"PHENOTYPIC DETECTION OF SELECTED VIRULENCE FACTORS OF CANDIDA SPECIES ISOLATED FROM WOMEN OF REPRODUCTIVE AGE WITH VULVOVAGINAL CANDIDIASIS"

By

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To,

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Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"PHENOTYPIC DETECTION OF SELECTED VIRULENCE FACTOR OF CANDIDA SPECIES ISOLATED FROM WOMEN OF REPRODUCTIVE AGE WITH VULVOVAGINAL CANDIDIASIS"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

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LIST OF ABBREVATION

BC	-	Before century
VVC	-	Vulvovaginal candidiasis
RVVC	-	Recurrent vulvovaginal candidiasis
NAC	-	Non albicans Candida
HIV	-	Human Immunodeficiency Virus
AIDS	-	Acquired immunodeficiency syndrome
GIT	-	Gastrointestinal system
DM	-	Diabetes mellitus
IUCD	-	Intrauterine contraceptive device
Ab	-	Antibody
Ag	-	Antigen
IL	-	Interleukin
ME	-	Microscopic examination
NICU	-	Neonatal intensive care unit
IVS	-	Idiopathic vestibulitis syndrome
LP	-	Lichen planus
VLBW	-	very low birth weight
SAP	-	Secreted aspartyl proteinase
Spp	-	Species
WHO	-	World Health Organisation

ABSTRACT

Background: Overgrowth of *Candida* species in the female lower genital tract, the vulva & the vagina results in vulvovaginal candidiasis (VVC). Production of virulence factors like biofilm & various enzymes (hemolysin, phospholipase, esterase) contributes towards its pathogenicity. Hence, in view of these factors contributing to pathogenic nature of the species & emerging resistance to antifungals; the study was taken aiming to detect the virulence factors of *Candida* species isolated from women of reproductive age group.

Objectives: 1. Phenotypic detection of selected virulence factors (Biofilm production, Hemolytic activity, Phospholipase activity, Esterase activity) of *Candida* species isolated from women of reproductive age with Vulvo-vaginal Candidiasis.

2.To detect the anti-fungal resistance of the isolates.

Material & Method: A total of 69 *Candida* spp. isolated from clinically suspected cases of VVC in the reproductive age group women. Different virulence factors (biofilm production, hemolytic activity, esterase activity, phospholipase activity) were detected by standard methods.

Results: Hemolytic activity was shown by 53(76.81%) *Candida* isolates, biofilm production in 32(46.37%) *Candida* isolates, phospholipase & esterase activity in 38(55.07%) & 27(39.13%) *Candida* isolates respectively. 93% of *Candida albicans* strain has shown phospholipase activity. All of the strains of *C. krusei* has shown esterase activity & ability of biofilm production. Esterase activity was observed in 73% of *C. tropicalis*, *C. albicans* (30%) & 27% of *C. glabrata*. Biofilm production was seen 61% (14) of *C. glabrata* strains followed by 55% (6) of *C. tropicalis*.

Among non albicans *Candida* Fluconazole resistance was observed more compared to voriconazole.

Conclusions: The study indicates that the virulence factors which were restricted to *Candida albicans* are now being expressed in non albicans *Candida* & that these factors may contribute to increased Antifungal resistance among *Candida* species. Our study also shows that antifungal resistance among *Candida* species has shown more association with biofilm production.

Keywords: *Candida*, Vulvovaginal Candidiasis, Antifungal resistance, Biofilm production, Hemolytic activity, phospholipase activity, esterase activity.

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INTRODUCTION

Approximately 75% of adult women may experience vaginal candidiasis at some point in their lives. It is the most prevalent fungus infection of the vulva and vagina[1]. Overgrowth of *Candida* species in the female lower genital tract results in vulvovaginal candidiasis (VVC). *Candida albicans* is considered as most virulent Candida species though non-albicans *Candida* (NAC) species have lately been recovered with increasing frequency[2]. Infections caused by Candida species represent a significant global public health concern [3].

Candida – a commensal of the healthy mucosal surfaces of the vagina, gastrointestinal tract, and oral cavity. These pathogens are true opportunistic in nature, taking advantage of the host's debilitated status to enter deeper tissues and the circulatory system [4]. However, specific circumstances or diseases, such as extended antibiotic use that changes the balance of microorganisms in vaginal environment, pregnancy, diabetes, immunosuppressive medications & HIV infection, may cause overgrowth of *Candida* [1].

Curdy white vaginal discharge and vaginal itching are two common clinical signs of vulvovaginal candidiasis. Patient commonly gets dyspareunia and dysuria. The tissue around vulva-vagina may swell and turn red [5]. A thorough evaluation, thorough history, physical examination, pelvic examination, and laboratory confirmation of the infection are all required for the diagnosis of vaginal candidiasis [6]

Presence of virulence factors indicates how pathogenic the organism is. The ability to evade host defence, adhesion to the surface epithelium, biofilm production, hemolytic activity, and invasion by the production of extracellular enzymes such as

phospholipase, proteinase, and esterase are the main virulence factors of *Candida* species relevant to the genitourinary tract that are responsible for its pathogenicity [2]. Extracellular hydrolytic enzymes infiltrate the host by promoting tissue penetration and adhesion. Iron acquisition by hyphal invasion is made easier by hemolysin. The pathogenesis of candidiasis depends on biofilms, which are microbial populations linked to the extracellular matrix and the outermost layers for adherence to the surface of the host.

In the reproductive age group, 50–72% of women experience vulvovaginal candidiasis [7]. Refractory vaginitis brought on by *C. albicans* that is resistant to azole medications has been more common recently[8].

VVC is managable with help of of antifungals. Few of which drugs are available over-the-counter. Many women self-medicate based on their symptoms, which encourages the spread of drug-resistant organisms.

In order to address the unmet clinical need, novel therapeutic approaches and extensive pathogenomics research are needed as the incidence of VVC is expected to increase globally over the coming decade. Hence, in view of these factors speciation, pathogenic factors contributing to the species and emerging resistance to antifungals; the study was taken aiming to detect the virulence factors of species *Candida* isolated from women of reproductive age group.

OBJECTIVES

1. Phenotypic detection of selected virulence factors (Biofilm production, Hemolytic activity, Phospholipase activity, Esterase activity) of Candida species isolated from women of reproductive age with Vulvo-vaginal Candidiasis.

2. To detect the anti-fungal resistance of the isolates.

REVIEW OF LITERATURE

History:

The first description for *Candida* infection is found in Hippocrates' "Epidemics' from 4th century BC. Rosen von Rosenstein identified Candidialinfection in pediatric patients and framed description in modern medicine.In 1844, the fungus was isolated from the sputum of a patient with tuberculosis by Bennett. It was isolated from vaginal infections by Wilkinson in 1849.Later it was isolated from blood and brain by others. It was named as 'Oidium albicans' in 1853 by Robin. `Oidium albicans` was derived from the word *Candida* which describes the *white Robe* (*Toga*) worn by Roman senators.[9]

In 1923 Berkhout structured *Candida* genus. Because of its genus name as monilia, it is also called moniliasis. In 1978 previously called as Torulopsis glabrata was renamed as *Candida*. *Candida dublinensis* was described as a new *Candida* species in 1995 from Dublin, Ireland. [10]

NORMAL VAGINAL SECRETIONS:

Physiology

Normal vaginal discharge in women of reproductive age ranges from 1-4 ml in a 24-hour period and is transparent white & odorless. It is most obvious during pregnancy, during ovulation, and when taking oral contraceptives.

Normal vaginal discharge is formed by

- Transudation from vaginal epithelium and desquamated cells of the cornified layer.
- Sweat and sebaceous gland secretions of vulva.
- Bartholin's gland secretions
- Mucous secretions from endocervical glands.

VAGINAL pH:

Healthy adult woman A childbearing age possesses lactobacillus, which are sugar-fermenting, Gram-positive bacteria.[9] It causes the vaginal pH to become acidic by converting the vaginal glycogen into lactic acid. pH of Vagina changes according to age; in Newborn it is five point seven, in Children- six to eight, during Reproductive age & pregnancy – three point eight to four point five & in Menopause-seven.[11]

DEFINITION:

Infection of vulvo-vaginal mucosa brought on by spp. of the genus *Candida* is known as vulvovaginal candidiasis. When balance between fungi & the host shifts in favour of the fungus, *Candida* spp. are able to induce superficial lesions in the vaginal mucosa.[12]

The infection can be acute or persistent and exhibit a variety of clinical symptoms.

