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# Prevalence and resistance pattern of candida isolated from vulvovaginal candidiasis of antenatal women

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**Abstract**
Vulvovaginal candidiasis is caused by the overgrowth of *Candida* species. About 75% of all women, experience at least one episode of candidiasis in their lifetime. *Candida albicans* is the most common infectious agent followed by Non-*albicans Candida* species are emerging pathogens and relatively higher resistance to antifungal resistance. Thus the aim of this study was to determine the prevalence of *Candida species* in antenatal women and their susceptibility patterns of antifungal drugs. The study was conducted in the department of O&G and Microbiology, SRM MCH & RC, Kattankulathur, Chennai, India. A total of 39 *C. albicans* and 66 NAC species were isolated. *C. albicans* had shown the highest resistance against ketoconazole 10% and NAC had shown the highest resistance against clotrimazole 6%. There was a clear shift in the prevalence of infection by *Candida albicans* to those by NAC and emerging as a potential threat for causing cause infection and posing a therapeutic challenge.

**Keywords:** Candida, Vulvovaginal candidiasis, Resistant, Clotrimazole, Ketoconazole

## 1. Introduction
Vulvovaginal candidiasis (VVC) is a fungal infection caused by the overgrowth of *Candida* species affecting the genital tract as an opportunistic pathogen. VVC is a common type of vaginitis, a gynaecologic disorder with a white discharge, soreness, dyspareunia, irritation and itching [1]. VVC is a common complaint among women of different age groups, regardless of their sexual activities and can be a possible risk for other diseases e.g. HIV/AIDS [2-5]. This infection progresses as colonization, superficial infection and hematogenous dissemination to different organs [3, 6, 7]. Reports show that about 75% of all women, experience at least one episode of which physician approved to be candidiasis in their lifetime [5, 8, 9]. VVC is a frequent companion of pregnancy, which greatly complicates the course of the pregnancy and threatens the health of both mother and child [10]. The incidence of VVC is almost doubled (particularly in the second and third trimester) among pregnant women, due to high production or changes in the levels of sex hormones and deposition of glycogen in the vagina during pregnancy [11]. Among *Candida* species, *Candida albicans* is the most common infectious agent. Non-*albicans Candida* (NAC) species are emerging pathogens and can also colonize on mucocutaneous surfaces [12]. *Candida tropicalis* and *C. glabrata* are the most important of the NAC infections [13, 14]. Although some NAC species like *Candida parapsilosis*, *Candida lipolytica*, *Candida lusitaniae*, *Candida kefyr* and *C. krusei* also can cause VVC [10, 15, 16]. Relatively higher antifungal resistance rate of NAC *species* may contribute to higher rates of recurrent infections. This increase in NAC vulvovaginitis has been attributed to overuse of antifungal therapy, which has resulted in the elimination of the more sensitive *C. albicans* [17, 18]. Secretory acid proteinase, phospholipase, esterase production has been associated with yeast pathogenicity [19]. These enzymes are implicated in the persistence and colonization of the vaginal tract [20-22]. They also facilitate tissue penetration and cleavage of immunoglobulin A (IgA), which is an important factor in vaginal immunity [23]. The lack of specificity of symptoms and signs, therefore, precludes a diagnosis that is based on history and physical examination without the corroborative evidence of laboratory tests (CDC, 2010) [24]. Thus, over 80% of antenatal women, referred by physicians with a putative diagnosis of VVC were found to have some other cause of vaginitis, and therefore most patients fail to respond to antifungal therapy cause of incorrect diagnosis [25]. Consequently, rapid and specific identification of *Candida* species will help to choose the suitable antifungal and improve patient care. This immune imbalance is caused by a number of factors, such as excess stress, allergies, indiscriminate use of antibiotics, steroids,

birth control pills and hormonal drugs and nutrient deficiency [10, 26]. Diabetes mellitus, pregnancy, and the use of tight nylon underwear also enhance the overgrowth of *Candida* in a manner that cannot easily be controlled by the body's defence mechanisms [10, 27-31]. The dynamic environment inside the host may include factors such as temperature, pH, and the normal flora and its metabolic products. These factors, along with the oxidative stresses associated with host defence mechanisms, require physiologic adaptation in the pathogenesis of *C. albicans*. Temperature enhances the virulence of several bacteria and influences fungal morphogenesis [32]. Increased temperature, pH, and steroids have also been shown to induce the yeast-to-hyphal transformation of *C. albicans* [33]. Thus the aim of this study was to determine the prevalence of *Candida species* in antenatal women and their susceptibility patterns of antifungal drugs.

