

Speciation and susceptibility testing of *Candida* isolates from vaginal discharge

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RESEARCH

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ABSTRACT

Background

Vaginal discharge is the frequently narrated complaint in the female sexually transmitted infection (STI) clinic. Vulvovaginal candidiasis (VVC) is the second most common reason for vaginal discharge. Treatment and prevention of VVC among the female population can reduce HIV acquisition and pregnancy related complications, in addition to improvement in their quality of life.

Aims

This study aims to detect the percentage of VVC among patients with vaginal discharge, as well as speciate and perform antifungal susceptibility tests for the *Candida* isolates.

Methods

Two high vaginal swabs were collected from 130 female outpatients with complaints of vaginal discharge. Potassium hydroxide (KOH) mount, Gram's stain and culture in Sabouraud's dextrose agar were performed. Antifungal susceptibility tests were done by the disc diffusion method, Hicomb strips and broth microdilution method.

Results

Isolation of candida was 39.23 per cent in our study. *Candida albicans* (45.83 per cent) was the commonest species followed by *Candida glabrata* (22.91 per cent). Amphotericin B was 100 per cent effective for all *Candida* isolates.

Conclusion

Though *C. albicans* was the commonest species, more than 50 per cent of isolates were non-albicans *Candida*. Therefore identification of species is important for appropriate anti-fungal therapy. Speciation also helps to prevent treatment failure and dissemination of drug resistance.

Key Words

Antifungal susceptibility test, candida, vaginal discharge

What this study adds:

1. What is known about this subject?

Vaginal discharge is a common complaint among the female population that imparts physical and emotional stress and even impairs their quality of life.

2. What new information is offered in this study?

Half of *Candida* isolates from vaginal discharge were non-albicans *Candida*, for which treatment has to be appropriate since intrinsic resistant to fluconazole is very common.

3. What are the implications for research, policy, or practice?

Vaginal candidiasis is usually diagnosed clinically and treated. It is necessary to culture, speciate and perform susceptibility testing to avoid treatment failure and recurrences.

Background

Candida is a normal commensal that takes the role of a pathogen under compromised conditions. Increased longevity of human life and immunocompromised conditions together paves the way for the increase in

opportunistic infections like candidiasis. Vulvovaginal candidiasis (VVC) is the second most common cause of vaginal discharge next to Chlamydia.¹ VVC is the leading cause of abnormal vaginal discharge due to microbial causes. Untreated VVC is a possible risk factor for acquisition of HIV.² Prevention of sexually transmitted infections (STIs) including VVC can reduce the transmission of HIV since STIs and HIV have the common mode of transmission and one enhances the risk of infection with the other. Early diagnosis and treatment of candidiasis can reduce the pregnancy related morbidity.³ Emergence of non-albicans species of *Candida* and widespread use of antifungals has increased the chances of treatment failure and development of drug resistance. This study has been carried out to study the commonness of VVC, of various species of *Candida* and to perform anti-fungal susceptibility test for the *Candida* isolates.

Method

This study has been conducted in the department of microbiology in association with the department of sexually transmitted diseases from February 2012 to January 2013.

Inclusion criteria: Sexually active female outpatients who presented to the STD clinic with complaints of vaginal discharge and pruritus were included in the study.

Exclusion criteria: Extremes of age, menstruation, history of antimicrobials intake within two weeks prior to the visit were excluded from the study. Ethical and research clearance were obtained from the Institutional Ethical and Research Committee. Informed consent was obtained from the patient before participation. Statistical analysis was done using simple percentage calculation.

Patient was asked to lie in the dorsal position and two high vaginal swabs were collected from each patient. During speculum examination, nature, colour, consistency and odour of the discharge were observed. One swab was used for Gram's stain, while another was used for culture using Sabouraud's dextrose agar (SDA). Discharge from the speculum was used for wet mount and potassium hydroxide (KOH) mount examination. Gram's stain showing gram positive budding yeast cells with or without pseudohyphae, KOH mount showing yeast cells with or without pseudohyphae and Sabouraud's dextrose agar that showed pasty, cream coloured circular colonies were considered diagnostic of VVC. Species identification and antifungal susceptibility tests were performed as per standard protocols. Gram's stained smears were also graded by the Nugent score for diagnosis of bacterial vaginosis. Wet

mount examination was done to look for motile trophozoites of *Trichomonas vaginalis*.