PREVALENCE

VVC is an exceedingly common mucosal infection of the lower female reproductive tract. It is estimated that 75% of women have at least one episode of vaginal Candidiasis in their lifetime.[13]

EPIDEMIOLOGY:

Candida, a dimorphic fungus (a part of the endogenous human microbiota) that lives in the mucosae of healthy people (30-70%) in GIT, oral & genital tract. *Candida* act as an opportunistic pathogen when the immune system is suppressed; it can harm terminally sick people and causes oral-pharyngeal infections, notably in AIDS patients. *Candida* is a 1eading causative agent of VVC in healthy women, the pathology of the fungus is not as of classical opportunistic pathogen in this scenario since VVC is not always related to an immunodeficient state.[14]

Recurrent Vulvovaginal Candidiasis is defined as the occurrence of ≥4 VVC episodes every year & requiring antifungal therapy continuously. CDC 2021 According to recent survey, which included 284 non-pregnant women and was done between February 2016 and May 2018, 78% of the women reported having a history of Vulvo-vaginal Candidiasis , with 34% being classified as having RVVC.[15]

Most of VVC/RVVC are of endogenous nature due to *C. albicans'* ability to enter the vaginal canal and exposure to secretions from d nearby gut and anus.[14]

Milder symptoms seen with NAC spp. compared to VVC by *C. albicans*. Willems and colleagues, who used a VVC mouse model, demonstrated that regardless of the NAC spp. studied, damage and neutrophil recruitment were considerably

decreased as comparison with *C. albicans*, though equal levels of colonization. The VVC treatment is made more challenging by the fact that NAC spp. may be more resistant to antifungals. There are common & species-specific *Candida* pathogenicity pattern, according to a more recent study. Particularly, vaginal epithelium has been seen to exhibit a biphasic host response. Such a response is characterized by an early mitochondria-induced type I IFN signaling activation that is common by all the spp. taken into consideration; the reaction only turns species-specific at later stages of infection. *Candida* has an ergosterol-containing, stiff cell wall formed of chitin. N acetyl glucosamine - a polysaccharide found in long chains in chitin. The cell wall also includes beta-glucans, another type of polysaccharide, and mannans, which are the main component of an antigen. Antifungals act selectively by targeting fundamental variations in membrane sterols.[16]

ETIOLOGICAL FACTORS:

Host factors:

1. Pregnancy entails a high risk of 30–40%, especially in the third trimester. (17) There are specific variations in levels of female sex hormones like estrogens, that increase a woman's risk of developing a yeast infection. Recurring infections are also m2re typical. No conclusive evidence exists to support the claim that treating asymptomatic vvc ↓es the risk of preterm birth during pregnancy. Additionally, the acidic environment of the pregnant vagina suppresses other microbes that generally prevent *Candida* from growing. An acidic vaginal pH <5 is more advantageous for the development of germ tubes and mycelia, even though a high pH (6-7) enhances the organism's initial adhesion to the vaginal epithelia.
2. Diabetes mellitus is one of the causes that might lead to recurrent vulvovaginal

candidiasis. The attachment of *C. albicans* onto vaginal epithelial cells is facilitated by elevated blood glucose levels. DM is thought to ↑se vulnerability by raising tissue glucose, decreasing phagocytosis, and altering yeast adhesion.[18]

3. The amount of glycogen in the vaginal epithelial cells is increased by oral contraceptive pills with high estrogen contents (>50 g), which is a valuable carbon source for *Candida'*s growth & germination. Additionally, this hormone expedites the growth of pseudo-hyphae. Progesterone & lactation are safe variables.[19]
4. Drugs: Frequen antibiotic use facilitates Candidial colonisation by removing the protecting natural vaginal flora. The likelihood of infection rises with the overall length of antibiotic therapy but is unrelated to a particular antibiotic class.[20] By impairing immunological response, systemic steroids raise candidiasis susceptibility. Corticosteroids and vulvovaginal candidiasis have a 19.83% association, according to a study conducted at a tertiary care facility. Tamoxifen use increases Candidial infection in postmenopausal women.[21]
5. Spermicide, diaphragm usage, IUCD, douching, perfumes, and feminine hygiene products will cause minimal damage to vulva and change the bacterial flora in the vagina. Douching is considered as risk factor for females who have Candida glabrata-induced recurrent vulvovaginal candidiasis.
6. Infection susceptibility is enhanced by endocrine conditions such hypothyroidism, hypoparathyroidism, Cushing syndrome, and polyendocrinopathy.
7. Immunosuppressive conditions include HIV infection, organ transplantation, neutropenia and congenital immunodeficiencies show impaired neutrophil and

macrophage killing, which makes people more susceptible to infection.

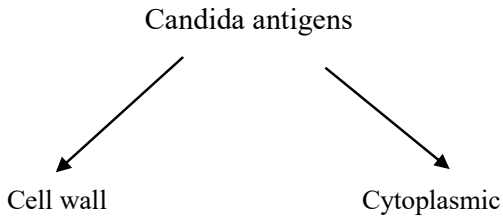
8. Skin maceration and occlusion brought on by tight dressings: Perspiration brought on by inadequately ventilated underwear or tightly fitting clothing elevates local temperature and moisture levels. Infection may also be exacerbated by mechanical irritation of the vaginal region brought on by clothing or sexual activity.
9. Nutritional factors: Nutritional deficits may change the integrity of the epithelial barrier or the host's defence mechanisms, allowing for more adhesion or penetration. Oral candidiasis is brought on by iron insufficiency, but unbound Iron promotes the growth of *C. albicans*. Keratinization is impacted by vitamin A deficiency. Higher infection rates are linked to deficiencies in the B complex vitamins, vitamin C, and folic acid. Chronic GI sickness brought on by zinc deficiency can result in recurrent vulvovaginal candidiasis. Host defence is compromised by protein insufficiency.
10. Genetic factors: Women who do not secrete Lewis antigen - a glycoprotein that prevents *Candida* from adhering to the vaginal mucosa, are at risk for developing vulvovaginal candidiasis again.[22] Risk factors include genetic polymorphisms and innate yeast hypersensitivity.[23,24] In individuals with recurrent vvc, the vaginal epithelial cells have reduced levels of anti-*Candida* activity compared to those in healthy women.[25]
11. Atopy: Women with RVVC were occasionally found to have atopy. In a research where 95 patients with RVVC and 100 controls were compared, allergic rhinitis was more common in the RVVC patients (74% vs. 42%).[26] Few case report indicated that ↑sed titers of eosinophils & IgE specific to *Candida* were present in vaginal washes, suggesting that there may be

contribution of local immune response to RVVC.[27,28] However, the proportion of RVVC patients who had IgE or eosinophil evidence was significantly lower than that of healthy females.[29,30]

12. Sexual factors: The spread of microbes might be aided by orogenital sex. [31] Among male sexual partners some may suffer with itching, erythema and burning in genitalia minutes- hours after sexual activity, the function of sexual transmission is debatable. When treating male sexual partners with ketoconazole, a randomized controlled trial found that the treatment groups' recurrence rates at six months and a year were comparable to those of the untreated partner groups.
13. Serum factors: Transferrin and clumping factor are examples of serum components that stimulate the development of germ tubes and mycelia, which facilitate invasion.[32] These substances also impair T cell function, can be reversed with antifungals.
14. HIV and vaginal candidiasis: In females with HIV infection, VVC, 1 of the most prevalent fungus infections, recurs frequently. Even if systemic fungal infection and mortality are only a very remote possibility, Candidial vulvovaginitis must be prevented and treated to enhance quality of life for HIV-positive patients. *Candida* infections are most frequently seen in HIV-positive people as oral, esophageal, and vulvovaginal candidiasis. They are precursors of systemic opportunistic infections as well as markers of the severity of immunological dysfunction. The immunodeficiency in HIV-positive people is directly correlated with the prevalence of vaginal candidiasis. This is brought on by immune suppression & regular use of antibiotics for a variety of bacterial opportunistic illnesses.

15. Immunology: Innate and acquired immunity are both essential for maintaining the organism in commensal state and to prevent it from entering into the bloodstream. Neutrophils play significant role in defence against systemic infections, whereas Th1-CD4+ T-lymphocytes' cell-mediated immunity (CMI) promotes defense against mucosal infections. The effectiveness of antibodies against the *Candida* infection is debatable. Epithelial cells from healthy people's saliva and vaginal lavages have been shown in recent investigations on mucosal innate resistance to suppress growth of *Candida* (invitro). OE cells taken from HIV+ Candidates with oral candidiasis have much less activity. Studies indicate some protective role for the epithelial cells suggesting that immunity to *Candida* is site-specific.

Fungal infections are typically mild & self-limiting which indicates that most individuals have a high level of innate protection against harmful fungi. The principal barriers to infection are mucosal surfaces and healthy skin. The main contributors to host resistance include epithelial cell turnover, skin fatty acid concentration, and low vaginal pH. Bacterial flora of skin & mucosa competes with fungus & prevent their unrestricted growth. Any breach in the natural barrier and changes in the bacterial flora promote illness. The outcome is determined by the organism's virulence, inoculum size, and host defence effectiveness. Protection from pathogenic Candidial colonization is provided by cell-mediated immunity. Phagocytosis is primary mode of elimination of infection. Ab along with complement have a role in eliminating fungi from the body.



These Ags are useful for serological test. The main antigenic element of the cell wall is mannan.

PATHOGENESIS:

Pathogenicity of Candida spp. is mediated by a number of virulence factors that include adhesion, biofilm formation, extracellular hydrolytic enzyme production, hyphal formation, phenotypic switching.

