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Original Research Article

STUDY ON ISOLATION, SPECIATION AND ANTIFUNGAL SUSCEPTIBILTY TESTING OF CANDIDA IN PATIENTS WITH VAGINAL DISCHARGE ATTENDING GYNAECOLOGY OPD IN A TERITIARY CARE HOSPITAL IN SOUTH INDIA

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ABSTRACT

Background: Vulvovaginal candidiasis (VVC) is the most commonly encountered gynaecological condition worldwide. C. albicans is the most commonly causative pathogen of VVC. However, non-albicans candida species like C. glabrata are also common which usually do not respond to the traditional drugs used for VVC. Hence drug susceptibility testing is important.
Materials and Methods: A prospective observational study was done in the Department of Microbiology which included 50 patients with complaints of vaginal discharge.
Results: 30% of the study sample had positive growth on SDA. C. glabrata was the most commonly isolated organism. Ketoconazole was the drug with highest resistance. Flucytosine, econazole had highest sensitivity.
Conclusion: Identification of appropriate antifungal agent in cases with uncomplicated or recurrent infections is impertinent.
Keywords: Candida, vulvovaginal candidiasis, drug susceptibility testing.

INTRODUCTION

Vulvovaginal candidiasis is responsible for one third of vagina related complaints globally.[1] Candidal species have been a part of the normal genital flora and are found in 20-30 % of healthy asymptomatic non-pregnant women. Approximately 70% of women experience atleast one episode of vulvovaginal candidiasis at some point in their lifetimes and 40-50% experience recurrence of episodes.[2]

Women of reproductive age group are more at risk, owing to host related factors like hyper-estrogenic state (pregnancy, hormone replacement therapy; obesity); diabetes mellitus; immunodeficiency states; antibiotic usage; treatment with glucocorticoids; genetic predispositions; behavioral factors such as use of birth control pills; intrauterine device; spermicides; condoms; hygiene habits like wearing tight-fit clothing and sexual behavior.[3] Although, vulvovaginal candidiasis is highly common in sexually active women, there is no evidence of it being transmitted sexually.[4]

The most common pathogen responsible for candida infection is C. albicans (85-95% of cases). However, other Candidal species, grouped under Non-albicans candida include C. glabrata, C. tropicalis, C. parapsilosis, C. krusei, C. dubliniensis, with C. glabrata as the predominant species.[5,6]

Candida species being commensal of genital flora, depending upon host factors, can penetrate the superficial mucosal lining and incite an inflammatory response. Patients usually present with vaginal discharge which is thick in consistency and adherent to vaginal walls. It is associated with vaginal itching, external dysuria, vaginal burning, dyspareunia, swelling and excoriations.[4]

Local examination of pelvis and vagina, vaginal pH testing, wet vaginal preparation and potassium hydroxide (KOH) mount are necessary for diagnosing vaginal candida infection and to rule out other etiologies of vaginitis. Presence of budding

yeast cells after KOH application is confirmatory for candida infection.[4]

Uncomplicated VVC can be treated using a variety of short-course topical treatments which include polyenes, imidazoles, or ciclopirox olamine.[7,8] In contrast, complicated VVC requires a more prolonged course of therapy and is often difficult to achieve successful results.[9] NAC species most commonly cause complicated VVC than uncomplicated cases and these species are often resistant to traditional antifungal agents.[10] Resistance to traditional antifungal agents has posed a major challenge in public health problems, especially in cases where the patient has been treated with an azole group of antifungal agent in the past; the likelihood of microbiological resistance increases. Therefore, identification and antifungal susceptibility testing are impertinent for selecting appropriate antifungal agent.[9,10]

This study was done with an aim to identify the prevalence of NAC species and test for antifungal sensitivity in patients with vulvovaginal candidiasis.

MATERIAL AND METHODS

This prospective study was conducted over a period of 6 months (from February 2022 to July 2022) in the Department of Microbiology in Modern Government Maternity Hospital, Petlaburj, (affiliated to Osmania Medical College, Telangana). A total of 50 non pregnant women above 20 years of age who presented to Gynaecology OPD with complaints of vaginal discharge and those who gave consent were included in the study.