## 2. Material and Methods

A total of 308 pregnant women attending the antenatal clinic of SRM MCH & RC, Kattankulathur, Chennai, India, were screened for *Candida* in a routine examination. The samples were collected for 12 months, starting from January 2018 to December 2018. The age of the women was between 18-45 Years.

### 2.1. Sample Collection and processing

A total of 308 samples of high vaginal swab were collected from asymptomatic and symptomatic pregnant women between the ages of 18-45 years attending antenatal clinic. All women who gave a history of taking anti-fungal agents during the earlier 2 weeks prior to the study were excluded from the study. Consent was obtained from each participant and also a questionnaire was completed with demographic data and possible risk factors such as age, occupation, diabetes, history of vaginitis, abortion, antifungal use and smoking. Collection of the vaginal swab was done by exposing the posterior fornix with a sterile vaginal speculum. All genital swabs collected in Amies transport medium received from women during the study period were included. Two vaginal swabs were collected from each patient and transported to the microbiology laboratory at SRM MCH & RC Hospital for processing. Each sample was examined for colour, appearance and odour and described as whitish or whitish-grey colour, cottage cheese-like discharge and odour or odourless. One sample was used for microscopic examination by wet preparation and gram stain while the other one was used for culture in Sabouraud's dextrose agar (SDA) and incubated at 37 °C for 18–24 hours to see the growth of creamy, greyish moist colonies.

### 2.2. Species identification

Identification of *Candida* species was done by Gram stain, KOH/Wet mount and germ tube test to differentiate the *C. albicans* from NAC (Figure 1 - 5) [34], chrome agar for speciation of *Candida* based on colour production (Figure 6), Dalmau plate culture to demonstrate the chlamydospore formation (Figure 7) [35], sugar fermentation tests were done to speciate *Candida* (Figure 8) [36].

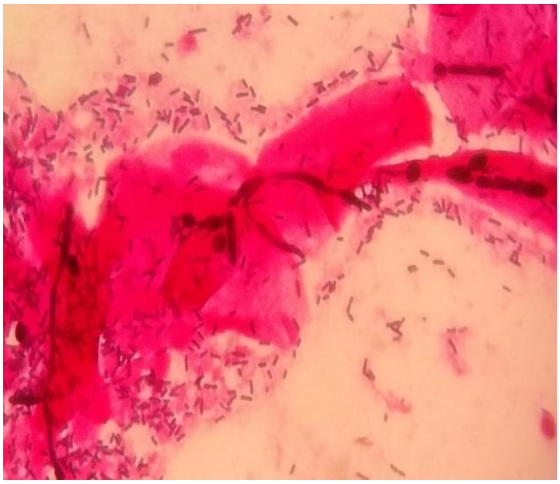

**Fig 1:** Direct microscopy of Gram-stained showing Gram-positive budding yeast cell with pseudohyphae, Gram-positive bacilli and epithelial cell (100X)