Speciation: *Candida* isolates were first subjected to a germ tube test to differentiate *Candida albicans* and non-albicans *Candida*. Germ tube positive *Candida* isolates were *C. albicans* and germ tube negative isolates were non-albicans *Candida*. They were inoculated into Hichrom *Candida* agar and corn meal agar to identify the species based on colour and difference in growth pattern along with chlamydospore formation (Table 1).⁴ Species confirmation was done by a carbohydrate assimilation test using Himedia carbohydrate discs (Table 2),⁵ (Figure 1).

Antifungal susceptibility testing: All the *Candida* isolates were tested for susceptibility to fluconazole, itraconazole and amphotericin B using three methods. Susceptibility was first performed by the disc diffusion method using Mueller-Hinton agar with 2 per cent glucose and 0.5µg/ml methylene blue as per Central Laboratory Standards Institute (CLSI) guidelines (M44- A2) (Figure 2). For azoles, zones were measured up to colonies of normal size and for amphotericin B, clear zone with no visible growth was measured. Minimum inhibitory concentration (MIC) values were determined by Hicomb strips. For amphotericin B, reading was taken at complete inhibition of all growth. For azoles, reading was taken at the first point of significant inhibition (so-called 80 per cent inhibition). Broth microdilution was done using RPMI-1640 medium (with glutamine without bicarbonate) supplemented with 0.2 per cent glucose and pH of 7.0 with 0.165mol/L MOPS (3-[N-morpholino] propane sulfonic acid) according to CLSI guidelines (M 27 -A3). MIC for amphotericin B is the lowest concentration with a score of 0 (optically clear). MICs for the azoles are the lowest concentrations with a score of 2 (prominent decrease in turbidity).

Results

One hundred and thirty female outpatients participated in our study. They were categorized into decades based on their age which showed 31-40 years was the commonest age group in our study. Analyses of educational status of the patients showed that one third of them were illiterates. High vaginal swabs collected from them were subjected for KOH mount, Gram's stain and culture for detecting the presence of VVC. Among the three methods, culture was the most sensitive, which detected 51 cases of candidiasis (Table 3). Speciation was performed using a germ tube test, corn meal, Hichrom agar and assimilation tests which showed that *C. albicans* (22) was the commonest species among the *Candida* isolates (Table 4). There was no

discrepancy found between the three methods used for susceptibility testing of candida isolates. Antifungal susceptibility tests showed that none of the Candida species showed resistance to amphotericin B (Table 5). Itraconazole and fluconazole were associated with 16.66 per cent and 12.50 per cent resistance respectively (Table 6).

Discussion

Candida is a part of the lower genital tract flora in about 20-50 per cent of asymptomatic individuals.⁶ Chronic or persistent vaginal candidiasis can lead to physical and mental stress impairing the quality of life. This study has been carried out to detect the percentage of VVC among patients with a vaginal discharge, various species of candida and their susceptibility pattern.

Our study subjects were in the age of 15-45 years. About 56.92 per cent of individuals were 31-40 years which is different from the study done by Nwadioha *et al* which showed 21-30 years as the commonest age group in their study accounting for 39 per cent followed by 31-40 years accounting for about 27 per cent of individuals.² Educational status has a direct impact on knowledge regarding general care, menstrual hygiene and adoption of contraceptive measures. Analysis of literacy of the subjects showed that 34.61 per cent of females were illiterates, 30 per cent had middle school level of education and 23.84 per cent had primary school education. About 3.07 per cent of females in our study were diabetic and 3.84 per cent of females were taking oral contraceptive pills. Nearly half of females (42.30 per cent) in our study were using fabrics during the menstrual cycle. These factors could have predisposed to VVC in those subjects. Among 130 subjects, 102 had foul smelling, homogenous vaginal discharge, 26 of them had curdy white discharge and two had green coloured, frothy discharge. Among the 102 clinically suspected cases of bacterial vaginosis, the Nugent score could confirm only 19 (14.61 per cent) as bacterial vaginosis. In addition to the 26 clinical considered VVC patients, 25 other samples also grew candida isolates (39.23 per cent). Trichomoniasis was suspected in 2 cases but wet mount identified 7 cases of trichomoniasis (0.54 per cent). Multiple infections were seen in 20.7 per cent of subjects.

