



## Species Detection of Vulvovaginal Candidiasis in Adult Females and their Antifungal Susceptibility Pattern

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**Abstract:** *Background:* Vulvovaginal candidiasis is a major public health problem worldwide and its incidence and prevalence has increased in recent years. Materials and *Methods:* This cross-sectional type of descriptive study was conducted on 100 females over a period of one year from July 2021 to June 2022 in Rajshahi Medical College hospital. Before commencement of the study, ethical clearance was obtained from the Ethical review committee of Rajshahi. Data were collected by a semi structured questionnaire and data analysis were done by SPSS software, version-24. *Results:* The most common isolates were *C. albicans* 27 (27.00%) followed by *C. tropicalis* 8 (8.00%) and *C. cruise* 4 (4.00%). Compounding was the most sensitive and fluconazole was the most resistant antifungal drugs. Non-*albicans Candida* species showed more antifungal resistance than *C. albicans*. *Conclusion:* Both *Candida albicans* from other *Candida* species showed a wide range of susceptibility towards different antifungal agents and fluconazole was found to be fewer sensitive drugs.

**Keywords:** *Candida albicans*, Non-*albicans Candida* and Vulvovaginal candidiasis.

### Original Researcher Article

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### Article at a glance:

**Study Purpose:** The purpose of this study was to isolate the candidal species from vaginal swab and determine their antifungal susceptibility patterns.

**Key findings:** The majority of Vulvovaginal candidiasis cases were caused by *C. albicans* species.

**Newer findings:** Compounding was the most sensitive and fluconazole was the most resistant antifungal drugs.

**Abbreviations:** DNA: Deoxyribonucleic acid and PCR: Polymerase chain reaction.



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## INTRODUCTION

*Candida* is the shortened name used to describe a class of fungi that includes more than 150 species of yeast. In healthy individuals, *Candida* exists harmlessly in mucous membranes such as ears, eyes, gastrointestinal tract, mouth, nose, reproductive organs, sinuses, skin, stool and vagina, etc.<sup>1</sup> It is known as “beneficial flora” and has a useful purpose in the body. When an imbalance in the normal flora occurs, it causes an overgrowth of *Candida albicans* and it is called

Candidiasis. The predisposing factors to candidiasis increase with host-related factors such as malnutrition, immunosuppression, diseases related to the endocrine system such as diabetes mellitus, cancer, autoimmune diseases, changes in the amount of saliva, changes in the epithelial cell layer, habit of eating carbohydrate-rich foods, age, poor oral hygiene and the use of steroids, chemotherapeutic drugs, prosthesis and cigarette smoking.<sup>2</sup> The most common agent of candidiasis is *Candida albicans*. However, *C. tropicalis*, *C. cruise*, *C.*

*Para psilosis*, *C. glabrata* and *C. sublimesness* have also been reported. Vulvovaginal candidiasis is an infection in the vagina and vulva when conditions change in this organ that leads to encourage its growth. Hormonal change, use of some medicines or suppression of the immune system can make vulvovaginal candidiasis frequently.<sup>3</sup> Vulvovaginal candidiasis most commonly occurs in reproductive-aged women and responsible for about one-third of cases of total vulvovaginitis.<sup>4</sup> Recurrent vulvovaginal candidiasis is also common. If *Candida* present as a normal flora in vagina, women are often asymptomatic. But vulvovaginal candidiasis requires both the presence of *candida* in the vagina and vulva as well as presence of the symptoms of irritation, dysuria, itching etc.

The most common responsible pathogen for vulvovaginal candidiasis is *C. albicans* responsible in 90% of cases followed by non-*albicans candida* such as *Candida glabrata*, *C. tropicalis* and *C. krusei*.<sup>5</sup> Because of its widespread availability of over-the-counter treatments, many patients with vulvovaginal candidiasis do not present for care. Although vulvovaginal candidiasis is more common in sexually active women but there is no evidence that the infection is sexually transmitted.<sup>5</sup> Accurate diagnosis is essential for proper treatment and to avoid the development of resistance. The diagnosis of vaginal candidiasis is based on recognition of the lesions on clinical examination which is confirmed by the microscopic identification of *candida* in the samples and/or isolation in culture in the study. Currently used methods for the isolation and identification of *Candida* species are different conventional culture media specially chromogenic culture media, germ tube test, manual and automated methods of assimilation of carbohydrates and nitrogen (auxanogram), chromogenic culture media and molecular techniques.<sup>6</sup> Culture in Sabourou Dextrose Agar (SCA) media takes 24-48 hours to grow. The germ tube test is the gold standard of laboratories for the identification of *C. albicans*<sup>7</sup> because it is cheap, quick and simple. Chromogenic media are frequently used in direct and rapid identification of yeasts because different *Candida* species produce unique colors on these media. Detection and

identification of fungal DNA by PCR is one of the most powerful and popular tools for detection and identification of *Candida* species.<sup>7</sup>

