with regard to other risk factors including menopause, genital hygiene, vaginal discharge, and other infectious diseases. While the use of douching and intravaginal medications was infrequent among these patients, this subgroup represents an important target for reproductive health education. Community-based interventions should prioritize educating women on proper hygiene

Table 6: Antifungal susceptibility patterns of various antifungal agents against isolated

Antifungal agents	Susceptibility	*Candida albicans* (n=110) (%)	*Candida non-albicans* (n=50)				p-value
			Candida glabrata (n=19) (%)	*Candida parapsilosis* (n=18) (%)	*Candida krusei* (n=6) (%)	*Candida tropicalis* (n=7) (%)	
Itraconazole	Sensitive	28 (46.67)	13 (68.42)	13 (72.22)	2 (33.33)	4 (57.14)	p<0.001*
	Intermediate	82 (82.00)	-	-	-	-	
	Resistant	-	6 (31.58)	5 (27.78)	4 (66.67)	3 (42.86)	
Fluconazole	Sensitive	14 (45.16)	6 (31.58)	9 (50.00)	-	2 (28.57)	0.001**
	Intermediate	-	-	-	1 (16.67)	-	
	Resistant	96 (75.00)	13 (68.42)	9 (50.00)	5 (83.33)	5 (71.43)	
Clotrimazole	Sensitive	108 (68.35)	19 (100)	18 (100)	6 (100)	7 (100)	1**
	Intermediate	1 (100)	-	-	-	-	
	Resistant	1 (100)	-	-	-	-	
Miconazole	Sensitive	41 (51.89)	16 (84.21)	16 (88.89)	1 (16.67)	5 (71.43)	p<0.001**
	Intermediate	64 (84.21)	3 (15.79)	2 (11.11)	5 (83.33)	2 (28.57)	
	Resistant	5 (100)	-	-	-	-	

"-": No cases recorded within the species indicate 0% prevalence

*Chi-square test

**Fisher's exact test

practices to reduce the incidence of genital infections, thereby aligning with the principle of preventative care.

Notably, the distribution of clinically isolated *Candida* species was consistent with previous studies in either Vietnam or other countries. A recent report by Nguyen Thu *et al*. indicated an even higher prevalence of 18% for *C. glabrata* [17]. Another research conducted by Alizadeh *et al*. illustrated *C. albicans* to be the most prevalent species (58.5%) using MALDI-TOF mass spectrometry [18]. An investigation carried out in Vietnam by Anh *et al*. reported a prevalence of 51.37% of *C. albicans* among reproductive-aged women [19]. Undoubtedly, *C. albicans* has been consistently confirmed as the predominant species isolated from vaginal specimens [17,20].

In view of the antifungal susceptibility assay, four antifungal agents used in this study fall into the azole class of five-membered heterocyclic compounds. However, clotrimazole and miconazole belong to the subclass containing two nitrogen atoms in the azole ring while fluconazole and itraconazole are in the sub-class featuring three nitrogen atoms in the azole ring. Generally, azole antifungals act by inhibiting the enzyme 14-alpha-demethylase, which converts lanosterol to ergosterol. Ergosterol, the main sterol in the fungal cell membrane, is thus disrupted leading to the fungal cell membrane damage resulting in cell death. Clotrimazole and miconazole are commonly administered as an intravaginal suppository. The standard regime for VVC involves a 200 mg dose for three consecutive days [21] or a single dose of 500 mg clotrimazole. Fluconazole is typically administered as a single oral dose of 150 mg for VVC, which is known as the common regime prescribed by Vietnamese clinicians [19,20]. Itraconazole, a newer-generation triazole antifungal agent, is prescribed for VVC, typically at an oral dose of 200 mg daily for three consecutive days [22].

Remarkably, the efficacy of older-generation antifungal agents is increasingly compromised by rising resistance. As an example, research by Wang *et al*. in 2016 indicated that *C. albicans* resistance rates to miconazole increased dramatically from 2.4% in 2006 to 59.8% in 2013. Another study reported an increasing prevalence of fluconazole-resistant *Candida* spp. with high rates observed in *C. glabrata* and *C. krusei* [23]. Recent studies have reported an increasing prevalence of broad-spectrum azole drug-resistant VVC [23,24]. In general, the mechanisms of azole drug resistance can be briefly described as follows: (1) drug target alteration; (2) formation of *Candida* biofilms; (3) alteration in ergosterol biosynthetic enzyme; (4) up-regulation of drug transporters; and (5) activation of membrane-related efflux pumps [25-29].