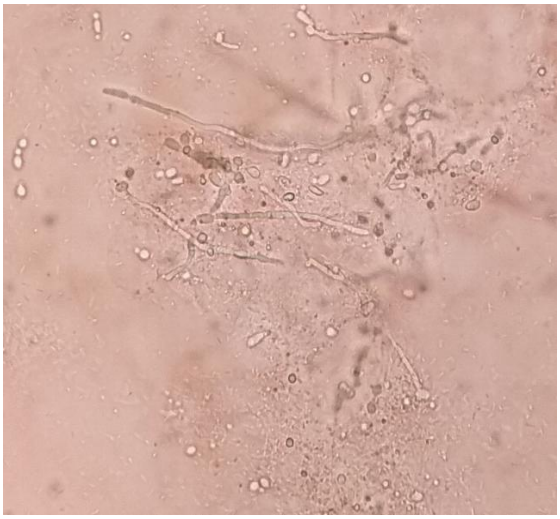

**Fig 2:** KOH /Wet mount showing budding yeast cell with pseudohyphae (40X)

**Fig 3:** On SDA *Candida* has grown as smooth, creamy white pasty colonies.

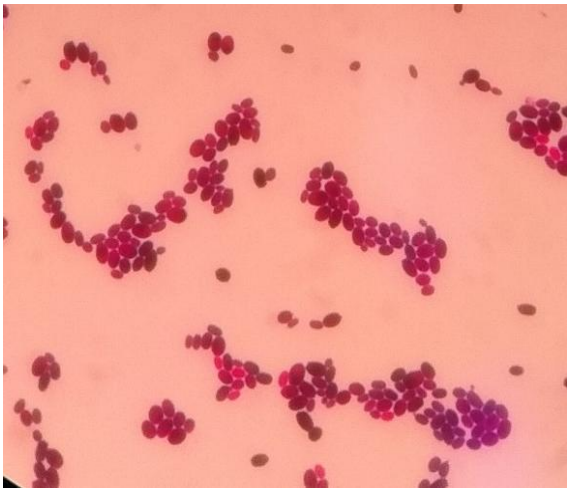

**Fig 4:** Gram-stained from a culture showing Gram-positive budding yeast cell (100X)

**Fig 6:** On chrome agar, *C. albicans* produced light green and *C. krusei* produced a pink colony

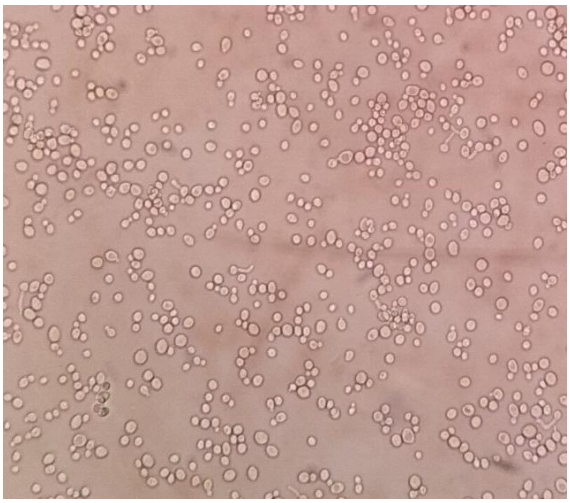

**Fig 5:** Germ tube formation by *Candida albicans* (40X)

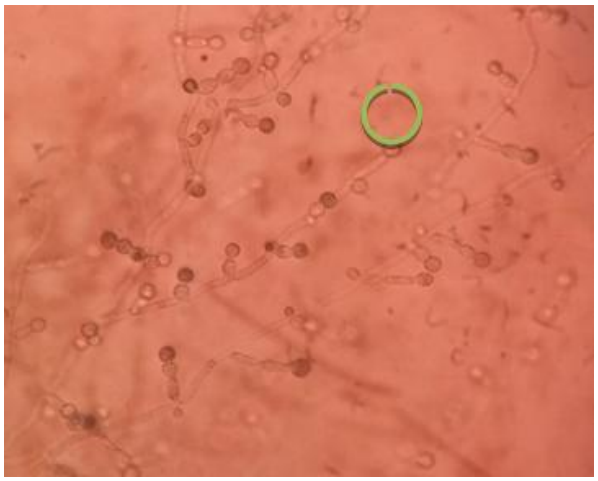

**Fig 7:** Chlamydospore formation by *C. albicans* on cornmeal agar (10X)

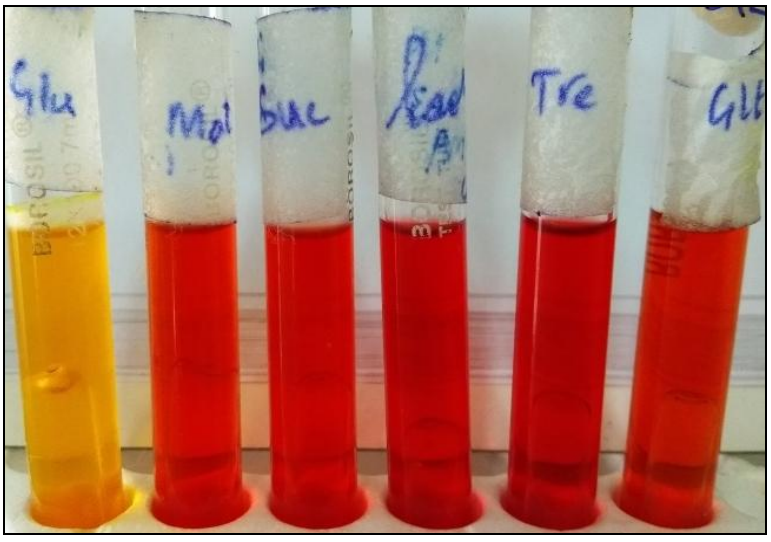


**Fig 8:** Sugar fermentation test of *Candida* species

### 2.3. Susceptibility testing

The isolates of *Candida* (308) were also tested for their *in vitro* susceptibility towards miconazole, clotrimazole and ketoconazole in accordance with the proposed guidelines for antifungal disk diffusion susceptibility testing of yeasts contained in the CLSI document M44-A [37]. As per the "CLSI" (M44-A) procedures "Mueller Hinton Glucose agar" with 2% glucose and 0.5 µg/ml of methylene blue were used for susceptibility testing. To standardize the inoculums 0.5 McFarland standard were used. *C. albicans* ATCC90028 and *C. Parapsilosis* ATCC22019 were used as a control. Following antifungal disks were used: fluconazole (10 mcg), ketoconazole

