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Original Research Article

Speciation of Candida isolates from vulvovaginal candidiasis cases, detection of virulence markers of Candida and antifungal susceptibility by disc diffusion method

Prathiba Mahadevaiah[1], **Raksha Yoganand**[1,*], **Soorashetty Manohar Rudresh**[1], **Ravi G S**[1]

[1] *Dept. of Microbiology, ESIC Medical College & PGIMSR, Bangalore, Karnataka, India*

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ABSTRACT

Introduction: Species belonging to the genus *Candida* are ubiquitous in nature and many of them form the normal microbial flora in human and animal bodies. A wide variety of infections on mucosal surfaces under certain conditions can be caused due to the *Candida* sp**.**

Methodology: A total of 40 isolates of Candida, isolated from vaginal swabs among 476 patients with clinically suspected VVC. Antifungal susceptibility testing done using Fluconazole and Voriconazole. Virulence markers Phospholipase activity, Proteinase activity, Biofilm Productionwere detected by standard methods.

Results: Out of 40 isolates, the following species were isolated, *Candida albicans* (52.5%) was the most common species isolated from vaginal swabs. The *Non Candida albicans* species isolated were *C.tropicalis* (27.5%), *C.parapsilosis* (15%), *C.glabrata* (5%).

Intense phospholipase activity was observed in 12 (30%) samples, there was no activity in 18 (45%) samples. A moderate phospholipase activity was observed in 6 (15%) isolates, poor activity in 4 (10%) samples.

Proteinase activity was strongly positive in 33 isolates which was identified as *C. albicans*and *C. tropicalis. C. glabrata*.

Strong biofilm formation was observed in 9 isolates, moderate in 8, weak in 11 and negative in 12 isolates. All 40 isolates are susceptible to Fluconazole and Voriconazole.

Conclusion: From this study it is observed that *C. albicans*and *C. tropicalis*are the major species of *Candia* causing VVC. Virulence marker analysis such as that for phospholipase, proteinase and biofilm formation will help to analyse the pathogenicity and further research required to elucidate the mechanisms involved in these pathways.

1. Introduction

Species belonging to the genus *Candida* is ubiquitous in nature and many of them form the normal microbial flora in human and animal bodies. *Candida* species is present in the mucosal surface of the skin, gastrointestinal, urinary, respiratory and genital tracts. The balance between *Candida* species and the lactobacilli residing in vaginal tract of women allow the persistence of these yeasts as commensal organisms.[1] These commensals may turn into opportunistic pathogens when there is any change in the normal environment in these regions and cause various fungal infections.[2] Vulvovaginal candidiasis (VVC), defined as symptomatic vaginitis, is such an infection of the vagina and

* *Corresponding author*.
E-mail address: rakshayoganand@gmail.com (R. Yoganand).

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vulva of the female genital tract by the *Candida* species normally residing in the vaginal mucosa. Vulvovaginal candidiasis (VVC), is symptomatic vaginitis, infection of the vagina and vulva of the female genital tract by the *Candida* species normally residing in the vaginal mucosa. 80% of the VVC cases are caused by *C. albicans* and 20% is by non *C. albicansCandida* (NCAC) species such as *C. glabrata.*[3] Other species which may cause VVC are *C. tropicalis*, *C. parapsilosis* and *C. krusei.* VVC is classified into complicated and uncomplicated; among which uncomplicated VVC is exhibited less than four episodes per year with mild severity whereas complicated VVC will be characterised by severe symptoms. Recurrent VVC (RVVC) which may occur more than four episodes per year due to increased risk factors are also considered as complicated VVC. VVC is the second most common cause of vaginitis reported, first being bacterial vaginitis second followed by Candida.

Presence of glandular structure cannot be observed in the normal vaginal mucosa. Normally, vaginal mucosa has a biological balance in the microorganisms present in it. Prevention of Growth of the pathogenic microorganisms is carried out by the Lactobacillus species. Hydrogen peroxide(H_2O_2) produced by the Lactobacilli creates an acidic medium in the vagina., This transforms glycogen present in vaginal epithelium to lactic acid that discourages the growth of other microorganisms. Alteration in this biological balance results in the clinical picture of itching, burning pain and vaginal discharge.[4]

The degree of expression of virulence such as hyphal forms, Specific Secreted Aspartyl Proteinases (SAPs), adherence, surface recognition molecule, extracellular hydrolytic enzymes, phospholipase production, acid phosphatase, peptidases, alkaline phosphatase, beta glucosidase, leucine amino peptidase, plasma coagulase, hemolytic factors, siderophores, metallopeptidases etc. varies significantly among different phenotypes of *Candida sp*. Each species of Candida spp. exhibit different degree of virulence factors.[5]

Candida species are responsible for around 80% of fungal infections in the hospital environment and 10-15% of UTI'S are caused by these microrganisms. Although the majority of infections are caused by *Candida albicans*, non *C.albicans* (NCAC) species including *C.glabrata*, *C.tropicalis* and *C.parapsilosis* are emerging as important nosocomial pathogens with a predilection for the urinary tract.