All patients were asked regarding the demographic details, any significant previous medical history, antibiotic usage, contraceptive use and sexual history. General examination was done. Local pelvic examination using speculum was done since all females were married. Vaginal secretions were smeared on slides. Wet mount, KOH mount and gram staining was done for the slides. Presence of budding yeast cells on microscopic examination, confirmed the presence of candidal infection.

The vaginal swabs were inoculated in Sabouraud's dextrose agar and incubated at 37 °C for 48 hours after which they were observed for any growth. Colonies appearing visually as yeast were confirmed by Gram staining and microscopic examination. Colonies from SDA suspected to be candida were confirmed by the Germ tube test. Short germ tube tests induced were induced in serum at 37o C to classify the isolates as albicans and non albicans.

CHROM agar is a method used to identify various species depending on the pigmentation. It is based on the principal of differential release of chromogenic breakdown products from various substrates following differential exoenzyme activity.[11] Using an inoculating needle, a single colony from a pure culture was seeded into CHROM agar media and incubated at 35 °C for 48 hours after which colour changes were noted.

Chlamydospore production on Corn Meal Agar (CMA) was used to determine the isolates ability to produce chlamydospore. Test strains were inoculated on CMA plates by slide culture technique. The 48-hour old yeast colony were streaked and stabbed into the media and covered with sterile cover slip and incubated at 25 °C for 72 hours. The arrangement of hyphae, pseudohyphae, blastospores, and chlamydospores were noted after incubation of 72 hours. Chlamydospore production was examined after staining with lactophenol cotton blue.[11]

All the isolates were subjected to antifungal susceptibility testing for fluconazole, econazole, nystatin, miconazole, amphotericin B, flucytosine and ketoconazole by disc diffusion method.

The basic investigations like blood sugar, renal function tests, urine routine and complete hemogram were done to look for precipitating factors. The cases were treated with antifungals as per sensitivity report and followed up after first and second week.

All the collected data was entered in Microsoft Excel. The baseline characteristics of participants were summarized using the mean, median or proportion as appropriate. Proportions were generated to demonstrate the distribution of Candida species. The susceptibility patterns were generated using proportions for each drug type per Candida species isolated.

RESULTS

In present study, 50 patients with vaginal discharge were included. The mean age of study sample is 36.08 years with the youngest being 21 years old and the oldest being 60 years old. 32 % (n= 16) of the patients were aged between 21- 30 years, 42% (n=21) were between 31-40 years, 24% (n=12) were aged between 41-50 years and one patient was aged 60 years (2%).

All 50 patients presented with complaints of white discharge and lower abdominal pain.

Swabs from vaginal secretions were taken and stained with potassium hydroxide (KOH). 40% of patients had epithelial cells on KOH staining (n=20); 30% had clue cells and 30% had budding yeast cells with pus cells.

On gram staining, 15 patients (30%) had gram positive budding yeast cells detected; 15 patients (30%) had gram negative bacilli with clue cells; and the remaining 20 patients (40%) had epithelial cells with gram positive bacilli. Positive cultures were predominantly found in age group of 31-40 (n=9); followed by 41-50 years (n = 3) and 21- 30 years (n=3).

On SDA, 15 patients (30%) had growth observed as white pasty colonies, suggestive of candida; while the rest 35 patients (70%) had no growth observed.

Growth from SDA was inoculated in germ tubes at 37o C, out the 15 patients, two patients had positive germ tube test (GTT) which is suggestive that the colonies belongs to C. albicans. The test was negative in the rest 13 patients, which is suggestive of NAC species.

The isolates from SDA were inoculated in corn meal agar at 27o C for 72 hours, 11 patients had blastospheres without pseudohyphae, one patient had clusters of blastoconidia with pseudohyphae, and two patients who had positive GTT had terminal chlamydospores.

Colonies from SDA were inoculated into CHROM agar and observed. 12 patients had cream to white colonies suggestive of C. glabrata, two patients had light green colonies which are suggestive of C. albicans and one patient had teal blue colour colonies which is suggestive of C. tropicalis.