(30 mcg) and miconazole (30 mcg). All the antifungal disks, control strains and culture media were parched from Hi-Media, Mumbai, India. The plates were incubated at 35 °C, and inhibition zone diameters were measured after 24 and 48 hours. The zones of inhibitions were as resistant (R), susceptible dose-dependent (SDD) and sensitive (S) as per the CLSI guidelines and referred from published paper (Table 1) [37- 40].

**Table 1:** Interpretive breakpoint of different antifungal agents

| Drugs with concentration (mcg) | Susceptible (mm) | Susceptible dose-dependent (mm) | Resistant (mm) |
|---|---|---|---|
| Fluconazole (10 mcg) | ≥19 | 15-18 | ≤14 |
| Ketoconazole (30 mcg) | ≥28 | 21-27 | ≤20 |
| Miconazole (30 mcg) | ≥20 | 12-19 | ≤11 |

Mcg=micrograms, mm=millimetre

## 3. Statistical analysis

Microsoft (MS) Excel 2010 software was used to store the data and analysed by using R version 3.5.2 software. Z test has been used to find the difference in proportions and a 5% level of significance has been used.

## 4. Results

A total of 308 antenatal women were screened for VVC and 105(34%) *Candida* species were isolated. Out of 105 *Candida* species 39(37.2%) were *C. albicans* and 66(62.8%) were NAC, among NAC 28(26.6%) *C. glabrata*, 20(19%) *C. tropicalis*, 11(10.4%) *C. parapsilosis*, 07(6.6%) *C. krusei* as showed in figure 9.