Epidemiological surveys reveal that *C tropicalis* and *C glabrata* are the second most frequent species isolated from oropharynx in case of former and from vagina and GIT in the latter.

VVC is the second most common cause of vaginitis reported, first being bacterial vaginitis. Although VVC affects millions of women globally, the estimation of the incidence of VVC is not accurate due to the lack of proper reporting and improper diagnosis which is attributed to the varied and non-specific clinical symptoms of the disease. Incidence of VVC is higher in women of reproductive age than at menopause.

VVC causes severe pain, mental distress, discomfort, and lower self-esteem, increase anxiety which may in turn affect normal functioning of professional and personal life, and interfere with sexual relations. It may cause financial loss and may lead to other complications such as pelvic inflammation and abscess, infertility, ectopic pregnancy, abortion or menstrual irregularities if left untreated. The risk factors for VVC are pregnancy, immunosuppression, hormonal changes/ treatments, use of antibiotics, oral contraceptives, diabetes or conditions in which the pH of the vaginal tract is altered.

Candida species are responsible for around 80% of fungal infections in the hospital environment and 10-15% of UTI'S are caused by these microrganisms. Although the majority of infections are caused by *Candida albicans*, non C.albicans (NCAC) species including *C.glabrata*, *C.tropicalis* and *C.parapsilosis* are emerging as important nosocomial pathogens with a predilection for the urinary tract.

In recent years molecular biological techniques such as pulse field gel electrophoresis, randomly amplified polymorphic DNA analysis and restriction fragment length polymorphism have been applied to diagnose *Candida* infections.

In addition to overall increase of *candida* infection epidemiological shifts in relative prevalence of different candida spp and emergence of new pathogens have occurred. An important factor for these shifts is introduction of anti fungal drugs into extensive use has resulted in selection of specific species that are inherently less susceptible to specific drug. And demographic parameters, specific genetic susceptibility have also been suggested as contributing factors.

The relative incidence of vaginitis caused by fungi other than *C. albicans* appears to be increasing. Recurrent disease (accounting for 21% of recurrent vs. 12% of initial infections) and with HIV infection (22% of infections in HIV-positive vs. 12% in HIV-negative women), especially in HIV-infected women who receive prophylaxis with imidazoles or triazoles can be credited to the non albicans infections. Short courses of topical antifungal agents make the selection of non-albicans yeasts least susceptible to these agents than is C. albicans.

In the culture, during the phenotypic switch, it was observed that the C. albicans exhibited both yeast form and hyphal form(12). Adherence of C. albicanswas demonstrated to be more as an effect of the high percentage of yeast cells. Especially in the HIV infected individuals, the infection due to C.albicansoccurs as an effect of elevated

levels of high frequency phenotypic switching.[6]

Resistance against antifungal fungal compounds in humans were demonstrated by the *C. albicans* and related species which are pathogenic. As a consequence of the expression of efflux pumps, there will be reduction in the drug accumulation. The alteration of structure or concentration of antifungal target proteins and by alteration of membrane sterol composition are observed as well. The clinical consequences of the antifungal resistance can be seen as treatment failure in patients and as the change in prevalence of Candida sp. which cause infection.[7]

Objective of the present study was to speciate Candida isolates causing Vulvovaginitis, detect virulence factors and check antifungal susceptibility to Fluconazole and Voriconazole.

We observed *C. albicans* as the most common causative agent of VVC in our hospital.

2. Materials and Methods

The study was approved by Institute Ethical Committee. 476 patients with clinically suspected vulvovaginal candidiasis attending Obstetrics and Gynaecology OPD at ESIC Hospital, Bangalore were recruited for the study. The study was conducted from November 2018 to May 2019, 18 months in the Department of Microbiology, ESICMC & PGIMSR.

2.1. Inclusion criteria

Patients clinically suspected to have vulvovaginal candidiasis were included in the study.

2.2. Exclusion criteria

Patients on any anti-fungal treatment were excluded from the study as it may interfere with the results.