Antifungal sensitivity

All colonies on SDA were subjected to antifungal sensitivity for fluconazole, econazole, nystatin, miconazole, amphotericin B, flucytosine and ketoconazole by disc diffusion methods.

33.3% of colonies were resistant and 67% were sensitive to Amphotericin B; 47% were resistant and 53% were sensitive to Nystatin; 6.7% were resistant and 93.3% sensitive to Flucytosine; all 100% were sensitive to Econazole; 73% were resistant and 27% were sensitive to Ketoconazole; all 100 % were sensitive to Miconazole; 40% were resistant and 60% were sensitive to Fluticonazole. [Table 1]

Table 1: Antifungal sensitivity

	Amphotericin B	Nystatin	Flucytosine	Econazole	Ketoconazole	Miconazole	Fluticonazole
No. of resistant isolates	5	7	1	0	11	0	6
No. of sensitive isolates	10	8	14	15	4	15	9

Table 2: Species wise antifungal sensitivity

		C. glabrata	C. albicans	C. tropicalis
Amphotericin B	Resistant	5	0	0
	Sensitive	7	2	1
Nystatin	Resistant	7	0	0
	Sensitive	5	2	1
Flucytosine	Resistant	1	0	0
	Sensitive	11	2	1
Econazole	Resistant	0	0	0
	Sensitive	12	2	1
Ketoconazole	Resistant	9	2	0
	Sensitive	3	0	1
Miconazole	Resistant	0	0	0
	Sensitive	12	2	1
Fluticonazole	Resistant	5	1	0
	Sensitive	7	1	1

C. tropicalis was sensitive to all antifungals.

DISCUSSION

Vulvovaginal candidiasis is a commonly encountered infection in up to 60 - 70% of females of reproductive age group. This prospective study was done in the Department of Microbiology, that included 50 patients presenting to Gynaecology OPD with complaints of vaginal discharge.

Majority of the study sample belonged to the age group of 31-40 years (42%). The mean age was 36.08 years. Most of the culture positive patients also belonged to this age group. This might be owing to the fact that majority of the study sample was from this age group. In the study done by Do Ngoc Anh et al,[12] the majority of study sample belonged to 31-40 years of age group. The mean age of this study was comparable to present study.

30% of the study sample had positive yeast cells on microscopic examination and when they were cultured on SDA, all had growth on suggestive of candida infection. Krishnapriya et al,[13] also had similar findings in their study. Presence of pus cells doesn't confirm fungal infection. This was supported by Sulaiman et al.[14]

Growth from SDA was inoculated in various media (CHROM agar, corn broth agar, germ tube test). The most predominantly isolated species was C. glabrata (positive in 12 out of 15 patients) followed by C. albicans and lastly C. tropicalis. Similar such species distribution was observed in studies done by Krishnapriya et al,[13] Goswami R et al,[15] Mohanty S et al,[16] and Ray D et al.[17] However many studies observed C. albicans as the predominant species. Presence of NAC in present study might probably be as a result of complicated and recurrent cases of VVC presenting to tertiary care hospital.

Antifungal testing

Antifungal sensitivity testing of Candida species identifies the susceptibility patterns of isolates so as to guide appropriate therapy. In present study, ketoconazole was the most commonly drug which

showed resistance, followed by Nystatin and fluconazole.

Miconazole and Econazole had shown the highest sensitivity (100%) in present study.

C. tropicalis was sensitive to all the tested drugs. Both C. albicans and C. glabrata had highest resistance against ketoconazole. C. albicans was highly sensitive to amphotericin B, Nystatin, Econazole, Flucytosine, and Miconazole.

C. glabrata had high sensitivity to Econazole, Miconazole and Flucytosine. Study done by Panchal et al,[18] also observe ketoconazole resistance to be predominant. Unlike present study, Krishnapriya et al13 had observed highest resistance to fluconazole in C. albicans.

CONCLUSION

Antifungal susceptibility testing guides in identifying clinical response, predicting treatment failure and developing local antibiograms to select appropriate antifungals. 12. With a diverse range of antifungals use, it has at present turned out to be indispensable to perform antifungal susceptibility testing and make information accessible to the physicians for effective therapy.

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