Antifungal resistance is now an increasing problem all over the world. *Candida* infections may resist antifungal drugs, making them difficult to treat.<sup>8</sup> About 7% of all *Candida* blood samples tested at Centers for Disease Control (CDC) are resistant to the antifungal drug Fluconazole. Although resistance is most common in other species particularly *Candida auris*, *Candida glabrata* and *Candida Para psilosis* than *Candida albicans* which is the most common cause vulvovaginal candidiasis.<sup>9</sup> Now the primary treatment option is amphotericin B, a drug that can be toxic for patients who are already very sick. Antifungal susceptibility testing has become an important tool for physicians faced with making difficult treatment decisions with vulvovaginal candidiasis.<sup>10,11</sup> The aim of this study was to isolate and identify *Candida albicans* and non *albicans Candida* species by different method from Volvo-vaginal infections in adult females attending in Rajshahi Medical College

## METHODS

A cross-sectional descriptive study was performed over a period of 1 year from July 2021 to June 2022 on 100 females with clinically suspected vulvovaginal candidiasis attending in outpatient departments of Rajshahi Medical College Hospital (RMCH). Microbiological works were done in the laboratory of the department of Microbiology, Rajshahi Medical College, Rajshahi. Vaginal swab from adult females were collected as specimens after taking informed written consent. Data were recorded in a partially structured pre-tested questionnaire. Isolation of *Candida* species is done by direct microscopy with wet mount, gram stain, LPCB stain preparation and culture in SDA media incorporate with chloramphenicol and gentamicin. Identification of different *Candida* species were done by germ tube test, subculture in chromogenic agar media and carbohydrate fermentation test. Antifungal drug susceptibility test of *Candida* species was done by disk diffusion method according to CLSI M44-A2 document.

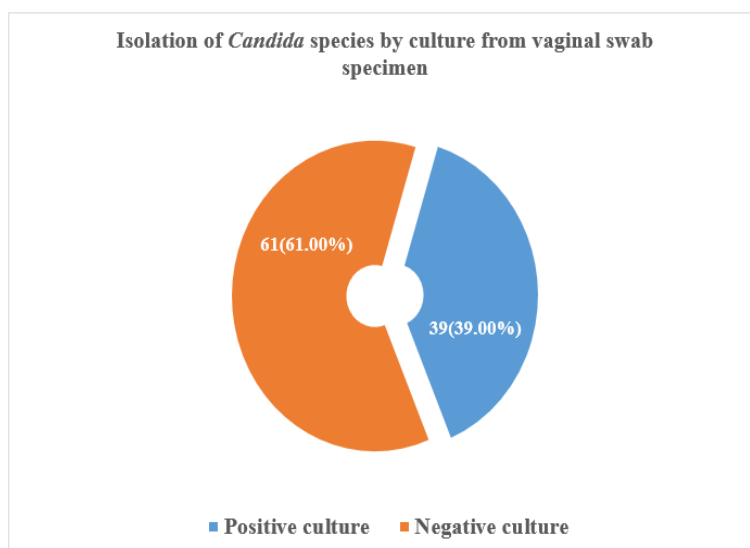
## RESULTS

**Table 1: Age distribution of the patients (n=100)**

Age group of the respondents in years	Frequency	Percentage
< 29	11	11.00%
30-39	35	35.00%
40-49	20	20.00%
50-59	16	16.00%
60-69	10	10.00%
≥ 70	8	8.00%
<b>Total</b>	<b>61</b>	<b>100.00%</b>

Out of 100 patients, 35 (35.00%) were within the age group of 30-39 years, 20 (20.00%) were 40-49 years, 16 (16.00%) were 50-59 years, 11

(11.00%) were < 29 years, 10 (10.00%) were 60-69 years and only 8 (8.00%) respondents were ≥ 70 years of age (Table-01).



**Figure 1: Isolation of *Candida* species by culture from vaginal swab specimen (n=100)**

Out of 100 vaginal swab specimen, 39 (39.00%) were culture positive and 61(61.00%) were culture negative (Figure-1).