Steps in pathogenesis:

- Adherence
- Invasion
- Host immune response

Additional studies established the critical function cytokines and interleukins play in candidial infection. Through toll-like receptor 2, *C.albicans* phospholipomannan causes an inflammatory response in keratinocytes. Interferon-gamma-induced protein-10 expression in human keratinocytes is decreased by *Candida albicans*. Lack of interleukin 12 receptor type 1 makes people more susceptible to candidiasis, most commonly mucocutaneous candidiasis.[11]

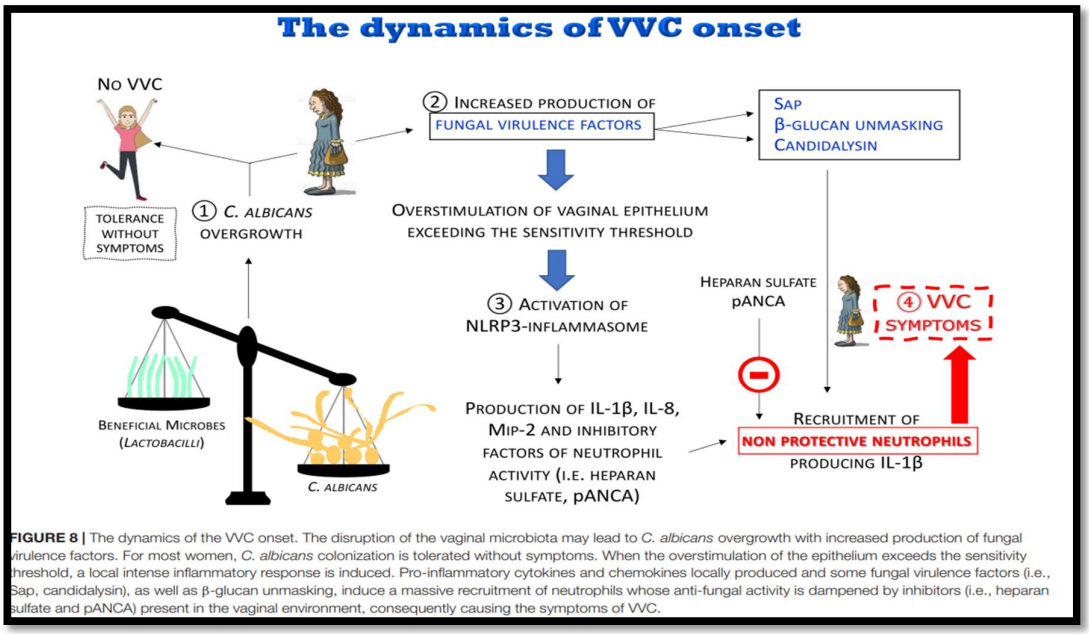


FIGURE 8 | The dynamics of the VVC onset. The disruption of the vaginal microbiota may lead to *C. albicans* overgrowth with increased production of fungal virulence factors. For most women, *C. albicans* colonization is tolerated without symptoms. When the overstimulation of the epithelium exceeds the sensitivity threshold, a local intense inflammatory response is induced. Pro-inflammatory cytokines and chemokines locally produced and some fungal virulence factors (i.e., Sap, candidalysin), as well as β-glucan unmasking, induce a massive recruitment of neutrophils whose anti-fungal activity is dampened by inhibitors (i.e., heparan sulfate and pANCA) present in the vaginal environment, consequently causing the symptoms of VVC.

Fig 1: The dynamics of VVC onset[33]

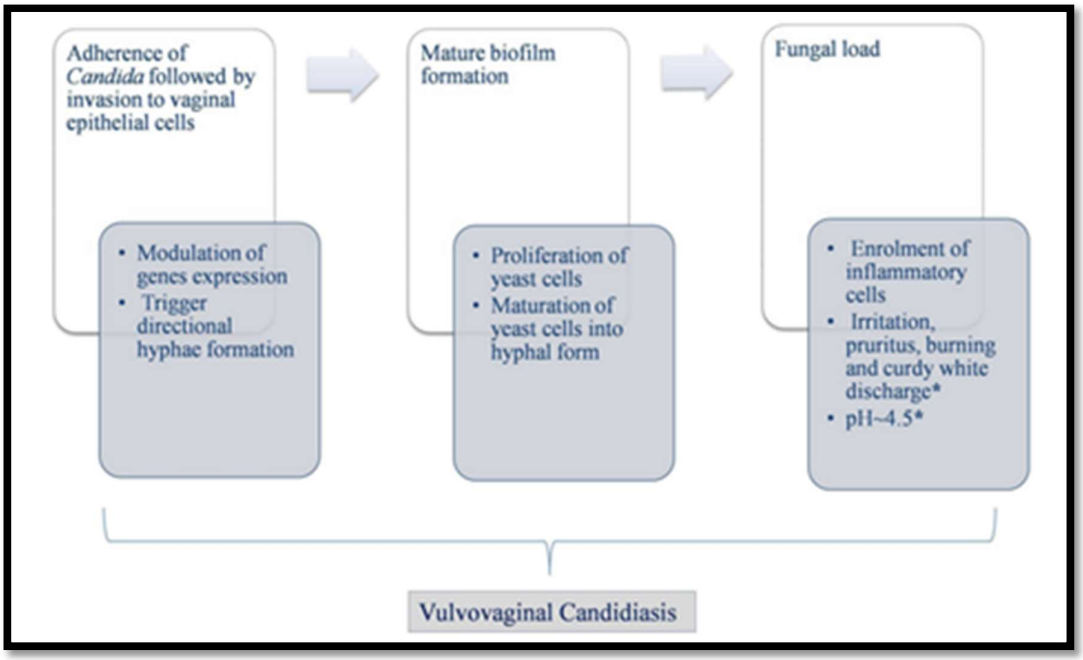


FIG 2: Pathogenesis of *Candida* & clinical symptoms[34]

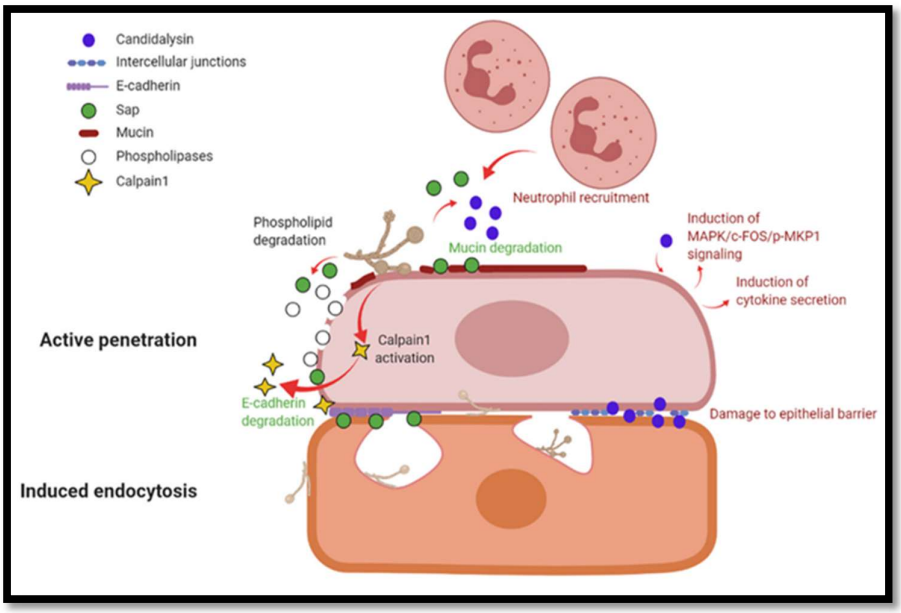


FIG 3: Depicting invasion by *Candida*: active penetration & induced endocytosis[33]