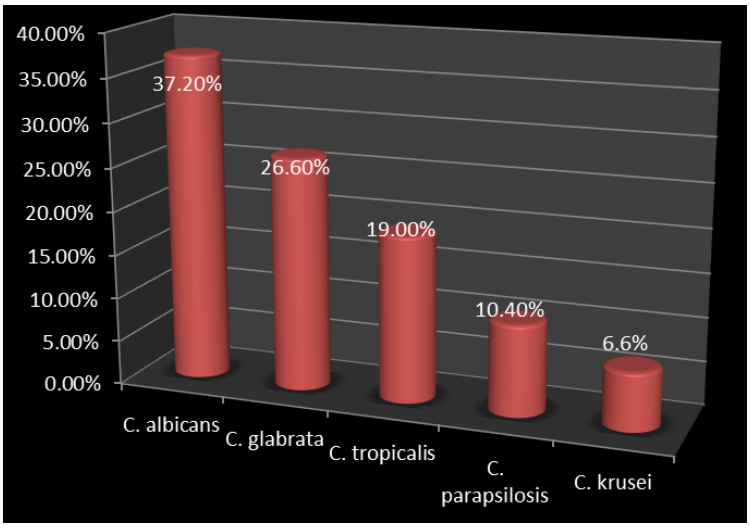


**Fig 9:** Distribution of *Candida* species isolated (n=105).

The highest prevalence of VVC was seen in between young age groups 22-25 followed by 26-28 age group as the data showed in table 2.

**Table 2:** Age group distribution in years (n=308)

| Age group in years | Total, n=308 | Positive for VVC, n=105(%) | P-value |
|---|---|---|---|
| 18-21 | 51 | 16(31.3) | 0.001* |
| 22-25 | 104 | 42(40.3) | 0.000* |
| 26-28 | 91 | 31(34) | 0.000* |
| 29-31 | 34 | 09(26.4) | 0.06 |
| 32-35 | 17 | 05(29.4) | 0.106 |
| 36-39 | 11 | 02(18.1) | 0.773 |

* Statistically significant ($p$<0.05)

The highest prevalence of VVC was observed in housewife followed by the teacher. The demography data of antenatal women are presented in table 3.

**Table 3:** Demographic characteristics of antenatal women (n=308)

| Occupations | Number of antenatal women screened(n=308) | Positive for VVC, n=105(%) | P-value |
|---|---|---|---|
| Housewife | 237 | 80(33.7%) | 0.000* |
| Employee | 54 | 18(33.3%) | 0.000* |
| Nurse | 14 | 06(42.8%) | 0.004* |
| Doctor | 03 | 01(33.3%) | 0.38 |

* Statistically significant ($p$<0.05)

The highest prevalence of VVC was observed in 2nd trimester of pregnancy 52.3% (55/105) followed by 1st trimester of pregnancy 29.5% (31/105) and 3rd trimester of pregnancy 18% (19/105) are presented in figure 10.

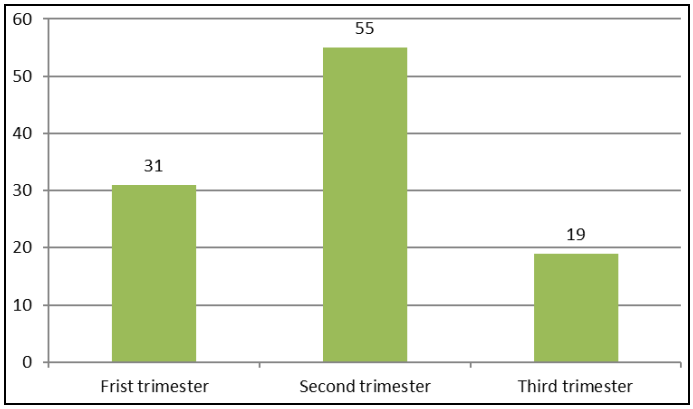


**Fig 10:** Distribution of VVC based on gestational age (n=105)

**Table 4:** Number of ANC visit (n=105)

| Number of visits | Total of 308 Antenatal women screened | Number VVC positive, n=105(%) | P-value |
|---|---|---|---|
| 1st time | 101 | 24(23.7) | 0.001* |
| 2nd time | 125 | 49(39.2) | 0.000* |
| 3rd time | 49 | 17(34.6) | 0.001* |
| 4th time | 33 | 15(45.4) | 0.001* |

* Statistically significant (p<0.05)

Out of 138 primigravida women, 41(29.7%) were positive for VVC and 64(37.6%) were positive from 170 multigravida women (Table 5).