**Table 2: Detection of *Candida albicans* from other *Candida* species by Germ tube test and chromogenic agar (n=39)**

Species	Germ tube test		Chromogenic agar media	Percentage
	Positive	Negative		
<i>Candida albicans</i>	27	0	27	27.00
Non- <i>albicans candida</i>	0	12	12	12.00

(N.B.- Culture positive specimens were considered here)

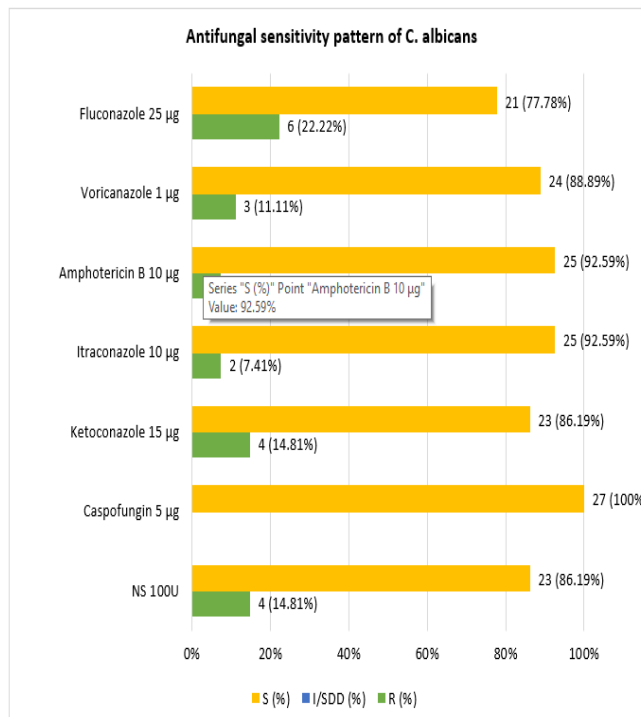
Germ tube test was positive in 27 isolates of *C. albicans* that was identified by chromogenic agar media. Chromogenic agar media also identified 12

species as non-*albicans candida* that were negative in germ tube test (Table-02).

**Table 3: Species distribution of culture positive vaginal candidiasis (n=39)**

Species distribution	Frequency	Percentage
<i>C. albicans</i>	27	27.00%
<i>C. tropicalis</i>	8	8.00%
<i>C. cruise</i>	4	4.00%
<b>Total</b>	<b>39</b>	<b>100.00%</b>

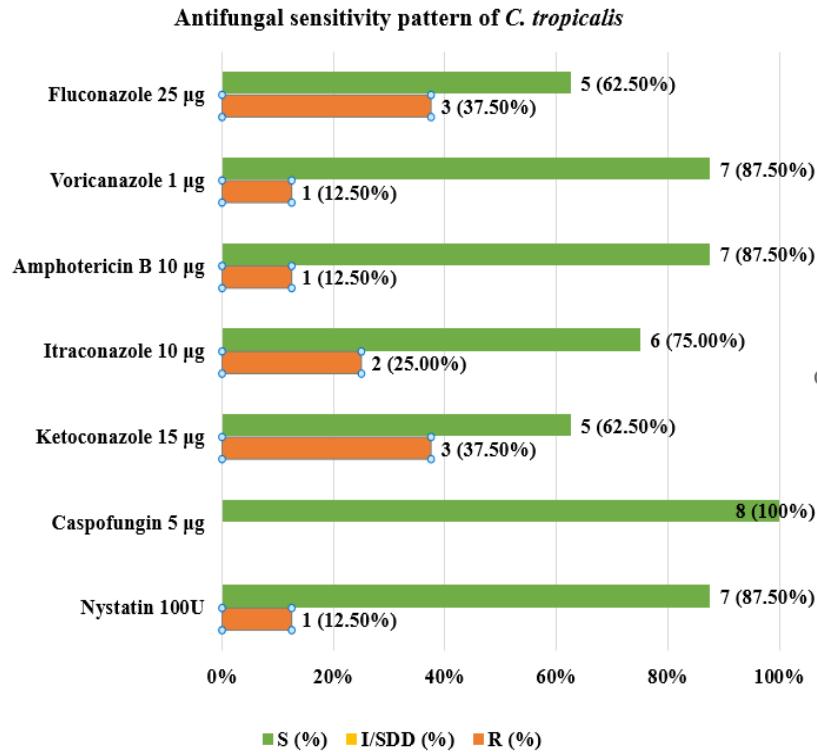
Out of 39 culture positive vaginal candidiasis 27 (27.00%) were *C. albicans*, 8 (8.00%) *C. tropicalis* and 4 (4.00%) *C. cruise* (Table-03).