Vaginal swabs collected from these patients attending the Obstetrics and Gynaecology OPD were subjected to various laboratory examinations for the presence of Candida species, speciation of the isolate and for the activity of different virulence factors. The vaginal swab was streaked onto SDA with antibacterials gentamicin and chloramphenicol tubes and incubated for 24-48 hr at 37ºC and examined for the presence of creamy white colonies. Any growth seen was identified to species level using standard laboratory techniques.

2.3. Speciation of Candida

2.3.1. Germ tube test

Germ tube test was used to differentiate *C. albicans*from other *Candida* sp. To a small tube, 0.5 ml of human serum was added. A single colony of test yeast cells from a pure culture was taken using a Pasteur pipette and emulsified in the serum and incubated at 37ºC for 2 hours in an incubator. After incubation a drop of the serum was transferred to a slide and covered with a cover slip. The drop was then observed in light microscope under low and high power objectives for the presence of any germ tube formation. *C. albicans*and *C. dubliniensis* forms a germ tube which is a short, slender, tube-like hyphal (filamentous) extension arising from the yeast cell without any constriction at the point of origin.[8] The elongated daughter cells from mother cells without constriction are called Germ tube which is positive in *Candida albicans and Candida dublinensis*. A constriction at the origin of mother cells is called pseudohyphae which is seen in other *Candida* sp. such as *C. tropicalis* and *C. glabriata* will interfere with formation of germ tube.[9]

2.3.2. Chlamydospore production on corn meal Agar (Dalmau plate)

*C. albicans, C. dubliniensis*and *C. tropicalis* produce chlamydospores on nutritionally deficient media. Chlamydospores are round, highly refractile and resistant asexual spores. Chlamydospore formation and its relationship to hyphae, pseudohyphae and other fungal structure can be studied by inoculating *Candida* isolates from primary culture on Corn Meal Agar (CMA). As CMA is clear media, the pattern of yeast growth can be examined directly by placing media plate on the stage of a bright field microscope. The pattern of growth on CMA can be used for speciation of *Candida* isolates. Addition of tween-80 (polysorbate) to corn meal agar enhances the chlamydospore formation. It also favors the development of pseudohyphae, hyphae and blastoconidia. In *C. dubliniensis* chlamydospores are often attached in pairs, triplets, or larger clusters to the same suspensor cell rather than singly at the hyphal (or pseudohyphal) ends in *C. albicans*.

Colonies from 16 hr SDA slant culture were inoculated into Cornmeal Agar (containing 1% Tween 80) in a 90 mm plate, using sterile straight wire yeast colony was lightly touched, and then 2-3 streaks were made around 3.5-4cm long and 1.2 cm apart. 22 x 22mm cover slip was placed over the control part of the streak. This will provide partially anaerobic environment at the margins of cover slip. The plate was incubated at room temperature in dark for 3 days. After 3 days plates were examined by placing the plate without its lid on the microscope stage. Using low power and high power terminal chlamydospores were looked near the edge of the cover slip. Morphological features like hyphae, pseudo hyphae, ascospores, blastospores, chlamydospores, basidiospores are noted.

2.3.3. Sugar assimilation test

The ability of each *Candida sp*. to utilize a particular carbohydrate as the sole carbon source can be used for speciation. Carbohydrate assimilation test is based on the use of carbohydrate free yeast nitrogen base (YNB) agar and

checking the presence of growth on carbohydrate containing media after incubation. Growth on the media and a change in the colour of an indicator indicates a positive test.[10]