Isolation of candida in our study was 39.23 per cent which was lower compared to the study done by Fathima *et al* which showed 63 per cent occurrence.⁷ According to our study, culture (39.23 per cent) identified more positives than Gram's stain (31.53 per cent) and KOH (27.69 per cent) (Table 3). This finding is in concordance with the study by Babic *et al* where culture detected 25.4 per cent of positives

which is more than microscopy positivity (23.8 per cent).⁸ Most common Candida species in our study was *C. albicans* (45.83 per cent) followed by *C. glabrata* (22.91 per cent) (Table 4). This is supported by Roppa *C et al* where the commonest species was *C. albicans* (50.7 per cent), but the second most common was *C. tropicalis* (28.6 per cent).⁹ This finding has been contradicted by the study done by Lakshmi *N et al* which showed that *C. parapsilosis* (33.3 per cent) was the predominant isolate followed by *C. albicans* (22.2 per cent).¹⁰ Overall occurrence of non-albicans Candida was 64.2 per cent in Lakshmi *et al* study. In our study, non-albicans Candida accounted for about 54.16 per cent. *C. dubliniensis* (1.6 per cent) is of low occurrence in the study performed by Babin *et al*.¹¹ In our study *C. dubliniensis* was not isolated at all. This could be due to the reason that methods used in our study (germ tube test, corn meal agar and carbohydrate assimilation test) cannot differentiate *C. albicans* and *C. dubliniensis* and based on Hichrom Candida agar, difference between light green and dark green is not of much use since subjective differences in appreciating the variation cannot be ruled out. Other than rapid identification, Hichrom Candida agar is useful in isolation of different species of Candida from mixed yeast infection.¹² Corn meal agar helps in identifying the isolate when colour interpretation in Hichrom agar is doubtful. Carbohydrate assimilation test is useful in confirming the species identification.

Results of the disc diffusion method correlated well with MIC determination by Hicomb strips and broth microdilution method. Disc diffusion can be used for assessing antifungal susceptibility in resource limited settings. If resistance is encountered, it should be crosschecked with MIC before reporting. All the Candida isolates were sensitive to amphotericin B. Overall resistance to itraconazole was seen in 16.66 per cent. More than half of *C. parapsilosis* and *C. krusei* isolates showed resistance to itraconazole and only one isolate of *C. albicans* showed resistance. Percentage of fluconazole resistance seen in our study was 12.50. Among the fluconazole resistant species, *C. krusei* was the commonest, followed by *C. parapsilosis* and *C. glabrata* (Table 5 and 6). In a study done by Araj *et al*, amphotericin B showed uniform susceptibility to all candida isolates irrespective of species¹³ which was the same finding seen in our study. Among the three drugs tested for susceptibility, itraconazole showed maximum resistance. Fluconazole showed 100 per cent susceptibility to *C. albicans* and *C. glabrata* was the commonest among non-albicans Candida to show resistance to fluconazole. According to the study done by Jithendra *et al*, all the fungal isolates demonstrated 100 per cent sensitivity to

voriconazole and 18 per cent of *C. albicans* showed resistance to fluconazole.¹⁴ But in our study, no resistance was detected for *C. albicans* against fluconazole, though non-albicans *Candida* were resistant to it. Initial treatment was given to the patients based on clinical assessment. They were advised to come for follow up after three days. If other infections were identified in laboratory tests, which were not treated during their first visit, they were treated during the second visit. Laboratory results of those who had persistence of symptoms were assessed for mixed infections or antifungal resistance and managed according to the laboratory reports.

Limitation of the study is that all the *Candida* isolates were speciated considering it a pathogen since they were from symptomatic females. No specific tests were done to differentiate it from commensal and colonizer. For detection of *Neisseria gonorrhoea* and *Chlamydia trachomatis*, endocervical sample has to be collected. They were not included in the study due to difficulties in the processing of samples for *Chlamydia trachomatis*.

