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Fungal aetiological agents in patients with vulvovaginitis - A hospital-based study in Puducherry

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Introduction

Vulvovaginalcandidasis is a common health problem of adult women mostly caused by candida species[1].In 20% of women it will be a normal vaginal flora & it gets multiplied in case of infection and hormonal changes leading to candidial infections[2].Vulvovaginal candidasis is considered to be the second common cause for genital infections among the women in reproductive age group between menarche and menopause[3]. Abnormal vaginal discharge is the characteristic feature of vulvovaginal infections (VVI)[4].

Among women of reproductive age group 75% of them will suffer with at least one or more episode of VVC, & 45%of women will have two or more episodes in their lifetime[1]. It is clinically characterized by curd like discharge, itching, burning, cracking, erythema &vulval oedema[5]. The risk is high in women with immune compromised state like diabetes mellitus, Human immunodeficiency virus, those with oral contraceptives, using broad spectrum antibiotics therapy & pregnant womens[6]If left untreated it leads to pelvic inflammatory disease resulting infertility in non- pregnant women, in pregnancy it leads to chorioamnionitis with frequent abortion, preterm labour and congenital infection of the neonates[7]. Among the candida species from the vaginal specimens C.albicansis most predominant followed by other non-candida such as C.glabrata, C. tropicalis, C.dubliniensis, C.parapsilosis and C.krusei.

Nowadays recurrentvulvovaginalcandidasis (RVVC) is more common & its infection rate will reach up to 180 million in the year2030[9]. This is mainly due to antibiotic overuse, diabetes,pregnancy cystic fibrosis &insufficient intake of anti-fungal drugs. RVVC is defined as four or more episodes of symptomatic VVC within one year & affects small percentage of women <5%[12] usually not responding to first line antifungal drugs[10]. Biofilm formation by candida isolates leads to treatment failure & cause subsequent RVVC[11].Single dose of flucanazole 150mg orally is highly recommended for RVVC for 6months.[10]Hencelaboratory-based diagnosis help us to

identify the species[6]. Which is essential for correct treatment and preventing resistance to antifungal drugs[5]. Chromogenic medium is highly recommended for detecting mixed fungal infection & it is mainly included for recurrent vulvovaginalcandidasis.

Anti-fungal susceptibility plays important role in preventing anti-fungal drug resistance in VVC& RVVC.

Aim& objectives of the study is to determine the fungal aetiological agents causing vulvovaginitis &To find out the antifungal susceptibility pattern among the candidial isolates.

Materials & methods

The present cross-sectional study is conducted in tertiary care hospital in department of microbiology for a period of 6 months. Study was conducted after getting approval from institutional ethical committee. A total of 50 high vaginal swabs received in Microbiology Lab (SVMC&RC) from clinically suspected cases of vulvovaginitis irrespective of age attending as both inpatient & outpatient to the department of OBG, SVMC& RC were included in this study. All identified & confirmed cases of bacterial infection S/o Bacterial vaginosis were excluded in this study. Two high vaginal swabs collected aseptically were subjected to battery of microbiological testing, one swab subjected for microscopy and other for culture. Under microscopy for direct smear examination gram stain,10 % KOH &calcofluor white stain were performed& looked for the presence of gram-positive budding yeast like cells with pseudo hyphae. Another Swab was inoculated in Sabouraud's Dextrose agar (Hi media) incubated aerobically at 37^0 c for 24hrs those plates showing no growth were further incubated for 48 to 72hrs and observed for characteristic colony morphology following standard guidelines.