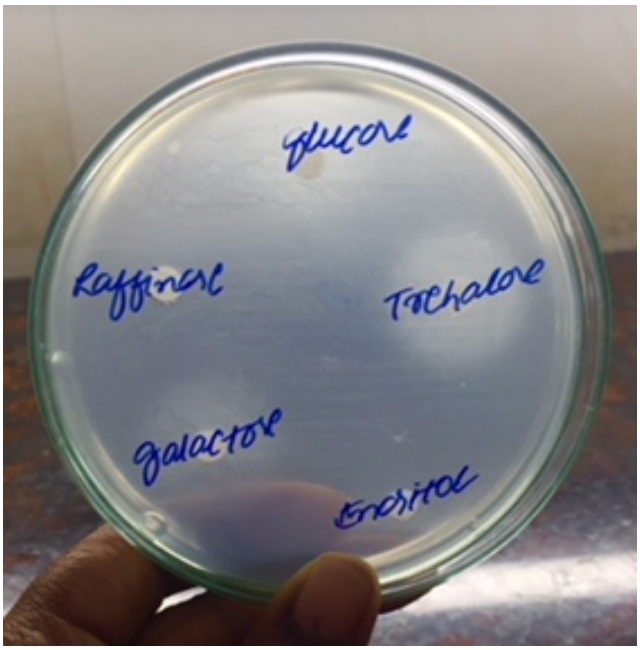

Fig. 1: Sugar assimilation test -1

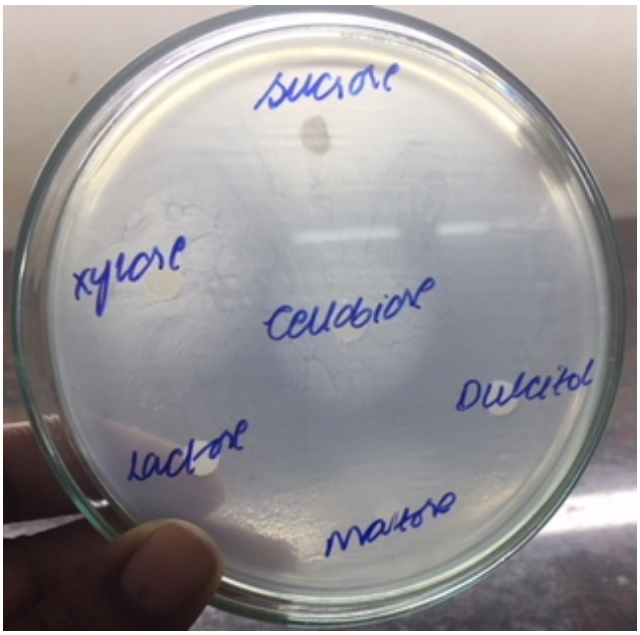

Fig. 2: Sugar assimilation test

Yeast Nitrogen Base (YNB)was obtained from Difco and YNB agar was prepared by adding 6.7 g to 100 ml distilled water and 20 g agar mixed in 980 ml distilled water. 18 ml of YNB agar was added to 18 × 150 mm screw-capped tubes and autoclaved at 121 °C and stored at 4 °C. A yeast suspension was prepared from a 24-48 hr old culture in 2 ml of YNB by adding a heavy inoculum matching 5 Mc Farland Standard. This suspension was added to the 18 ml of molten agar (cooled to 45°C) and mixed well. The entire volume was poured into a 90 mm petri plate and allowed to harden.

20% sugars were prepared- 2 g of sugar in 10 ml of distilled water taken in screw capped tubes, subjected for inspissation.

Discs were aseptically placed onto the surface of the agar plate using sterile syrine, and 5μl sugar solution is impregnated on to disc using sterile yellow tips and Micropippette. Plates were incubated at 37 °C for 3-4 days. The plates were examined for the presence of growth around the disc which was considered as positive for that particular carbohydrate.[11] Growth around glucose disc was recorded first which served as positive control (viability of yeast).

Fresh subculture was made on SDA for testing of virulence factor characteristics. It was suspended in normal saline which was matched to a concentration of 2 McFarland standard.

2.3.4. CHROM Agar

A rapid, plate based media used simultaneously for isolation and identification of various Candida species. This method identifies various Candida species by colour as a result of biochemical reactions. It shortens the time for Presumptive identification of organisms. It is Possible to identify Candida species by their morphological characteristics within 24hrs. This is based on direct detection of specific enzymatic activities by adding certain substrates of flurochromes to media.[12] C.albicans produces light green, C.parapsilosis produces white to pale pink, C.glabrata produces white large glossy pale pink, C.tropicalis produces dull blue to purple color.

2.3.5. Phospholipase activity

Candida species isolated from the samples were analysed for the production of phospholipase secretion by growing them on SDA containing egg yolk. The egg yolk agar was prepared by dissolving 65g SDA, 58.4g NaCl, 5.5g $CaCl_2$ in 980 ml distilled water and sterilized at 121 °C for 15 minutes. 10% Egg yolk was centrifuged at 5000 x g for 30 min and 2 ml of the supernatant was added to the medium cooled at 45-50 °C. A 10 μl suspension of 24hr old yeast culture was inoculated onto this egg yolk agar medium and incubated at 37°C for 4 days to analyse phospholipase activity. Phospholipase activity was indicated by a zone of precipitation around the colony. The ratio of colony diameter to the diameter of the dense white zone of precipitation around phospholipase positive colonies (Pz value), correlated with hydrolysis of [14C] phosphatidylcholine[13] Pz value of 1 is considered as No phospholipase secretion, Pz score between 0.70 and 0.99