Conclusion

Germ tube test is useful to identify albicans and non-albicans *Candida* in a resource poor setting. If non-albicans *Candida* is identified, it should be speciated to provide appropriate antifungal therapy since few non-albicans *Candida* are inherently resistant to fluconazole.

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PEER REVIEW

Not commissioned. Externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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ETHICS COMMITTEE APPROVAL

Ethical and research clearance were obtained from the Institutional Ethical and Research Committee, Stanley Medical College

Table 1: Candida speciation by Hichrom and corn meal agar

Species	Colour on Hi chrom	Morphology in corn meal
<i>C. albicans</i>	Light green	Terminal and intercalary chlamydo spores
<i>C. glabarata</i>	Pink to purple	No pseudohyphae, only blastoconidia
<i>C. tropicalis</i>	Blue with pink halo	Branching pseudohyphae and blastoconidia
<i>C. parapsilosis</i>	Cream to pale pink	Curved pseudohyphae and blastoconidia
<i>C. krusei</i>	Pink	Pseudohyphae and blastoconidia resembles crossed match stick
<i>C. dublinensis</i>	Dark green	Terminal and intercalary chlamydo spores

Table 2: Speciation of *Candida* by carbohydrate assimilation

Species	Xyl	Lac	Suc	Mal	Mel	Cel	Tre
<i>C. albicans</i>	+	+	+	-	+	+	+
<i>C. tropicalis</i>	+	-	+	+	-	+	+
<i>C. glabarata</i>	+	-	-	-	-	-	+
<i>C. parapsilosis</i>	+	-	+	+	-	-	+
<i>C. krusei</i>	+	-	-	-	-	-	-
<i>C. dublinensis</i>	+	+	+	-	+	+	+

Xyl-xylose, lac-lactose, suc-sucrose, mal-maltose, mel-melibiose, cel-celibiose, tre-trehalose

Table 3: Positivity by KOH mount, Gram's stain and culture

Test	Number of positives	% of positives
KOH	36	27.69
Gram's stain	41	31.53
Culture	51	39.23

Table 4: Distribution of various species of *Candida*

Total <i>Candida</i> isolates (48)	Number	%
<i>C. albicans</i>	22	45.83
<i>C. glabarata</i>	11	22.91
<i>C. parapsilosis</i>	7	14.58
<i>C. tropicalis</i>	4	8.33
<i>C. krusei</i>	4	8.33

Table 5: Species wise antifungal susceptibility pattern

Species	Fluconazole Resistant		Itraconazole resistant		Amphotericin B Resistant	
	No	%	No	%	No	%
<i>C. albicans</i> (22)	0	0	1	4.54	0	0
<i>C. glabarata</i> (11)	1	9.09	0	0	0	0
<i>C. tropicalis</i> (4)	0	0	0	0	0	0
<i>C. parapsilosis</i> (7)	2	28.57	4	57.14	0	0
<i>C. krusei</i> (4)	3	75	3	75	0	0

Table 6: Overall antifungal susceptibility pattern of Candida isolates

Total isolates	Sensitive	Resistant
Candida (48)	Itraconazole (83.34%) Fluconazole (87.50%) Amphotericin B (100)	Itraconazole (16.66%) Fluconazole (12.50%) Amphotericin B (0%)

Figure 1: Carbohydrate assimilation test suggestive of *C. glabarata*

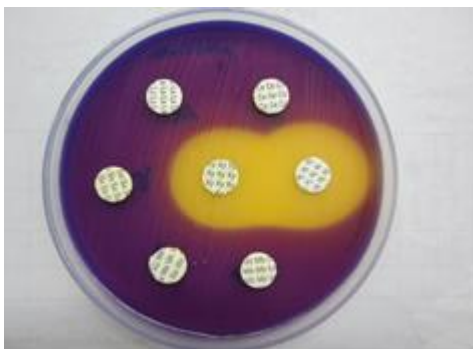


Figure 2: Antifungal susceptibility test by disc diffusion method