**Table 5:** Types of Gravida

| Types of gravida | Total, n=308 | Positive VVC, n=105(%) | P-value |
|---|---|---|---|
| Primi gravida | 138 | 41(29.7) | 0.001* |
| Multi-gravida | 170 | 64(37.6) | 0.001* |

* Statistically significant ($p<0.05$)

A total of 105 *Candida* species were tested for clotrimazole, ketoconazole and miconazole. Out of 39, *C. albicans* isolates 78%, 90% and 71% had shown susceptible to clotrimazole, ketoconazole and miconazole respectively. *C. albicans* had shown the highest resistance against ketoconazole 10% followed by miconazole 4%. Out of 66 NAC, 91%, 93% and 95% had shown susceptible to clotrimazole, ketoconazole and miconazole respectively. NAC had shown the highest resistance against clotrimazole 10% followed by ketoconazole 4%. For SDD data showed in Table 6.

**Table 6:** Profiles of antifungal susceptibility test by disk diffusion method

| *Candida* species (n=105) | Clotrimazole | | | Ketoconazole | | | Miconazole | | |
|---|---|---|---|---|---|---|---|---|---|
| *Candida* species | S | SDD | R | S | SDD | R | S | SDD | R |
| *C. albicans*(n=39) | 78% | 21% | 1% | 90% | - | 10% | 71% | 25% | 4% |
| NAC(n=66) | 91% | 3% | 6% | 93% | 2% | 5% | 95% | 3% | 2% |

Resistant (R), Susceptible Dose-Dependent (SDD) and Sensitive (S)

## 5. Discussion

VVC is one of the real reasons that lead ladies to search out an obstetrician or gynaecologist. In spite of therapeutic advances, VVC remains a typical issue around the world, influencing all stratus of society [41]. VVC is an important cause of morbidity in pregnant women. In pregnant women, vaginal candidiasis has been related to emotional stress and suppression of the immune system which steps up the risk of *Candida* species overgrowth and its pathogenicity [42]. It can cause abortion, chorioamnionitis and preterm delivery [43]. Transmission of *Candida* infections can occur from the vagina of the infected mother to the new-born, giving rise to congenital *Candida* infection [44]. Therefore, early detection, early diagnosis with adequate pharmacotherapy and avoidance of predisposing factors would resolve VVC in short period of time.

In the present study, the prevalence of VVC is 34%. The prevalence reported in this study is higher than the 26% reported in Ibadan [45], 30.7% reported in Jamaica [46], and the rate of 30% reported in Nnewi, a town in Nigeria [47]. This prevalence rate is almost double the rate reported in Maroua, Cameroon 55.4% [48]. In the age group of 22-25 years, the highest incidence of vaginal candidiasis was found followed by the age group of 26-28. Ako *et al*., in 1993 confirmed the most common occurrence of vaginal candidiasis in the age group between 20-25 years [49]. Sehgal *et al*., in 1990 reports also revealed the highest prevalence of vaginal candidiasis in the age group 21-30 years [50]. In the present study, there was a statistically significant *(p<0.05)* prevalence in the age group 18-28 years. The age group includes younger women who have minimal vaginal defence mechanisms against *Candida* species and who are sexually active [51]. They often have the habit of constantly using the oral contraceptives to avoid getting pregnant; therefore women in the reproductive age group are much more prone to candidiasis in the vagina. Results of this study revealed that 37.6% of women were multigravida. The findings of this study are consistent with other studies which showed increase prevalence of VVC in multigravida than primigravida. Longer sexual history and use of birth control pills are enforced in multigravida as vital risk factors responsible for increased incidence of VVC [52]. In the current study, 52.3% prevalence was detected in the 2nd trimester followed by 29.5% in 1st trimester and 18% in the 3rd trimester. Deepa *et al*., in 2014 reported the prevalence of VVC as 54% in the second trimester, 30% in the third trimester and 16% in the first trimester [53]. Compared to this study, although the prevalence orders are different, the second-trimester prevalence rates are the same. Likewise, Oyewole, *et al*., in 2013 also found the highest rates of vaginal candidiasis among pregnant women in their second trimester (61%) [54].