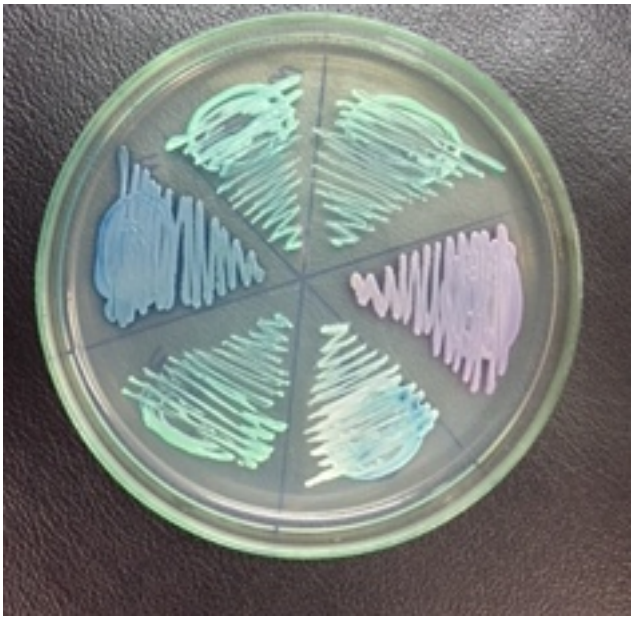

Fig. 3: Growth on Chrom Agar

was taken as positive and the Pz score ≤0.70 was taken as strong positive.

Phospholipase secretion, i.e. Pz value was calculated using the equation:

Pz = Colony diameter (mm)/Colony diameter (mm) + Diameter of precipitation zone (mm)

2.3.6. Proteinase activity

Protein production of *C. albicans* was induced using 1% Bovine Serum Albumin (BSA). 1% BSA medium supplemented with 2% dextrose, 0.1% KH_2PO_4, 0.05% $MgSO_4$ and 2% agar. An 18 hr yeast suspension was prepared. 5 μl of the suspension was inoculated onto BSA agar plate. The plate was incubated in a moist chamber at 37 ºC for 5 days. The plate was flooded with naphthalene black 1.25% solution in methanol/water 90% v/v for 15 min and decolourised with 15% acetic acid for 36 hr.[14] Pr_d score of 1,considered negative; Less than 1-was taken as positive and those with ≤ 0.63 was taken as strong positive.

Proteinase production (Pr_d value) was calculated as follows:

Pr_d= Diameter of the zone of proteolysis around the colony (mm)/ Colony diameter (mm)

2.4. Biofilm production

Microtitre plate assay was used to determine the biofilm producing ability of isolates. Sabouraud's dextrose broth (6% glucose; pH 6) was used to induce the biofilm production in microtitre plates, inoculated with 10^7 CFU/ml freshly subcultured *Candida* isolates. The plates were incubated at 30°C. Then washed with 0.15M phosphate