Suspected yeast colonies on SDA plates were identified by gram staining. For presumptive identification of candida albicans germ tube test was performed using 0.5 ml of pooled human serum incubated for 37c for 2-4 hrs. In that yeast cells which showed filamentous extension with no constriction at the neck were considered as germ tube positive. For further identification and differentiation candidalisolates weresubcultured onCHROM agar (Hi Media) is identified based on colony color, appearance & shape. Dalmau plate method is done using corn meal agar here isolates were streaked by cutting agar perpendicularly & a 22*22mm cover slip kept above it covering the upper half of streak line incubated at 25^0 c for 48hrs and examined directly through microscope under 10 & 40 x observed for chlamydospore formation & pseudohypae. Sugar assimilation & sugar fermentation test were done for further identification.

All the identified candida isolates were subjected to antifungal susceptibility test on Muller – Hinton agar (Hi Media) supplemented with 2% glucose & 0.5µg /ml methylene blue by disc diffusion method. Inoculum is prepared by using straight guaze wire 5 distant colonies were picked up and inoculated into 5ml sterile test tube containing 0.85% normal saline incubated to match the turbidity equivalent to MC Far land standard 0.5 following antifungal discs were used like nystatin (100mcg), fluconazole (25mcg) & voricanazole (1mcg) as per CLSI M44 –A2 guidelines[8]. Antifungal disks were placed aseptically over the lawn & incubated for 37^0 c for 24hrs plates were reincubated further for 24hrs (Fig - 1) if the plate doesn't show any zone of inhibition. Zone diameters of antifungal disks were read by using measuring ruler, zone size interpretated according to CLSI M44-A2 guidelines. Antifungal agents Zone

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diameter in mm Resistant (mm) Susceptible Dose Dependent (mm) Susceptible (mm) Fluconazole (25 µg) ≤14 15–18 ≥19, Voriconazole (1 µg) ≤13 14–16 ≥17, Nystatin (100 units) R≤16 I-17–24 S ≥25. [14] Quality control were ensured by using candida albicans ATCC 44374.

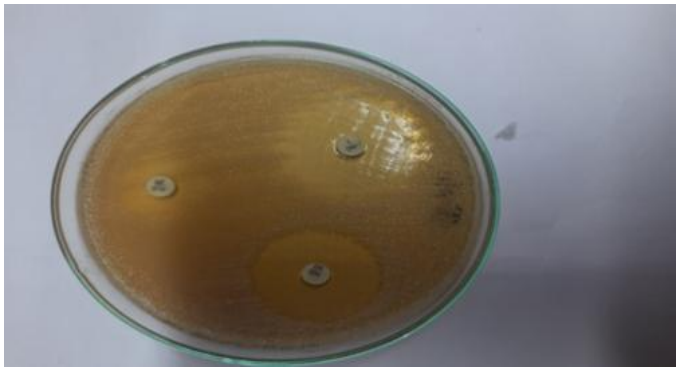


Fig 1: Showing the antifungal susceptibility testing by disc diffusion method.

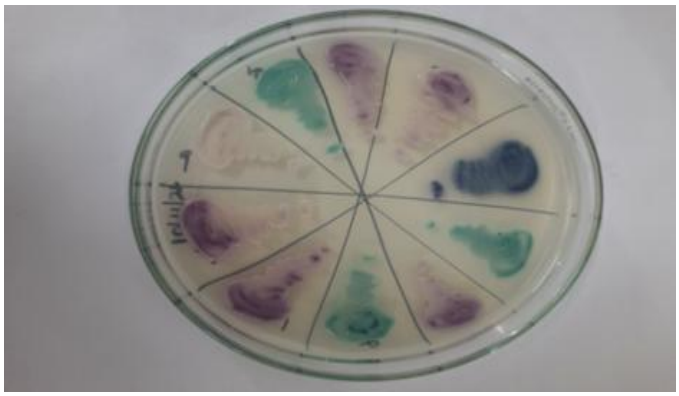


Fig 2: Showing the candida isolates with different colour pattern on HI chrome agar