**Note:** S-Sensitive, I-Intermediate, SDD-Susceptible dose dependent, R-Resistant,  
**Figure 2: Antifungal sensitivity pattern of *C. albicans* (n=27)**

Antifungal sensitivity pattern of *C. albicans* showed that compounding, Amphotericin B and Itraconazole were sensitive in 27 (100%), 25 (92.59%) and 25 (92.59%) of the isolates, respectively. Voriconazole was sensitive in 24 (88.89%) of the isolates. Both ketoconazole and

nystatin were sensitive in 23 (86.19%) of the isolates. Fluconazole was sensitive in 21 (77.78%) and resistant in 6 (22.22%) of the isolates. None of the antifungal drug was I/SDD to the isolates (Figure-2).

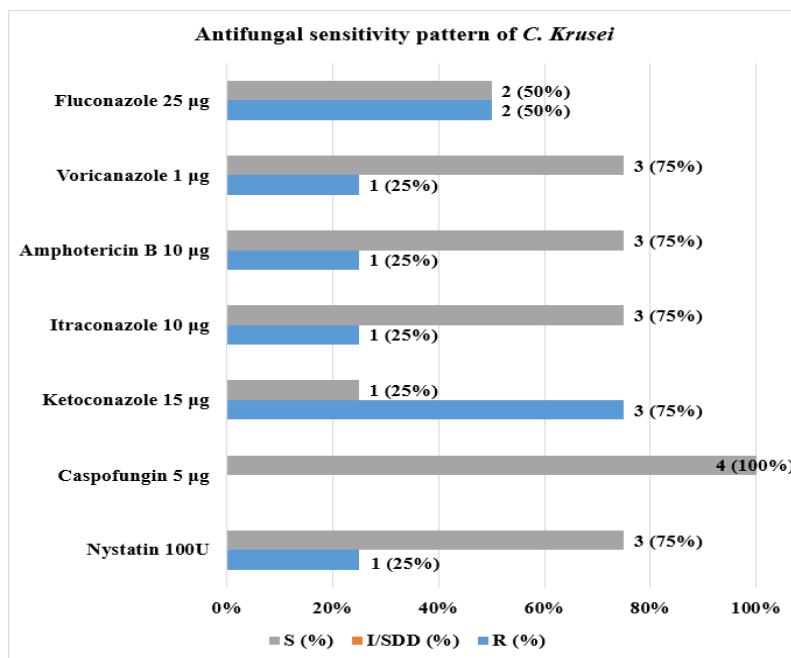


**Note:** S-Sensitive, I-Intermediate, SDD-Susceptible dose dependent, R-Resistant.

**Figure 3: Antifungal sensitivity pattern of *C. tropicalis* (n=8)**

Antifungal sensitivity pattern of *C. tropicalis* revealed that compounding was sensitive in 8 (100%) of the isolates. Nystatin, Voriconazole and Amphotericin B, all were sensitive in 7 (87.50%) of the isolates. Itraconazole was sensitive in 6

(75.00%) and resistant in 2 (25.00%) of the isolates. Both Ketoconazole and Fluconazole were sensitive in 5 (62.50%) and resistant in 3 (37.50%) of the isolates. None of the antifungal drug was I/SDD to the isolates (Figure-3).



**Note:** S-Sensitive, I-Intermediate, SDD-Susceptible dose dependent, R-Resistant,

**Figure 4: Antifungal sensitivity pattern of *C. Krusei* (n=4)**

Antifungal sensitivity pattern of *C. cruse* revealed that only compounding was sensitive in 4 (100%) of the isolates. Nystatin, Voriconazole, Itraconazole and Amphotericin B, all were sensitive in 3 (75%) and resistant in 1 (25%) of the isolates.

## DISCUSSION AND CONCLUSION

About 70% of reproductive age women are affected with candidiasis which is one of the most common vaginal infections. *Candida albicans* is the main of cause of vulvovaginal candidiasis, however, episodes due to non-*albicans* species of *candida* are also increasing now a day and treatment option for both groups are challenging due to recent increasing antifungal resistance. Out of 100 patients, 35 (35.00%) were within the age group of 30-39 years, 20 (20.00%) were 40-49 years, 16 (16.00%) were 50-59 years, 11 (11.00%) were < 29 years, 10 (10.00%) were 60-69 years and only 8 (8.00%) respondents were ≥ 70 years of age. Nearly similar findings were found in a study done by R *et al.*<sup>12</sup> in India where (26-35) age group women carried the highest frequency of vaginal candidiasis.<sup>12</sup> Findings were not similar in a study done by Sehgal *et al.*<sup>13</sup> where vaginal candidiasis occurs most frequently in the age group of 20- 25 years.<sup>13</sup>