In this study, however, clotrimazole and miconazole were effective against all tested *Candida* isolates as the low prevalence rate of resistance was recorded. Indeed, none of the isolated *C.* non-*albicans* exhibited resistance to clotrimazole and miconazole while the tested *C. albicans* exerted low resistance to clotrimazole and miconazole with a rate of 0.91% and 4.50%, respectively. In contrast to clotrimazole and miconazole, all tested *Candida* isolates were documented with a moderate to extremely high prevalence rate of resistance to fluconazole and itraconazole. Of the 110 *C. albicans* isolates, 82 were resistant to itraconazole and 96 to fluconazole accounting for 74.5 and 87.28%, respectively. *Non-albicans* isolates, on the other hand, showed different rates of resistance against fluconazole and itraconazole. *C. krusei* was recorded to be the highest resistance to itraconazole whereas the lower resistance came to *C. parapsilosis*. The same pattern was also observed in fluconazole, claiming the highest rate of 83,33% in *C. krusei* and 50% in *C. parapsilosis* as the lowest.

In addition to various factors partially responsible for the resistance of *Candida* isolates against antifungal drugs as mentioned above, the virulence of *Candida* spp. might be likely attributed to other factors such as the change in vaginal microbiota and the alteration of vaginal pH owing to physiological and biochemical factors [30]. It is noteworthy to know that appropriate nutrition plays a crucial role in maintaining vaginal health and defending the host against pathogenic microbial infections. On the other hand, abuse of using vaginal cleansing products might have adverse effects on vaginal health, owing to the disruption in the vaginal microbiota. The vaginal microbiome (also called vaginal microbiota or vaginal flora) refers to the micro-ecosystem of various microorganisms (such as bacteria, fungi, and parasites) that naturally colonize the vagina. The vaginal microbiome plays a vital role in maintaining a woman's reproductive health, significantly assisting in fertility and overall reproductive success. Furthermore, the vaginal microbiome can enhance the quality of life by defending against pathogenic microbial invasion causing urinary tract infections. Previous studies indicated that vaginal cleansing products might be likely to disrupt the vaginal microbiome by removing vaginal mucous and causing turbulences of bacteria living in the vaginal canal, leading to changes in vaginal pH and an imbalance in beneficial and harmful bacteria in the vaginal cavity.

As a consequence, this event particularly declines the total number of *Lactobacillus* species (commonly known as probiotics) which is the most predominant species colonizing the vagina. There has been accumulating evidence to insist that *Lactobacillus* species are beneficial to the host in various ways by (1) producing lactic acid to keep a low pH value (3.8–4.5); (2) producing hydrogen peroxide (H_2O_2) and bacteriocins (natural antibiotics); (3) preventing harmful bacteria from sticking to vaginal walls; and (4) helping reduce inflammation and enhance immune systems. Environmental factors affecting the virulence of *Candida* spp. thus should be taken into consideration,

thereby significantly contributing to the elucidation of the host-pathogen interactions paying the way for establishing an effective measure in preventing *Candida* infections.

CONCLUSION

This study underscores the significant prevalence of *C. albicans* as the primary etiological agent of VVC, while also highlighting *C. glabrata* and *C. parapsilosis* as the most frequently encountered non-*albicans* species. Notably, *C. albicans* demonstrated high susceptibility to clotrimazole but marked resistance to fluconazole. *C. krusei* exhibited the highest resistance levels to both itraconazole and fluconazole, contrasting with *C. parapsilosis*, which displayed the lowest resistance rates to these agents. These findings affirm the clinical utility of routine species identification and antifungal susceptibility testing in the effective management of VVC, empowering clinicians to prescribe targeted therapies aimed at reducing patient morbidity and mortality. Furthermore, the emergence of antifungal drug-resistant *Candida* species emphasizes the critical need for ongoing antifungal surveillance, assessment, and synthesis of new antifungal agents [31].

Nevertheless, the findings obtained from this study highlight the complexity and diversity of fungal infections in women. Therefore, in-depth research on this topic could potentially be supported and conducted in the near future with a larger patient cohort and more advanced methodologies such as polymerase chain reaction, MALDI-TOF, or sequencing for definitive species confirmation. Such comprehensive investigations are essential to extensively explore the molecular mechanisms underlying *Candida* species resistance to specific antifungal drugs, thereby providing significantly deeper insights into the pathogenicity of *Candida* species and considerably contributing to the establishment of robust clinical protocols for the effective treatment of invasive pathogenic fungal infections.

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CONFLICT OF INTEREST

The authors confirmed to have no conflict of interest.

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