1.Light green-coloured colonies S/O Candida albicans

2. Purple fuzzycoloured colonies S/O C.krusei

3. Pale pink-coloured colonies S/O C.parapsilosis

4. Blue purple-coloured colonies S/O C.tropicalis.

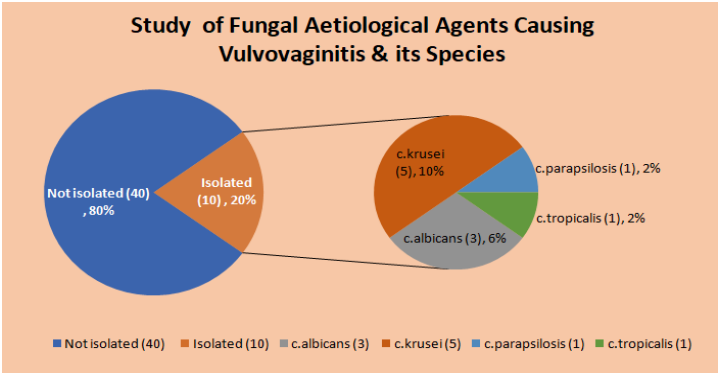
Results

During my study period**,** out of 50 clinically suspected cases of vulvovaginitis samples processed for culture 10/50(20%) candida species were isolated. Out of which the most common isolates were, 5C.krusei(6%), followed by 3C.albicans (6%) 1C. parapsilosis (2%) & 1 C.tropicalis (2%) species were identified. Culture confirmed cases were between age group of 20 to 40years.

All confirmed cases of candida albicans were germ tube test positive. Direct microscopic examination of high vaginal swab shows budding yeast cells in only 6% cases. Among confirmed cases C.albicans&C.krusei are most common isolates followed by C.parapsilosis& C.tropicalis.Anti-fungal susceptibility test were performed in all 8(16%) fungal isolates which shows 100% sensitivity to flucanazole,voricanazole& except two isolates showed resistance to nystatin.

Vulvovagnitis Samples.	Total count	Percentage (100%)	Total no of Candida species Identified
Total Samples	50	-	C.krusei (5)10%
Not Isolated	40	80%	C.albicans (3)6%
Isolated	10	20%	C.parapsilosis(1)2% C.tropicalis (1)2%

Figure 3: Showing total no of isolates



Discussion

VVC is a common public health problem among the women in reproductive age group presenting with a complaint of abnormal vaginal discharge. In my study out of 50 samples what we received in our lab, identified samples belongs to age group between 20-40yrs. Out of 50 high vaginal swabs tested 40 sample shows no growth only 10 samples were positive for fungal growth in that 10% 5 were C.krusei,NCA followed by 6%

isolates were C.albicans, 2% 1isolate each of C.parapsilosis, 2%C. tropicalisrespectively. This study shows the predominance of NCA among all isolates in thatC.krucei is the common isolate among our study.

Direct microscopic examination of high vaginal swab shows budding yeast cells in only 6% cases. This shows the cultureyield is more reliable than microscopic findings.Most of the isolates showed $> 10^5$ CFU/mlgrowth indicating infectious pathogen from colonizer. No mixed growth has been found. Incorporation of Candida HI CHROME agar helps us to identify candida species. Hence in my study NCA is common species isolated among 50 samples similar to the study done by KalaiarasanK,et al[1], Non candida albicans isolated more than C.albicans.

In a study done by RaoRP et al[5], states that Non candida albicans (NCA) is slightly higher in non-pregnant women than C.albicans.Bitew. A et al[13]study states that C.albicans is common than NCA contrast to our study result. NCA is common nowadays due to inappropriate treatment, over counter prescription of antifungal drugs. In order to prevent RVVC antifungal testing should be performed with candida ATCC controls, most of fungal isolates in my study shows sensitivity to all 3 antifungal drugs except 2 isolates resistant to nystatin. AFST should be done mainly because in some countries even in India antifungal drugs is prescribed to patients without identifying the common organism this leads to treatment failure & drug resistance. Patient might land up in infertility, preterm delivery .