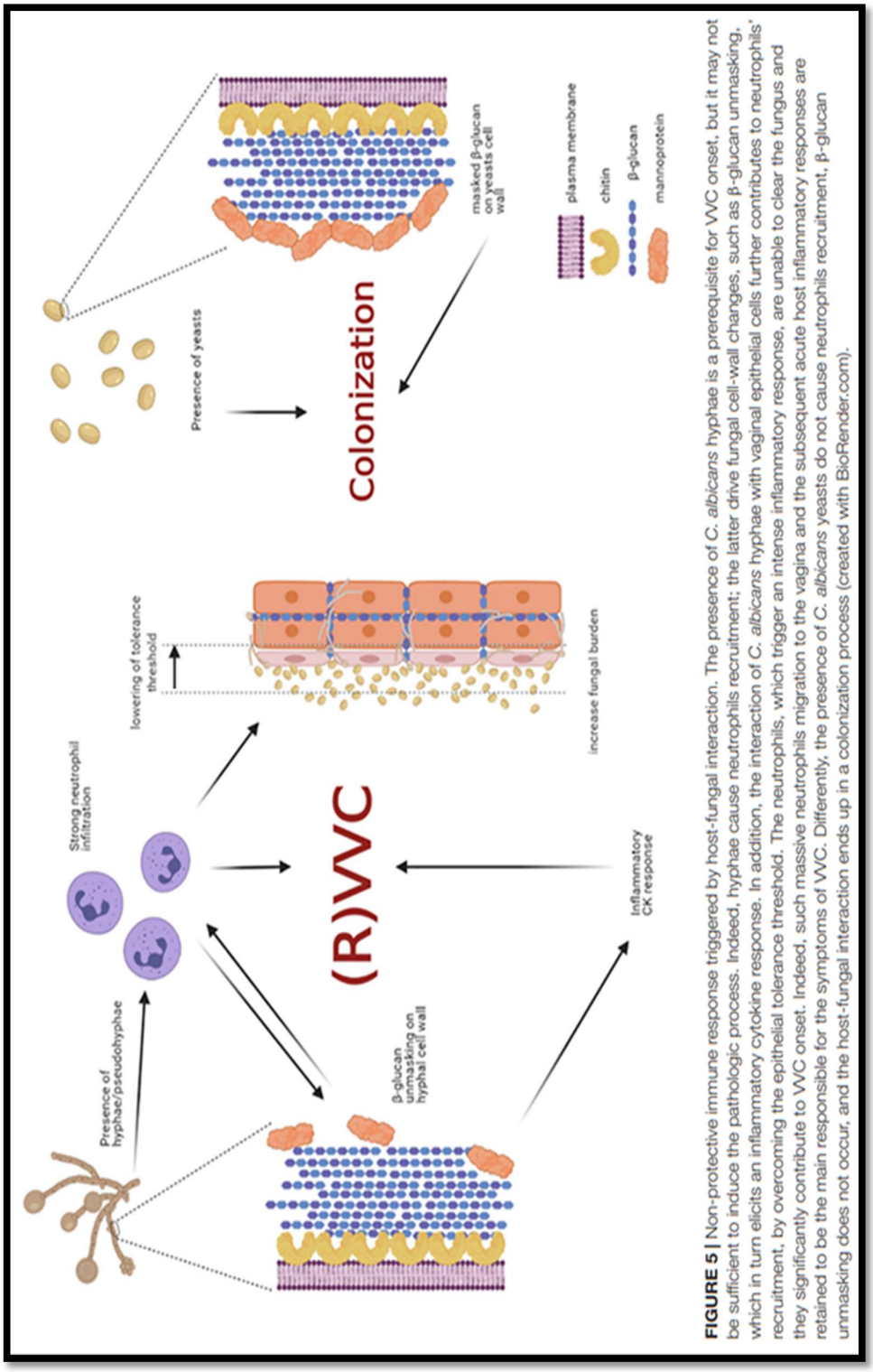


FIGURE 5 | Non-protective immune response triggered by host-fungal interaction. The presence of *C. albicans* hyphae is a prerequisite for VVC onset, but it may not be sufficient to induce the pathologic process. Indeed, hyphae cause neutrophils recruitment; the latter drive fungal cell-wall changes, such as β-glucan unmasking, which in turn elicits an inflammatory cytokine response. In addition, the interaction of *C. albicans* hyphae with vaginal epithelial cells further contributes to neutrophils' recruitment, by overcoming the epithelial tolerance threshold. The neutrophils, which trigger an intense inflammatory response, are unable to clear the fungus and they significantly contribute to VVC onset. Indeed, such massive neutrophils migration to the vagina and the subsequent acute host inflammatory responses are retained to be the main responsible for the symptoms of VVC. Differently, the presence of *C. albicans* yeasts do not cause neutrophils recruitment, β-glucan unmasking does not occur, and the host-fungal interaction ends up in a colonization process (created with BioRender.com).

FIG 4: Immune response: Host-fungal interaction [33]

CLINICAL FEATURES:

C/F differs in severe vvc & asymptomatic vvc.

Clinical classification**:**

CDC classified VVC in 2 groups.

UNCOMPLICATED VVC : Sporadic & ingrequent in non-immunocompromised with mild/moderate symptoms, causative organism in *Candida albicans*.

COMPLICATED VVC: Recurrent episodes (4 or more per year) ,severe, associated with pregnancy , poorly controlled diabtes & HIV infection by the non albicans *Candida* species.[6]

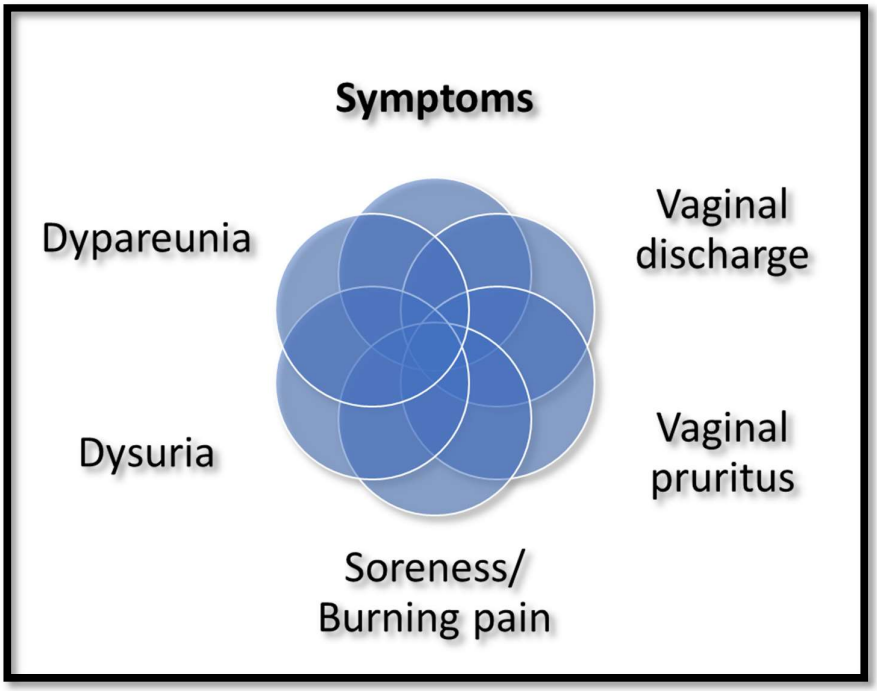


FIG 5: symptoms of vvc[6]

Signs:

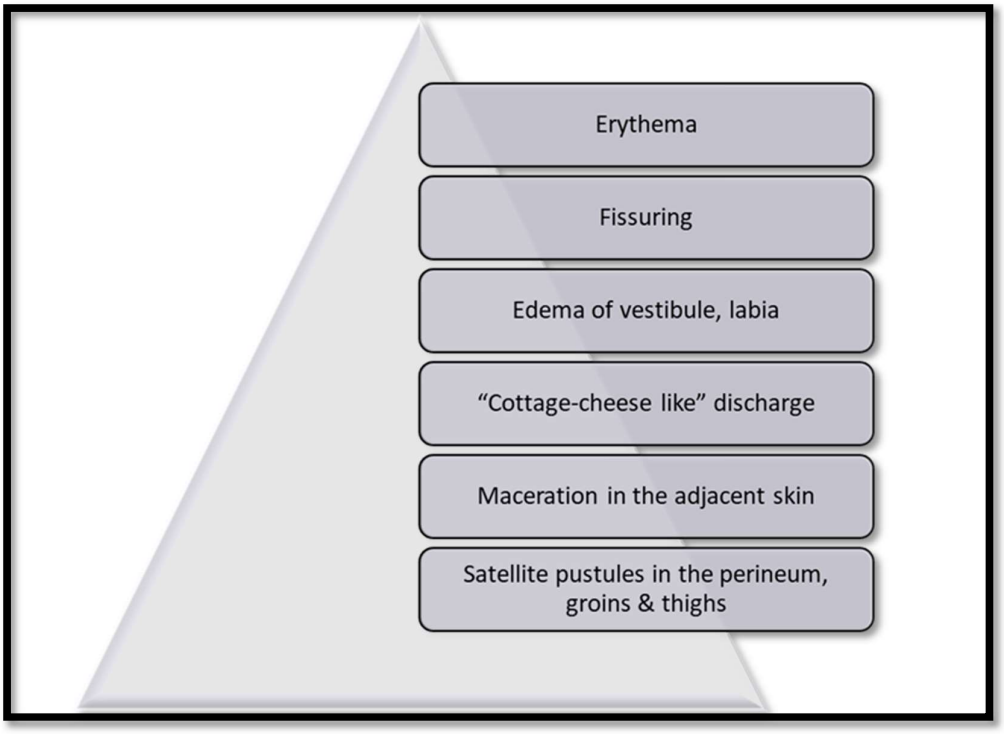


FIG 6: SIGNS OF VVC[6]

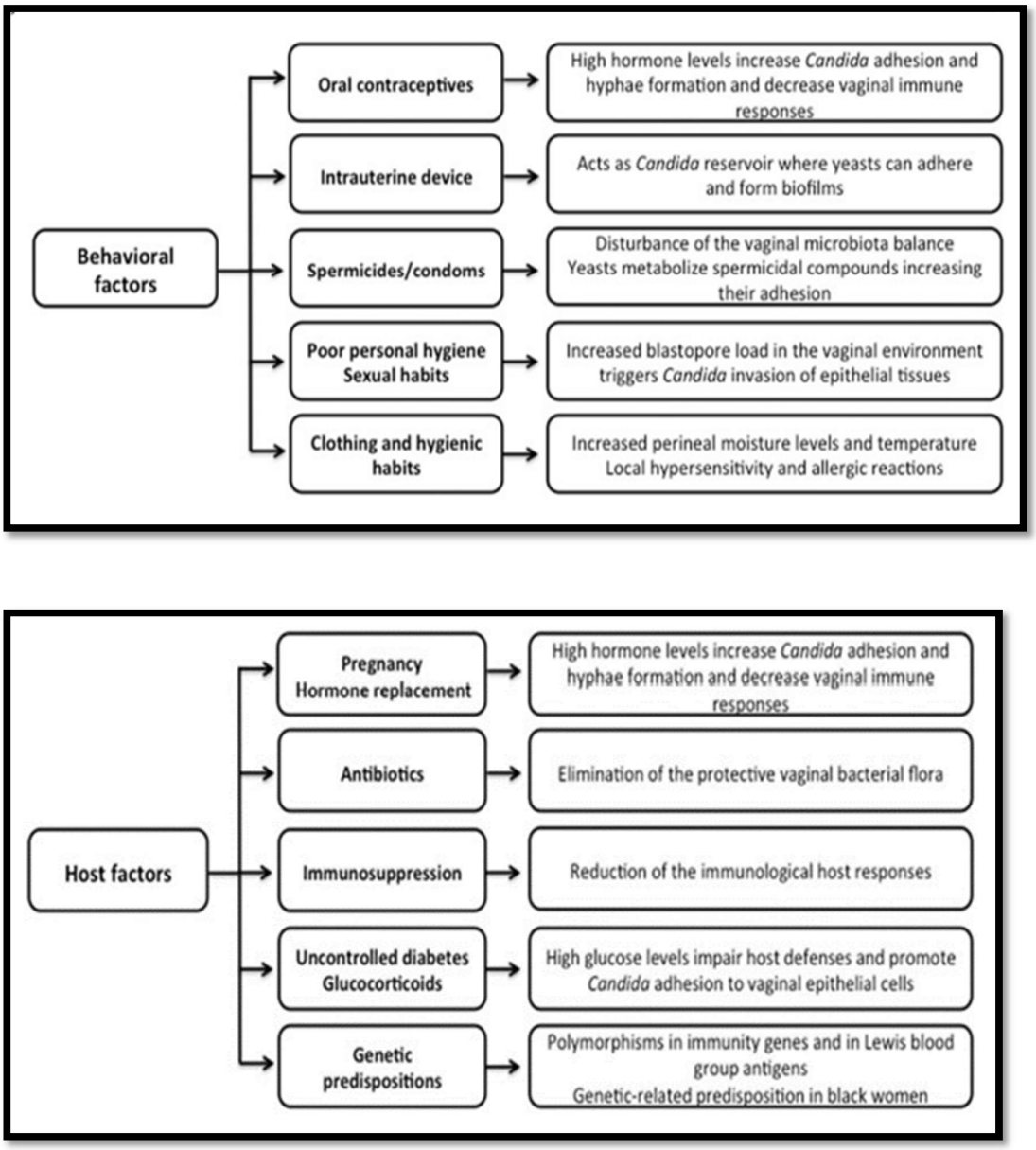


FIG-7 : Risk factors of vulvovaginalcandidosis[35]:

Differential diagnosis:

Infectious conditions:

- Infection with Chlamydia, Trichomonas or bacterial vaginosis
- Genital herpes
- Genital scabies

Allergic or Chemical vulvitis, IVS, LP, leucorrhea, Desquamative vaginitis, contact dermatitis are the noninfectious condition that may mimic like vvc.

Treatment regimens of VVC: The cell wall of *Candida* is a complex glycoprotein that depends on the biosynthesis of ergosterol. Azole compounds, found in antimycotic drugs, are believed to block ergosterol production, allowing topical antimycotics to achieve cure rates in excess of 80%. The only oral azole agent approved for this indication by the US Food and Drug Administration (FDA) is fluconazole, which also shows a high cure rate. Adequate therapeutic concentrations are found in vaginal secretions for 72 hours after the ingestion of a single 150-mg tablet.

In considering treatment, distinguishing between sporadic and recurrent episodes of vulvovaginal candidiasis is of great importance. Uncomplicated sporadic VVC is caused by C. albicans. These strains exhibit sensitivity to azole antifungals.

Studies have shown there is no marked difference in the clinical efficacy of various topical azole antifungals. So starting empirical treatment without waiting for culture results is advisable. In uncomplicated cases treatment is selected according to patient's preferences.

Ample number of antifungal regimens are available for the treatment of vulvovaginal candidiasis including oral and topical agents. But drug interaction with oral usage must be taken in to consideration. Hepatotoxicity secondary to ketoconazole therapy occurs in approximately 1 in every 10,000-15,000 individuals exposed to this drug. Side effects include nausea, abdominal pain, and headaches can also occur. Drug interactions occur with simultaneous use of warfarin, calcium channel antagonists, theophylline, rifampicin, cyclosporine, oral hypoglycemic agents, protease inhibitors and to name a few.[11]

As per CDC's STI Treatment Guidelines, 2021 treatment of VVC is as followed[6]

ANTIFUNGALS	**DOSAGE**
COTRIMAZOLE 1% cream CLOTRIMAZOLE 2% cream MICONAZOLE 4% cream TERCONAZOLE 0.4% cream	5g intravaginally daily for 3 days
MICONAZOLE 2% cream TERCONAZOLE 0.4% cream	5g intravaginally for 7 days
TICONAZOLE 6.5% ointment BUTOCONAZOLE 2% cream	5g intravaginally one day
VAGINAL SUPPOSITORY: 1200 mg MICONAZOLE 200 mg MICONAZOLE 100mg MICONAZOLE 80 mg TERCONAZOLE	 1 day OD for 3 days OD for 7 days OD for 3 days

Oral Agents Fluconazole 150 mg SD

Non – albicans VVC

Therapy duration is longer. It is needed according to antifungal susceptibility or preferably non azole drugs for 7-14 days.

Preventive measures in complicated VVC:

Lactose, sucrose intake may promote growth of yeast, cessation of OCP's shows reduced frequency of clinical episodes. Loose-fitting clothing & cotton underwear are advised. Glycemic control in diabetic patients.

***CANDIDA*:**

Candida spp. are unicellular organisms, round to oval in shape & size range from 2-6 μm. In *Candida* reproduction budding blastoconidia are formed by asexual methods, and by sexual methods produces ascospores, basidiospores. Budding forms elongate & form pseudohyphae. Pseudohyphae have cell wall constrictions.

Candida species - found in human, animals, packaged food. In humans *Candida* species are commensal as can be in GIT, oral cavity, genital area. Infections may be caused by endogenous invasion, if not may be acquired from external sources. Asymptomatic vaginal carriage has been seen in healthy women approx. 21-32%. Resistance for common antifungals are demonstrated by various recent studies.

Classification of *Candida*:

Candida Species:

Candida, Candida tropicalis, Candida kefyr, Candida stelloidea, Candida parapsilosis, Candida rugosa, Candida guiliermondii, Candida, C. krusei, C. dubliniensis, C. vishwanathii, *Candida lusitaniae* .

There are now 200 species of *Candida* known so far. The differences between these species can be found in colony traits, microscopy (morphology), metabolic processes like fermentation and sugar absorption & growth in various conditions.[36]

Adults and the elderly are more likely to contract *C. glabrata* infection than children. Patients with haematological malignancies, particularly those with neutropenic circumstances, are most likely to contract *C. tropicalis*[37] *C. tropicalis* was found in tissues that were surrounded by necrosis in mouse models and a few human studies, showing that the bacterium may enter the tissue effectively. The virulence factors present in *Candida* species, such as aspartyl proteases and acid proteinases, are responsible for this occurrence.[38]

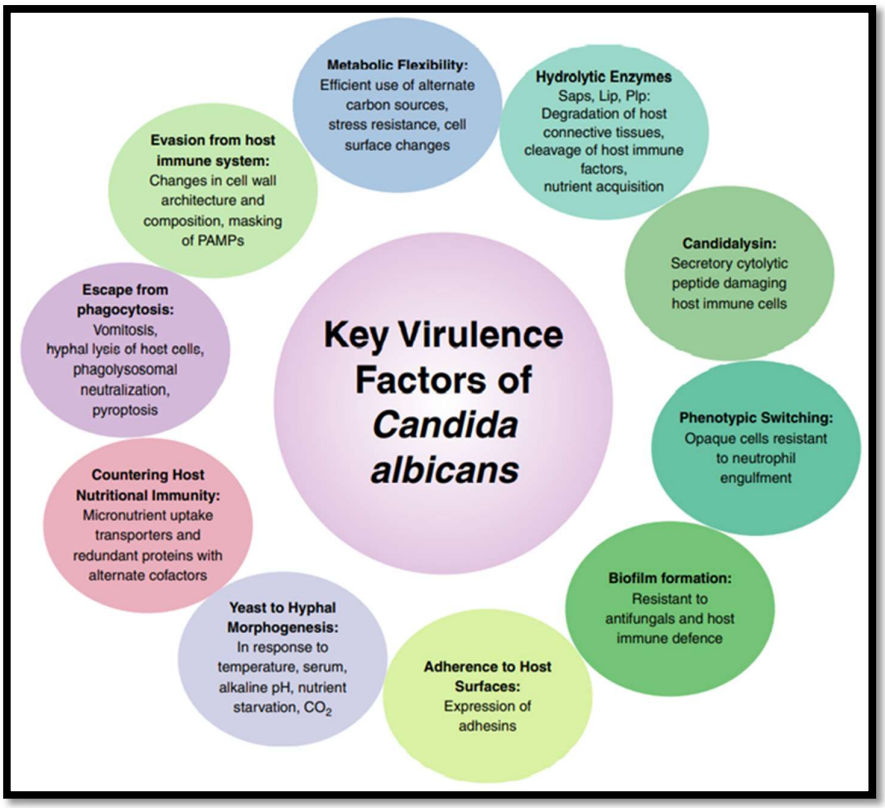
Azole antifungal resistance is linked to *C. krusei*. It can thrive in media devoid of vitamins.

Virulence factors:

Adhesin: These fungal molecules adhere to host buccal, dermal, vaginal epithelial, endothelial cells, oral, GI tissues & some inert substances such indwe1ling medica1 catheters.

1. 1.Severa1 adhesins are discovered so far: - mannans & chitin.
2. 2.Mannans are mannoproteins (proteinaceous substance).
3. 3.Different Candida spp. have different adhesins. Systemic ds. can be caused by *C. albicans* in patients with catheters & also prosthetic valves .[39]

Biofilm: The fungus proliferates to generate biofilm after initially adhering. Proteins and carbohydrates found in biofilm contribute to both the difficulty in clearing biofilm by host-defense system and the poor response to antifungal medications. All Candida species, especially *C. albicans*, can display it. The buildup of biofilm on catheters and artificial valves can result in systemic illness.[40]



Candida spp. secrete a number of extracellular hydrolytic enzymes that are vital for adhesion, tissue penetration, invasion & annihilation of host tissues (Schaller et al., 2o05). Haemolysins, phospholipases, lipases, and secreted aspartyl proteinases (Saps) are the enzymes most frequently linked to Candida pathogenicity (Silva et al., 2012).