In the present study A total of 308 antenatal women were screened for VVC and 105 (34%) *Candida* species were isolated. Out of 105 *Candida* species 39 (37.2%) were *C. albicans* and 66(62.8%) were NAC, among NAC 28(26.6%) *C. glabrata*, 20(19%) *C. tropicalis*, 11(10.4%) *C. parapsilosis*, 07(6.6%) *C. krusei.* An earlier study was done by El-Sayed *et al*. [55] has reported a higher prevalence rate of *C. albicans* in VVC as 86% and the prevalence rates of 59% were reported by Al-Hedaithy *et al.* [56]. In this study, there was an increasing rate of NAC species. The overall rates of 62.8% was reported in this study for NAC species which is higher than 34.6% reported by Donbraye-Emmanuel, *et al*; among pregnant women in Ibadan, Nigeria and 42% reported by Nwadioha, *et al*; among pregnant women at Aminu Kano Teaching Hospital, Kano, Nigeria [57, 58].

In this study, overall resistance was 1% with clotrimazole

followed by 4% for miconazole and 10% for ketoconazole by *Candida albicans* whereas resistance was 2% for miconazole followed by 5% for ketoconazole and 6% for clotrimazole seen in Non *albicans Candida*. Resistance to miconazole, ketoconazole and clotrimazole is of great concern as these are the first line azoles used for the treatment of vulvovaginal candidiasis. In this study, clotrimazole had shown good sensitivity to *C. albicans* and NAC which is quite high in the study reported by *Ajitha et al.* [59]. Another study conducted by *Sachin et al*., [60] reported 50% clotrimazole resistance to *C. parapsilosis* and 20% by *C. albicans*, whereas in our finding 6% clotrimazole resistance was seen in NAC. The results of the present study revealed 90% were ketoconazole sensitive to *C. albicans* and 93% were ketoconazole resistant against NAC, which was highest, while a study was done by *Sachin et al.*, reported 25% of *Candida* species was resistant to ketoconazole [60]. However, in the present study, as compared to *C. albicans* the majority of NAC had shown a high level of resistance towards clotrimazole (Table 6). A huge alteration in the epidemiologic patterns of *Candida* and furthermore the development of resistance among already susceptible species because of increased uses of over-the-counter antifungal agents. The prior study reported that there is a higher MIC value by most NAC species; consequently, it is very difficult to treat. An enormous report discovered that among NAC of vaginal isolates, *C. glabrata* is emerging as more resistant to azoles as compared to isolates from bloodstream infection [61, 62].

## 6. Conclusion

The study offers information about *Candida* species distribution and antifungal susceptibility activity of *Candida* isolated from antenatal women with VVC. In this study, there was a clear shift in the prevalence of infection by *Candida albicans* to those by NAC. NAC species are emerging as a potential threat for causing infection and posing a therapeutic challenge. Early empirical antifungal therapy and further research to improve diagnostic, preventive and therapeutic strategies are necessary to reduce the considerable mortality and morbidity in antenatal women. Appropriate selection of drugs for the treatment of *Candida* infections and antifungal susceptibility testing must be performed regularly. The study was directed on a low number of isolates and in a tertiary care hospital which is the limitation of the study.

## 7. Acknowledgement

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## 8. Author's Contribution Statement

Dr Maitrayee Sen designed the study, guided in conducting this research study, samples collected from antenatal women, drafted the manuscript and also reviewed the manuscript. Mr Kanishka Hrishi Das carried out the research study, evaluated the results and also drafted the manuscript.

## 9. Conflict of Interest

Conflict of interest declared none.

## 10. Funding

Authors did not receive any funding to conduct this research.

## 11. Ethics

The study was conducted after getting approval from the Institutional Ethical Committee (IEC, 1090/ IEC/2017).

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