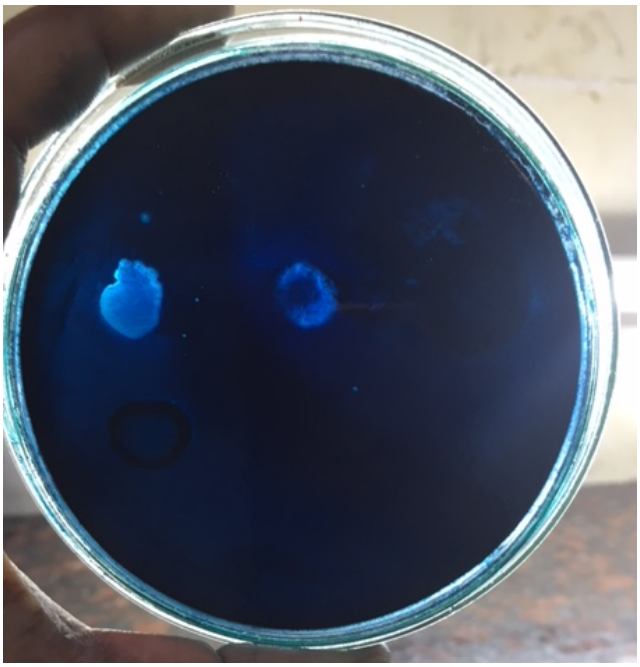

Fig. 4: Proteinase production

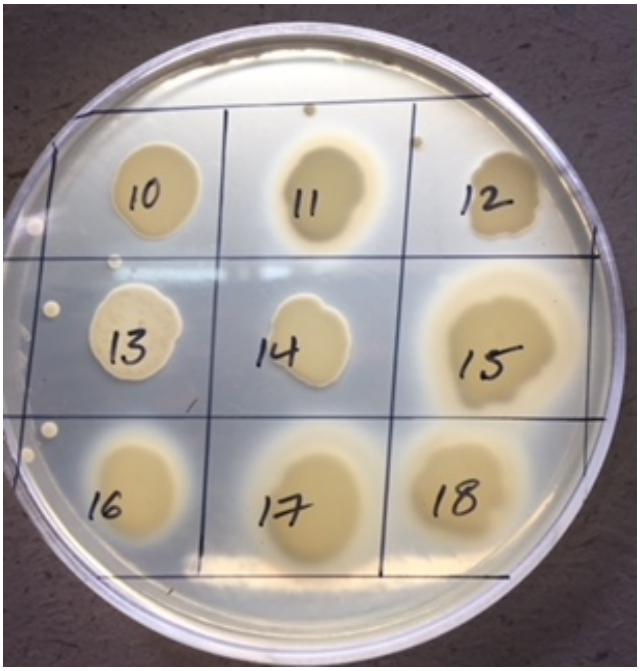

Fig. 5: Phospholipase production

buffer saline to remove the planktonic cells, and then stained with 0.1% crystal violet, 0followed by estimation of optical density at 540nm. For each isolate the biofilm production was tested in triplicate and an internal control *C.albicans* repeatedly giving positive result for particular test was used as standard control strain. The strength of biofilm production(weak, moderate, strong) was calculated using a method previously reported.[15]

2.5. Antifungal drug susceptibility testing using fluconazole (10 mcg) and Voriconazole (1 mcg)

Antifungal susceptibility test for Fluconazole(10mcg) and Voriconazole(1mcg) was done by Disc diffusion method on glucose methylene blue Muller Hinton agar (GM-MH). GM-MH was prepared by addition of 2% glucose and 0.5μg/ml of methylene blue to Muller Hinton agar. The agar medium should have a pH of 7.2-7.4 at room temperature after gelling.[16]

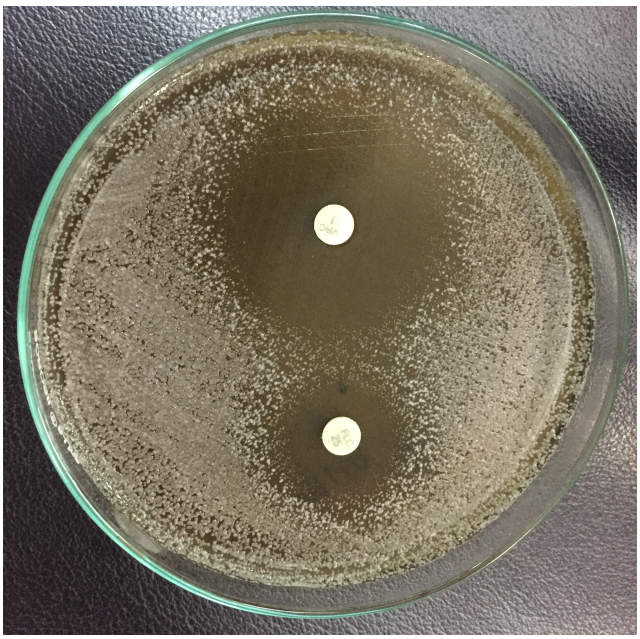

Fig. 6: Antifungal susceptibility

The inoculum was prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on SDA. Colonies were suspended in 5ml of sterile 0.85% saline. This suspension was adjusted to turbidity of 0.5 Mc Farland yielding 1x10^6 - 5x10^{6}cells/ml.

Table 1: Zone diameter interpretive standard

Antifungal agent	Disk content	Zone diameter, Nearest whole (mm)		
		R	S-DD	S
Fluconazole	10mcg	≤14	15-18	≥19
Voriconazole	1 mcg	≤13	14-16	≥17

2.6. Statistical analysis

The categorical variables were expressed as frequency and percentages. The association of virulence factors among Candida species was compared using graph pad prism software, online version.

3. Results

In this study, 476 patients clinically suspected for VVC were included among which 40 patients were confirmed for the VVC by the diagnostic criteria; age ranging from 19-48 years. Vaginal swabs were collected from these patients and analysed for the identification of the species of *Candida* using germ tube formation, sugar assimilation, chalmydospore formation and for the determination of various virulence factors present in the microorganism such as proteolytic activity, phospholipase activity, biofilm and Sensitivity to antifungal drugs such as flucanozole and voricanozole was tested using disc diffusion method for each isolate.