Saps are associated with alterations in the host immunological response and enhance adherence to and injury to host tissues. Ten SAP genes (SAP1-10) have so far been discovered in *Candida albicans.* 3 in *Candida parapsilosis* and at least four in *Candida tropicalis* (SAPT1-4) but most of d genes in NAC species are yet uncharacterized. Few investigations have shown *C. glabrata* create proteinases, however the specific typ of proteinase is yet unknown. Saps only exhibit proteinase activity in an acidic environment (Williams et al., 2011). Numerous investigations have shown that *Candida* spp. isolated from women wd VVC exhibit high expression

of SAPs and higher activity of proteinase than asymptomatic vaginal *Candida* carriers. Additionally, it has been shown that VVC and the expression of *C. albicans* SAP1-3 are strongly and specifically correlated. In glycerophospholipids, phospholipases hydrolyze one / more ester linkages. Phospholipases hydrolyze one /more ester bonds in glycerol phospholipids. Glycerophospholipids contain one or more ester linkages that phospholipases hydrolyze, resulting in host-cell membrane disruption and yeast adherence to host tissues. It has been discovered that there are more phospholipase-producing strains from vaginal isolates of individuals with candidosis than in resp. and cutaneous isolates, despite the fact that several *Candida* species can manufacture extracellular phospholipases. Seven phospholipase was identified in *C. albicans* (PLA, PLD1-2, PLC1-3, PLB1), and it was found that PLB1, PLB2 were expressed in the vaginal washes of both infected women & asymptomatic vaginal *Candida* carriers though at lower levels inn healthy women. Triacylglycerols are hydrolyzed by lipases, and lipase activity linked to *Candida* adherence, host tissue damage, and immune cell disruption (Stehr et al., 2004). Ten genes (LIP1–10) in *C. albicans* code for lipases, while two lipase genes have been found in *C. tropicalis* and C. parapsilosis, respectively (CpLIP1-2). Particularly in regards to their precise association with anatomical site of infection, these enzymes have received less research than Saps and phospholipases. By destroying haemoglobin, the haemolysins that *Candida* species create make it easier to collect iron, which is necessary for their survival, persistence in the host.

Production of these proteins has been described in several *Candida* species *(C. albicans C. glabrata C. parapsilosis & C. tropicalis*; the genetic expression of haemolytic activity is not understood clearly .[35]

Phenotype- switching: *Candida* can switch reversibly from unicellular budding

yeast to pseudo hyphae. Colony morph changes from smooth to rough and fuzzy. Slutsky et al. originally discussed this phenomenon in 1985 and 1987. Phase variation in bacteria is similar to this. The organism can adapt to different anatomical places in the human body by expressing fungal plasticity. This characteristic makes it easier for the organisms to get past the host's defenses.

Laboratory diagnosis: VVC diagnosis is often made clinically and t/t can be empirically started.

Direct microscopy

Direct ME : clinical specimens reveals budding yeast cells - blastoconidia, pseudohyphae with points of constrictions.

GTT: This experiment shows that yeast can grow germ tube. Suspected colonies are suspended in regular human or sheep serum for a period of two to three hours at 37°C. A suspension drop is dn taken on a slide and looked at under a microscope. Long tubes-like extensions known as germ tubes are visible protruding from yeast cells. The pseudohyphae's point of attachment is unconstricted. The REYNOLDS BRAUDE phenomenon refers to this test.

Chlamydospores: Cornmeal polysorbate 80 Agar is used for demonstration of chlamydospores. At 22°C-25°C for 48–72 hours, yeast generates chlamydospores. Chlamydospores are thick-wal1ed refractile cel1s that are generated at low temperatures in nutrient-poor, oxygen-deprived environments.[41]

Assimilation test

It represents best identification of NAC species depending on the capability to absorb coal hides (organic compounds).

Agar plates with *Candida* where paper discs impregnated with various carbohydrates are placed. Candidial development around a specific disc is a sign for assimilation of that specific carbohydrate.

Fermentation: It is carried out in liquid media that has been supplied with various carbohydrates and a colour indicator. The fermentation reaction's gas generation is indicated using a Durham's tube. Different colour changes serve as indicators of acid generation and pH variations.

Culture: The most typical media used for the culture of fungus, moulds, and yeasts is SDA. To avoid bacterial contamination, it is given with gentamycin, chlorheximide, and chloramphenicol in addition to glucose, neopeptone, and polypeptone agar. Nitrogen is provided by peptone agar and carbon by glucose. The media has a pH that leans slightly acidic. Clinical significance of the colony and microscopic morphology is minimal. Vaginal swabs are incubated at 25°C and 37°C after being inoculated with SDA agar. Within 24 to 48 hours, colonies start to appear

CHROM Differential agar: CHROM agar is a quick plate-based assay for isolating and simultaneously identifying several *Candida* spp. The media contains chromogenic material that specific enzymes released by various *Candida* species react with to produce a distinctive colony colour. It has a pH adjustment of 6.1 and contains glucose, peptone, agar, and chromogenic mix. Store it away from direct sunlight in a temperature range of 2 - 8℃. Samples must be injected and then incubated for 48 hours at 37° C.

Features of different *Candida* species:

***C.albicans*:** At 48 hours*, C. albicans* develops into medium-sized, smooth, green-dark meta1lic green colored moist colony

***C.tropicalis*:** Medium-dark meta1lic blue-colored smooth medium sized colonies appear in 24 hours

***C. krusei*:** Rough to crenated, pink colored dry colonies. They are flat large & spreading type.

***C.glabrata*:** Darker mauve center-colored colonies of medium size.

Other spp. – shows white/pink colored colonies

NON CULTURE METHODS:

SEROLOGICAL TESTS

Antibodies to mannan protein

Gel Immunodiffusion

Immunoelectrophoresis

ELISA to detect antibodies against enolace & heat shock proteins

Detection of arabinitol in body fluids of infected person

PCR: By using the PCR, a portion of the DNA molecule can be picked out and amplified. Although quite expensive, this method is far more precise than traditional ones. ≤10 yeast colonies can be detected in vaginal swab by PCR. It is extremely sensitive but not appropriate for separating asymptomatic candidiasis from other types. For the diagnosis of invasive candidiasis, PCR can be used to quantify *Candida albicans* DNA. Single, direct PCR, multiplex PCR, Dot blot hybridization detects *Candida* in sample blood by targeting specific rRNA sequences.

TREATMENT

The treatment of candidiasis is effective with a wide range of medications. Antifungals are the cornerstone of treatment for vulvovaginal candidiasis. An antifungal agent selectively removes fungal pathogens with little harm to the host. In addition to the antifungal agent, other factors that affect clinical response include the illness severity, the patient's compliance with the treatment, and the drug's pharmacological characteristics. The majority of strains of vulvo vaginal candidiasis respond to therapy, and treatment is quite straightforward. Systemic and topical therapies don't differ significantly, according to randomised research. Topical treatment might be used to treat milder types. Systemic therapy is necessary in moderate and severe instances.

In comparison to the discovery of antibacterial agents, that of antifungal agents are lagging. This is because the cellular constitution of the relevant species. As prokaryotic organisms, bacteria have a variety of structural & metabolic goals that are distinct from those of the human host. However, because fungi are eukaryotes, the majority of substances that are poisonous to them are also toxic to their hosts. Furthermore, fungi are more challenging to quantify than bacteria due to their slow development and frequent exhibition of different cellular forms. This makes it more difficult to conduct research intended to assess an antifungal agent's in vitro or in vivo qualities.

Development of major brand new azole antifungal agents gave treatment options for a lot of opportunistic & systemic fungal infections during the 199os. Fluconazole & also itraconazole have proven to be much safer than both ketoconazole & amphotericin B. Despite these advancements, systemic fungal inf. continues to be

hard to deal with, drug resistance to the readily available medications is awaiting. The use of more recent azole class of drugs combined with other antifungal agents with various action mechanisms is likely to offer enhanced efficacy. 2nd generation triazoles were created to offer broad coverage of opportunistic and developing fungal organisms and also overcome resistance to older medications.

Classification of antifungals:

CLASS OF DRUGS	**EXAMPLES**
Polyene	Nystatin Amphotericin B
Azoles	Imidazoles, Clotrimazole, Ketoconazole, Miconazole, Triazoles, Fluconazole, Itraconazole, Voriconazole
Allylamies	NAFTIFINE, TERBINAFINE
Benzylamines	Butenafine
Echinocandins	Caspofungin, Micafungin

Polyene Antifungal Drugs This group includes the antibiotics nystatin, amphotericin, and pimaricin (Natamycin). They cause osmotic instability by interacting with sterols in the fungal cell membrane. Small molecules leak from the inside of the fungal cell to the outside through channels formed by the ergosterol found in fungi and the cholesterol found in humans.

Amphotericin B:

It works across a wide spectrum against *Candida* and other severe fungi infections. With ↑sed serum concentrations, it's fungicidal. If given orally it is not absorbed. Poor CSF penetration is present. It is broken down in the liver and eliminated through the bile and urine. It strengthens Flucytosine's antifungal effects.

Azoles

Ketoconazole, fluconazole, along with itraconazole is owned by this particular team. They inhibit lanosterol engaged in biosynthesis of ergosterol, that is needed for integrity of cell membrane structure & function of fungus. Inhibition of this particular enzyme leads to abnormal sterol buildup inside the cell & additionally results in fungal cell death.

Allylamine

Inhibit ergosterol biosynthesis on the amount of squalene epoxidase. Naftifine, Terbinafine are included in this specific team. Amorolfin, morpholine drug, prevents the identical pathway at later step.