The women of childbearing age are more frequently vulnerable to vulvovaginal candidiasis. Low levels of protective cervical antibodies increased sexual activity and new influence of reproductive hormones are the main cause of high incidence in this age group. In the current study, out of 39 culture positive vaginal candidiasis 27 (27.00%) were *C. albicans*, 8 (8.00%) *C. tropicalis* and 4 (4.00%) *C. cruse*. Similar findings were found with the studies done by Mohanty *et al.*<sup>14</sup>, Visagie *et al.*<sup>15</sup>, R *et al.*<sup>12</sup>, Bitew *et al.*<sup>16</sup> and Lima *et al.*<sup>17</sup> In this study, 7 antifungal drugs named Fluconazole, Voriconazole, Nystatin, compounding, Itraconazole, Ketoconazole and Amphotericin B were tested by disc diffusion method against 39 isolates of *Candida*. compounding, Amphotericin B and Itraconazole were sensitive in 27 (100%), 25 (92.59%) and 25 (92.59%) of the isolates of *C. albicans*, respectively. Voriconazole was sensitive in 24 (88.89%) of the isolates. Both ketoconazole and nystatin were sensitive in 23 (86.19%) of the isolates.

Fluconazole was sensitive in 2(50%) and resistant in 2(50%) of the isolates. Ketoconazole which was sensitive only in 1 (25%) and resistant in 3 (75%) of the isolates. None of the antifungal drug was I/SDD to the isolates (Figure-4).

Fluconazole was sensitive in 21 (77.78%) and resistant in 6 (22.22%) of the isolates.

Data revealed that compounding was sensitive in 8 (100%) of the isolates *Candida tropicalis*. Nystatin, Voriconazole and Amphotericin B, all were sensitive in 7 (87.50%) of the isolates. Itraconazole was sensitive in 6 (75.00%) and resistant in 2 (25.00%) of the isolates. Both Ketoconazole and Fluconazole were sensitive in 5 (62.50%) and resistant in 3 (37.50%) of the isolates. 100% isolates of *C. cruse* were sensitive against compounding. Nystatin, Voriconazole, Itraconazole and Amphotericin B, all were sensitive in 3 (75%) and resistant in 1 (25%) of the isolates. Fluconazole was sensitive in 2(50%) and resistant in 2(50%) of the isolates. Ketoconazole which was sensitive only in 1 (25%) and resistant in 3 (75%) of the isolates.

None of the antifungal drug was I/SDD to the isolates. Test results revealed compounding was the most sensitive antifungal drug followed by Nystatin, Amphotericin B, Voriconazole, Itraconazole, Ketoconazole. Fluconazole was found to be least sensitive antifungal drug in this study *et al.*<sup>12</sup> reported that 91.6% for *C albicans* was sensitive to Voriconazole, 71.42 % to *C. tropicalis*, 50% to *Cruse*. Sensitivity to Fluconazole was seen in 91.6 % of *C. albicans* & 42.85 % of *C. tropicalis*. 75 % for *C. albicans* was sensitivity to Amphotericin B, 100 % to *C. tropicalis*, 100% for *C. cruse* & *C. glabrata*. Sensitivity to Nystatin was 91.6% for *C. albicans* & 100% for *C. tropicalis*, *C. cruse* & *C. glabrata*. Lima *et al.*<sup>17</sup> showed that all isolates were susceptible to Amphotericin band Voriconazole which findings were nearly similar with our study.

An increase in the predisposing conditions in recent years has resulted in an increasing incidence of *Candida* infections. Identification of *Candida* to the species level is important to provide information for a given area of study. The choice of antifungal is also dependent on the species of *Candida*. Therefore, the species level identification

of the *Candida* isolates can greatly influence the treatment options for the clinicians and therefore crucial for facilitating hospital control measures. Vulvovaginal candidiasis is now a growing medical concern and antifungal susceptibility is a time demanding issue. In this study, *Candida albicans* is the most common isolate followed by *C. tropicalis*, *C. cruise* and among the seven antifungal drugs used in this study, compounding is the most sensitive (100%) for all *Candida* species. So, to establish an effective treatment protocol and to reduce the alarming increase of antifungal resistance, it is advised to ensure regular culture practice and antifungal susceptibility testing in case of vulvovaginal candidiasis.

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### Declarations

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**Conflict of interest:** Authors declared no conflict of interest.

**Ethical approval:** Ethical approval of the study was obtained from the Ethical Review Committee, Rajshahi Medical College, Rajshahi. Informed consent was taken from all participants. All the study methodology was carried out following the relevant ethical guidelines and regulations.

**Consent for publication:** Taken.

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