Major risk factors encountered in the present study were Diabetes (15%), Pregnancy (22.5%) and patients on prior antibiotic treatment was(15%) and all diabetic patients presented with recurrent vaginitis.

Among 40 samples analysed from clinically suspected cases of Vulvovaginal Candidiasis, 21 were *C. albicans*, 2 were *C. glabrata*, 6 samples were *C. parapsilosis* and 11 were *C. tropicalis*. And were further tested for virulence activity.

Table 2: Speciation of *Candida* species

Species	No. of samples	Percentage
C. albicans	21	52,5
C. glabrata	2	5
C. parapsilosis	6	15
C. tropicalis	11	27.5

Phospholipase activity was observed in 55% of the isolates among which is an intense activity in 30%,moderate action in 15% of the isolate and poor reaction was seen in 10%,. Proteinase activity was strong in 82.5% of the samples and biofilm formation observed in 28 samples. Strong biofilm formation was present in 9 samples, moderate in 8 and weak formation in 11 samples. Proteinase and biofilm formation was observed in 16 samples; proteinase and phospholipase activity in 16 samples and phospholipase and biofilm formation in 7 samples. As each species had different range of response in virulence markers it is not classified separately. All the three virulence markers were present in 7 isolates. All isolates were sensitive to both the antifungal drugs flucanozole and voricanozole.

Intense phospholipase activity was observed in 12 (30%) samples and there was no activity in 18 (45%) samples. A moderate phospholipase activity was observed in 6 (15%) isolates and poor activity in 4 (10%) samples. However,

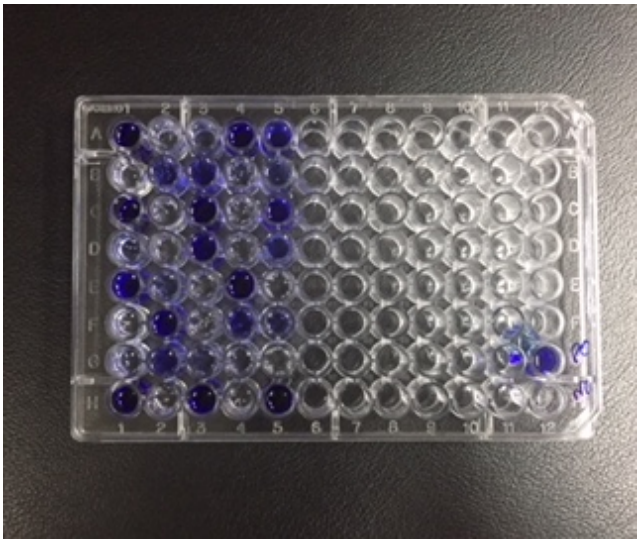

Fig. 7: Biofilm formation

statistical comparison of phospholipase activity showed there was no significant difference between albicans and non albicans species (z= 0.130; p=0.89). Proteinase activity was strongly positive in 33 isolates which was identified as *C. albicans*and *C. tropicalis. C. glabrata* isolated from one sample showed positive reaction in proteinase activity but less than other species. Comparison of the proteinase production between *Candida albicans* species and non albicans species indicates that the non-candida albicans species produce a statistically more significant phospholipase (z= 3.504; p = 0.00046). Strong biofilm formation was observed in 9 isolates, moderate in 8, weak in 11 and negative in 12 isolates. Proteinase and biofilm formation was present in 16 isolates; proteinase and phospholipase activity in 16 isolates; and phospholipase and biofilm formation was observed in 7 isolates. All three virulence markers- proteinase, phospholipase and biofilm formation was observed in 7 isolates.

Table 3: Significant association of virulence markers

Species	No. of samples	Phospolipase activity	Proteinase Production	Biofilm formation
C. albicans	21	12	21	16
C. glabrata	2	0	2	2
C. parapsilosis	6	3	0	3
C. tropicalis	11	7	11	8

Among the isolated Candida species, *C. albicans* (10) isolates had more than 1 Virulence marker, and (5) isolate had more than 2 Virulence marker, and among the isolated *C.tropicalis* (8) isolates had more than 1 Virulence marker, and (2) isolate had more than 2 Virulence marker. *C. parapsilosis* and *C. glabrata* (2) each had more than 1 Virulence marker.