Echinocandins along with cyclo peptides:

They're the newly found group of fungicidal agents. These non-competitively prevent fungal cell wall β-D-glucan synthesis via inhibition of the enzyme 1,3-β glucan synthase. Eg: Anidulafungin, micafungin and caspofungin. Negative events are nausea, vomiting, fever & infused-vein complications. They're less harmful drug. This drug causes embryotoxicity so contraindicated in pregnancy. It may be used in azole resistant *Candida* infection.

Antimetabolite Antifungal Drugs

5- Flucytosine acts as an inhibitor of both DNA and RNA synthesis via the intracytoplasmic conversion of 5-fluorocytosine to 5-fluorouracil. Flucytosine is available in the tablet form.

AZOLES:

Structure: Azoles are a group of nitrogen heterocyclic rings (5 nos) with at least one additional non-carbon element that is either of the following: nitrogen, sulphur, or oxygen. The fundamental chemicals have two double bonds and are aromatic. The analogues azolines and azolidines, which are sequentially decreased, have less. An azole's aromatic bond is created by a single pair of electrons which is from each hetero atom in the ring

Classification of azoles: Two kinds of azole antifungals—triazoles & imidazoles—have same mode of action and antifungal spectrum. In comparison to imidazoles, systemic triazoles metabolise relatively slowly and have less of an impact on human sterol production. Triazoles make up the majority of the novel medications now being developed due to these benefits. Among the azoles Clotrimazole, Miconazole, Oxiconazole, Ketoconazole, Setaconazole, Butoconazole are now available on the market. Triazoles include fluconazole, terconazole, itraconazole, voriconazole, posaconazole, and the investigational medication isavuconazole. Triazoles have less of an impact on cytochromes of mammalians than ketoconazole and miconazole, but both have more severe side effects. All of them carry a danger of hepatotoxicity, and there is also a chance of endocrine malfunction.

Mechanism of action: Primary impact of Triazoles & Imidazoles on fungi is suppression of a microsomal enzyme called 14-alpha-sterol demethylase at amounts reached after systemic injection. The manufacture of ergosterol for the cytoplasmic membrane is impaired by imidazoles and triazoles, which also cause a buildup of 14-alpha-methylsterols. These metabolic intermediates could damage the tightly packed Acyl chains present in phospholipids and interfere with the activities of some membrane-bound enzyme systems & preventing the fungi from growing. Azoles can directly ↑se the permeability of the cytoplasmic membrane of the fungus, although the necessary concentrations may only be attained topically.

Ketoconazole: It is the first imidazole that may be taken orally. Orally, it is readily absorbed. The pH has an impact on the absorption, which varies with diet. It takes 1-2 hours after a single dose for the plasma concentration to reach its peak. The medication does not enter the CSF & in c/o fungal meningitis, therefore its not used. The liver's cytochrome 3A4 enzyme breaks it down. It is eliminated through faeces and urine.

Fluconazole: Triazoles are antifungal. By substituting a nitrogen group for the imidazole group, the antifungal activity & resistance to metabolic degradation are improved. Inhibition of 14 demethylase in the fungal cell wall is the mechanism of action. It can be given intravenously, orally, and there are also eyedrops (o.3%) available. More than 90% of it is absorbed, and it is not reliant on the pH of the stomach (t1/2 is 25 - 30 hrs.) Metabolism in liver is less & 80% of the drug g e t s excreted in urine. It can cross BBB & thus useful for the t/t of fungal meningitis.

Itraconzole: It is a broad spectrum fungistatic, inhibits cyclic oxidase and peroxidative enzymes, causing an increase in peroxide production and the subcellular breakdown of fungi.

- Direct membrane damage caused by phospholipids in the membrane
- Oral solution form of hydroxypropyl beta cyclodextrin displays higher absorption on an empty stomach and in gastric environment with alkaline ph.

It is metabolised by the liver by cytochrome 3A4 and eliminated in the urine and feces [30%]. Because the medicine only reaches low serum levels in children under the age of 5, twice day administration is necessary. After ingestion, the medication is delivered to the skin, as well as the sebaceous, sweat, and nail and nail bed glands. It is detectable in the stratum corneum 3–4 weeks after the medicine has been stopped. CSF is not penetrated by it.

Voriconazole: It is a broad spectrum triazole of second generation that is effective against severe fungus infections. Candida infections in the mouth, the mucosa of the skin, resistant Candida, and fusarium infections

Clotrimazole: It comes in creams ranging from 1-2%.
Available as lotion, powder, spray, and solutions. It results in potassium efflux, nucleic acid breakdown, intracellular protein leakage, and ultimately cell death.

Nystatin: It is an antifungal polyene that comes from the bacteria Streptomyces noursei. Due to its toxicity when blood levels are raised, there are currently no injectable preparations of this medication available. Enzymes such as GDP-mannose dehydratase (nysIII) and glycosyltransferase, aminotransferase (nysDII), P450 monooxygenases (nysL and nysN) are involved in the compound's manufacture (nysDI).

Mechanism of action: Nystatin acts by binding to ergosterol, component of fungus cell membrane, just like natamycin & amphotericin- B do. Ergosterol is only found in fungi, thus the medicine does not effect animals or plants. When present, it generates pores in membrane which cause K+ leakage, acidity, and fungal death. However, a large portion of Nystatin's systemic harmful effects are linked to the drug's impact on human cells as a result of binding to sterols, specifically cholesterol.

It is this consequence that explains the nephrotoxicity seen at ↑sed serum level of Nystatin.

Uses:

Skin, vaginal mucosa & esophageal candidiasis all react favorably to nystatin therapy. It comes in a variety of formats. In high-risk people, oral nystatin tablets are frequently used as a prophylactic therapy.

HIV +ve people with a 1ow CD4+ and people using immunosuppressive medications are at risk for fungal infections. Although fluconazole is the recommended medication, also used as prophylaxis in newborns with VLBW (1500 g) to prevent invasive infections. When given to such infants, the medication also lowers mortality. According to current clinical recommendations, these medications should only be administered to NICU newborns with fungal infections who were born with extremely low birthweights (1000g).

Liposomal Nystatin demonstrated better in vitro activity than Amphotericin B colloidal formulations & has been found to be beneficial against various fungal infections that are resistant to Amphotericin B. Additionally, invasive aspergillosis and other systemic diseases are treated with it. Compared to Amphotericin B,

liposomal Nystatin appears to have a lower risk of severe nephrotoxicity.

Formulations :

- Oropharyngeal thrush is prevented or treated using an oral suspension form. The optimal dosage for GIT Candidal infections is a tablet.
- It can also be applied topically as a cream to treat superficial Candidal infections.
- Nystatin's liposomal formulation researched in the 1980s.

In the early 21st century with the goal of resolving issues brought on by the parent molecule's poor solubility and the resulting toxicity of free medicine.

Spectrum of Antifungal action: The fungi *Candida*, Coccidioides sp., Paracoccidioides are susceptible to the effects of azoles in clinical settings (dermatophytes). The susceptibility of Sporothrix schenckii, Fusarium, Scedosporium apiospermum (Pseudallescheria boydii), and Aspergillus spp. is intermediate. More resistant are the Mucormycosis-causing agents and Candida krusei. Posaconazole has marginally increased mucormycosis agent activity in vitro.

Resistance: As a result of the widespread usage of azoles, primary and secondary resistance have emerged, resulting in clinical failure. Also with advanced HIV infection along with esophageal / oropharyngeal candidosis demonstrates more treatment failure. The 14-alpha sterol demethylase gene, ERG11, has undergone an accumulation of mutations, which is the primary route of resistance in *Candida* albicans. The aforementioned alterations prevent heme from attaching to the azole while still allowing access to the enzyme's natural substrate, lanosterol.

In all azoles, cross-resistance is observed. Fluconazole resistance can be increased in *Candida albicans* and *Candida glabrata* by the azole efflux by ATP-binding cassette & major facilitator transporter. Another potential source of resistance is excessive synthesis of C14-alpha-sterol demethylase. ERG3 mutation in the gene (C5,6 sterol reductase) can also ↑se resistance.

Antifungal susceptibility: Its goal is to give the treating doctor the knowledge they need to choose the best antifungal medications. Because of past antifungal exposure or genetic changes, isolates of the same spp. may show variations in MIC. Common antifungals examined include amphotericin B, 5 flucytosine, ketoconazole, itraconazole, voriconazole, and fluconazole. Newer antifungals have been added to this list recently. A standard for testing for antifungal sensitivity has been established by the NCCLS. However, due to the fact that all azole resistant candidiasis cannot be accounted for by in vitro techniques, its applicability in clinical aspects is quite restricted. With species other than *Candida albicans*, resistance is more prevalent. The alternative solutions for the aforementioned issue include upping the amount of a current medication, switching to a different medication, and taking multiple medications. Common methods used for susceptibility are disc diffusion method & Microdilution method.[11]

MATERIAL & METHODS

STUDY DESIGN: Cross-sectional study

STUDY PLACE: The study was conducted in Department of Microbiology at Jawaharlal Nehru Medical College, Belagavi, Karnataka, 590010

STUDY PERIOD: January 2021 to December 2021.

ETHICAL CONSIDERATION: Approval for the study was obtained from the Institutional Ethical Committee, JNMC Institutional Ethics Committee on Human Subjects Research, J. N. Medical College, Belagavi before commencing it. Informed consent was taken from the study population.

All patients satisfying the inclusion criteria were documented. Patients were interviewed by structured questionnaire.

STUDY POPULATION: All women within reproductive age group (18-45 years) with clinically suspected VVC attending OBG Dept. at KLE'S & Medical Research Centre, Belagavi.