Conclusion

This study help us to understand the magnitude of the problem & to implement necessary treatment modalities. Culture positive results will be correlated clinically with definite diagnosis of VVC. Chromogenic medium helps in detecting the mixed fungal infection and included especially in case recurrentvulvovaginal candidiasis.Diagnosis by clinical picture might be misleading hence confirmation is done by microscopy or culture.Culture should be performed in case RVVC if patient is having some risk factors. Treatment should be started based on AFST & patient should be monitored if symptom persists. Better to avoid treating asymptomatic male partners.

References

1. Kalaiarasan K, Singh R, Chaturvedula L. Fungal Profile of Vulvovaginal Candidiasis in a Tertiary Care Hospital. J Clin Diagn Res. 2017 Mar;11(3): DC06-DC09

2. Genital / vulvovaginal candidiasis (VVC) | Fungal Diseases | CDC [Internet]. [cited 2016 Jun 20]. Available fromhttps://www.CDC.gov/fungal/diseases/candidiasis/genital/.

3. Unemo, Magnus, Ballard, Ronald, Ison, Catherine, Lewis, David, Ndowa, Francis. et al. (2013). Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus / edited by Magnus Unemo … [et al]. World Health Organization. https://apps.who.int/iris/handle/10665/85343

4. Pereira LC, Correia AF, da Silva ZDL, de Resend CN, Brandão F, Almeida RM, de Medeiros NoBrega YK. Vulvovaginal candidiasis and current perspectives: new risk factors and laboratory diagnosis by using MALDI TOF for identifying species in primary infection and recurrence. Eur J Clin Microbiol Infect Dis. 2021 Aug;40(8):1681-1693. doi: 10.1007/s10096-021-04199-1. Epub 2021 Mar 13.

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5. Rao RP. Study of vulvovaginal candidiasis among nonpregnant women attending a tertiarycare teaching hospital in Karnataka, India. J. Evid. Based Med. Healthc. 2019; 6(12), 951-954

6. AniRubita N.S, Uma Maheshwari S.S.M, Mahalakshmi.G, Saranya.P.V. Fungal profile of vulvovaginal candidiasis in a tertiary care hospital., Volume-9 | Issue-9 | September - 2019 | PRINT ISSN No. 2249 - 555X | DOI: 10.36106/ijar

7. NedaKia sat, Ali Rezaei –Matehkolaei, Ali ZareiMahmoudabadi, khadijehHamidavi, Mohamad pour.etal.Prevalence of vulvovaginalcandidasis in Ahvaz, southwest Iran: A semi –large scale study. Jundishapur J Microbiol.2019 Mar;12(3)

8. CLSI. Methods for antifungal disc diffusion susceptibility testing of yeasts;Approved Guideline-Second edition. CLSI document M44-A2. Wayne, PA:Clinical and Laboratory Standards Institute; 2009.

9. Waikhom et al., prevalence of vulvovaginalcandidasis among pregnant women in the Ho municipality, Ghana: species identification & antifungal susceptibility of candida isolates-BMC pregnancy & childbirth (2020) 20:266

10. Amir Arastehfar et al.,A high rate of recurrent vulvovaginalcandidasis& therapeutic failure of azole dervivatives among Iranian Women-fmicb.2021.

11. Nicolas Papon, Patrick Van Dijck-A complex microbial interplay Underlies Recurrent Vulvovaginal candidiasis pathobiology (2021) Sep/Octo (6)

12. Gandhi et al., /Indian journal of obstetrics and gynecology research 2022;9(3):328-334

13. Bitew, A., Abebaw, Y. Vulvovaginal candidiasis: species distribution of Candida and their anti-fungal susceptibility pattern. BMC Women's Health 18, 94 (2018). https://doi.org/10.1186/s12905-018-0607-z

14. Diagnostic a R. Suceptibility testing of yeasts 2011. Taastrup: RoscoDiagnostic a; 2011.

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