Table 4: Virulence marker in each species

Species	More than 1 virulence factor	More than 2 virulence factor
C. albicans (21)	10	5
C. parapsilosis (6)	2	0
C. tropicalis (11)	8	2
C.glabrata (2)	2	0

Table 5: Antifungal drug susceptibility

Drug	Sensitive	Percentage %
Fluconazole 10mcg	40	100
Voriconazole 1mcg	40	100

All isolates were sensitive to both voricanozole and flucanozole

4. Discussion

Candida is the most prevalent fungal pathogen in humans. Although the majority of infections are caused by *Candida albicans*, NCAC species including *Candida glabrata*, *Candida tropicalis* and *Candida parapsilosis* are emerging as important pathogens in humans.

Candida species express several virulence factors to escape from the host defence mechanisms. Expression of virulence factors may vary depending on the infecting species, geographical origin, type of infection, site of infection and host reaction. Virulence factors play an important role to understand the pathogenesis of candidiasis. We analysed the detection of several virulence factors of Candida species such as biofilm production, proteinase activity, and phospholipase activity.

Fluconazole is an azole group of antifungal agent, been widely used for both treatment and prophylaxis of fungal infections. Due to its limited spectrum of antifungal activity and resistance noticed in immunocompromised hosts, second generation triazoles like voriconazole have been developed.

Present study included 40 clinical isolates which showed gram positive budding yeast cells with or without pseudohyphae. Our study aimed to determine the invitro susceptibilities of various Candida species isolates to fluconazole and Voriconazole and to compare both.

Present study showed Candida infection more common in age group of 19-30 years, followed by age group of 30-45 years.

In present study association of risk factors were Diabetes (15%), Pregnancy (22.5%) and patients on prior antibiotic

treatment was 15% and all diabetic patients presented with recurrent vaginitis i.e. they presented with recurrent episodes of vulvovaginal candidiasis atleast 4 specific episodes in one year.

Incidence of Vulvovaginal Candidiasis increases in Pregnancy, Diabetes. In pregnancy incidence increases due to immunocompromised state, hormonal effects and nutritional factors. VVC incidence increases in poorly controlled diabetes, due to immunocompromised status.

In the present study Speciation of the *Candida* species was performed to identify the species which revealed that 21 isolates were *C. albicans*, 2 were *C. glabrata*, 6 samples were *C. parapsilosis*and 11 were *C. tropicalis.*Similar results were obtained on CHROM Agar, CHROM agar has the advantage of being rapid, simple and cost effective as compared to conventional methods which are slow, technically demanding.

Intense phospholipase activity was observed in 12 (30%) samples and there was no activity in 18 (45%) samples. A moderate phospholipase activity was observed in 6 (15%) isolates and poor activity in 4 (10%) samples. However, statistical comparison of phospholipase activity showed there was no significant difference between albicans and non albicans species (z= 0.130; P=0.89). Proteinase activity was strongly positive in 33 isolates which was identified as *C. albicans*and *C. tropicalis. C. glabrata* isolated from one sample showed positive reaction in proteinase activity but less than other species. Comparison of the proteinase production between *Candida albicans* species and non albicans species indicates that the non-candida albicans species produce a statistically more significant phospholipase (z= 3.504; P = 0.00046). Strong biofilm formation was observed in 9 isolates, moderate in 8, weak in 11 and negative in 12 isolates.

In present study all the isolates were sensitive to Flucanozole and Voricanozole.

Triazoles remain active against many isolates, Fluconazole is the first line antifungal agent for treating uncomplicated candidiasis.

Voriconazole seemed to be superior to fluconazole with a better susceptibility in the fluconazole resistant strains. However, in the present study there were no fluconazole resistant strains.

Changing trends in the antifungal susceptibility towards fluconazole recommends routine antifungal susceptibility testing of Candida isolates in clinical microbiology laboratories. Presumptive identification followed by confirmation of yeast species helps to initiate early appropriate antifungal therapy thereby reducing the morbidity and mortality.[17]

5. Conclusion

From this study it is observed that *C. albicans*and *C. tropicalis*are the major species of *Candia* causing VVC. The different species can be differentiated using various tests such as germ tube formation and sugar assimilation tests. Virulence marker analysis such as that for phospholipase, proteinase and biofilm formation will help to analyse the pathogenicity and further research required to elucidate the mechanisms involved in these pathways. This will help to treat the patients more effectively. As the patients were found to be sensitive to antifungal drugs such as flucanozole and voricanozole, these drugs can be used for the treatment.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Author biography

Prathiba Mahadevaiah, Senior Resident https://orcid.org/0000-0002-5623-8718

Raksha Yoganand, Professor and HOD https://orcid.org/0000-0003-2254-3893

Soorashetty Manohar Rudresh, Associate Professor https://orcid.org/0000-0003-0555-6748

Ravi G S, Professor https://orcid.org/0000-0002-8431-882X

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