INCLUSION CRITERIA: All new & recurrent cases of clinically suspected VVC presenting to Department of OBG, in the reproductive age group women.

EXCLUSION CRITERIA: Patients on corticosteroids & hormonal therapy (estrogen & estrogen-progestrogen therapy).

DATA COLLECTION: Data collection included Name, Age, Sex, IP number, Sample Id, Ward, Address, Date of admission, Diagnosis, Brief clinical history,

Presenting complaints, History of any interventions, Similar Past history & details of treatment; if any & other associated illness.

SAMPLE SIZE: The minimum sample size based on Prevalence is

$$n = 4pq/d^2$$

where p is the percentage of prevalence

q= (100-p) & d is the relative error.

With p=82% & d=10% of p = 8.2 ≈ **10**

The sample size is 60.

SPECIMEN COLLECTION: 2 High vaginal swabs were collected from each clinically suspected patient.

PROCESSING OF SPECIMENS:

After collection of the sample one swab was inoculated onto Sabouraud Dextrose agar plate & Chrom agar. From other swab direct smear was prepared & Gram staining was done. The inoculated plates were incubated at 35℃ for 24 hours inside Biological Oxygen Demand (B.O.D).

MICROSCOPY:

Gram Stain: The prepared smear was examined under microscope. Yeast cells & hyphae form observed.

CHROM agar: It is used for the identification of different candida species. Colony morphology, color has been we11 defined due to the chromogenic substrate within the medium when it was utilized to isolate the yeast straight from the clinical specimen CHROM - agar – medium could be utilized for presumptive identification and simultaneous isolation 0f various *Candida* – spp. as *C.albicans, C.parapsilosis, C.glabrata, C.tropicalis, C.krusei, C. dubliniensis.* Using this medium improves the speed for the presumptive detection of organisms and enables a lot easier detection of several yeast species with mixed infection found in the clinical specimen. CHROM agar candida displays the following shades of colonies following incubation for 48-72 hrs at 30℃.

SPECIES	**COLOR ON CHROM AGAR**
C. albicans	Light green
C. glabrata	Pink to purple
C. tropicalis	Blue
C. prapsilosis	Cream to pale pink
C.krusei	Pink

Biochemical reaction:

- Germ Tube Test

ANTIFUNGAL TESTING:

Antifungal susceptibility test was done by disc diffusion method. Zone of inhibition is interpreted as susceptible, intermediate, resistant as per standard guidelines.

PHENOTYPIC DETECTION OF VIRULENCE FACTORS BY FOLLOWING METHODS:

- Biofilm formation
- Esterase activity
- Phospholipase activity
- Hemolysin activity

BIOFILM ACTIVITY: Method by Branchini et al[42]

A loopful of test strain from SDA plate

↓

Inoculated into a tube containing 10 ml Subouraud dextrose broth

(Supplemented with 8% glucose)

↓

Incubated at 37℃ for 24 hours. After that the broth is aspirated out & walls of the tube stained with 1% Safranin and kept for 7 mins and then the stain will be removed. Then Biofilm production was graded as Negative, 1+,2+,3+.

ESTERASE ACTIVITY: [43]

Detected by TWEEN 80 medium as described by Slifkin with minor modification: Medium prepared by taking 1000 ml of distilled water with peptone (ten gram) + NaCl (five gram) + CaCl2 (0.1 g) and Agar (15 g). the prepared media is autoclaved and after cooling the autoclaved reagents to 50℃, 5 ml of tween 80 added to it. 25 ml of medium poured into 90 mm petri dish plate and stored in refrigerator. 10 micro lit test strain inoculated on tween 80 medium Plates incubated at 37 ℃ and observed upto 10 days. Production of esterase determined by presence of a halo around the inoculum. Observed against the transmitted light. Diameter of the colony and diameter of the zone along with the colony was measured. Ratio between colony and diameter of the zone and colony was calculated (Ez score); score of 1 taken as negative, 0.63-0.99 as moderate esterase activity & ≤ 0.63 as strong esterase activity.

PHOSPHOLIPASE ACTIVITY:(Method by Price et al) [44]

A volume of 10 microlit of test strain inoculated onto the surface of EGG -YOLK AGAR. Incubated at 37℃ x 7 days. To determine the activity precipitation zone; diameter around the yeast growth to be measured. The ratio between colony and the diameter of the zone and colony calculated known as Pz score. A Pz score of 1 is taken as negative, 0.63-0.99 as moderate phospholipase activity & ≤ 0.63 as strong phospholipase activity.

HAEMOLYTIC ACTIVITY:

(Method by Mann et al.) [45]

4ml fresh sheep blood + hundred ml of SDA supplemented with 3% glucose (pH 5.6)

↓

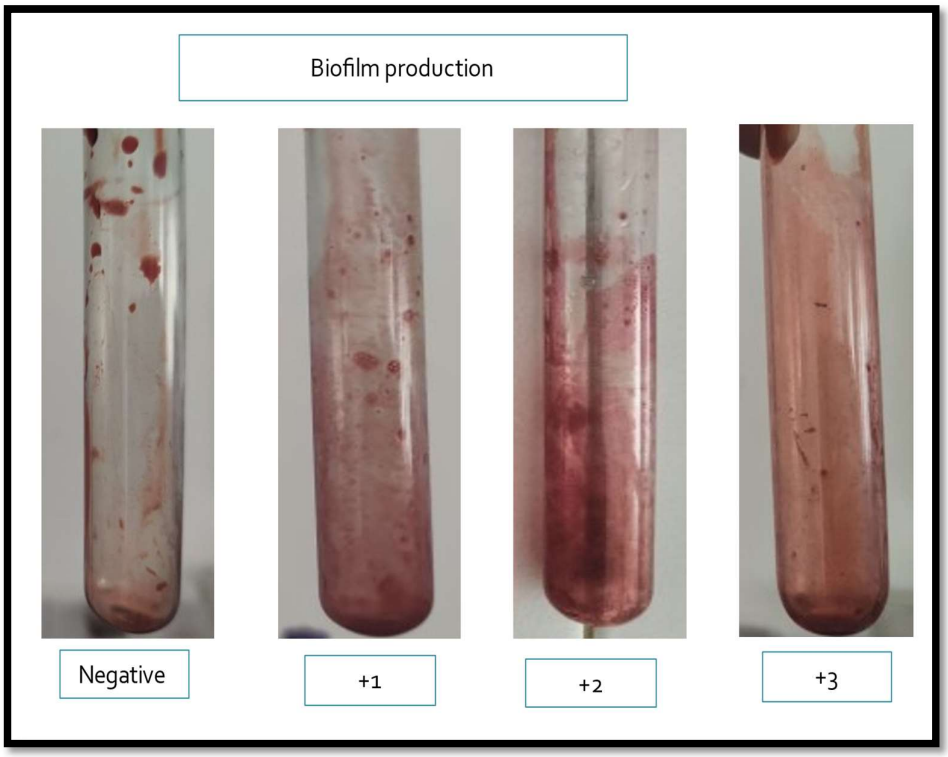
Stored in refrigerator

A volume of 10 micro lit of test strain inoculated on sugar enriched Sheep- Blood Agar medium

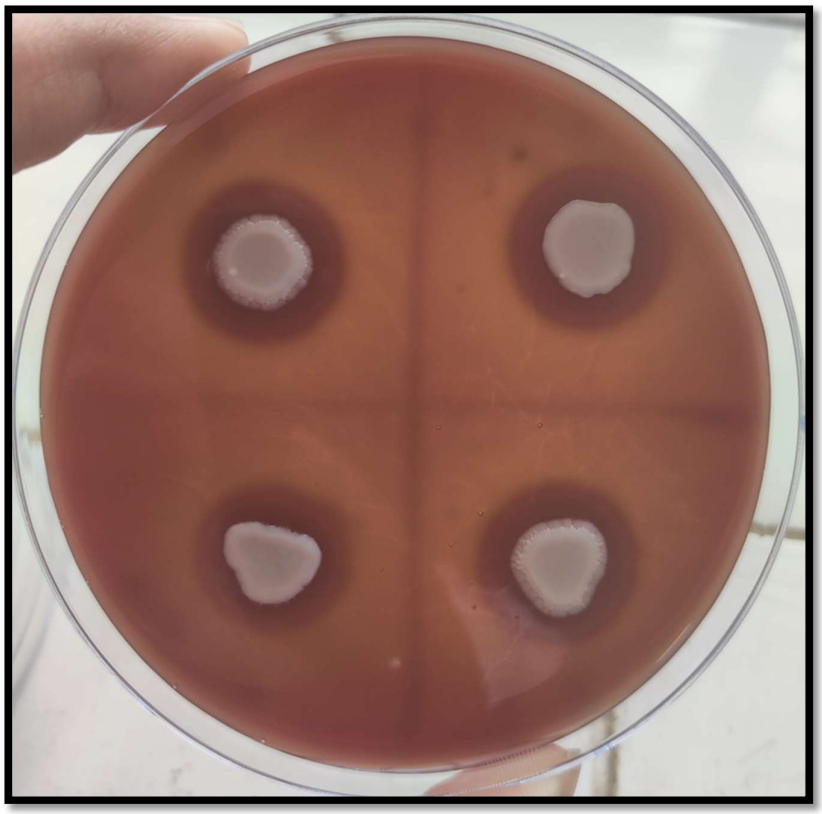
↓

Incubated at 37℃ in 5% CO2 for 48 hours

Haemolytic activity determined as distinct translucent halo around the inoculum site (against transmitted light).

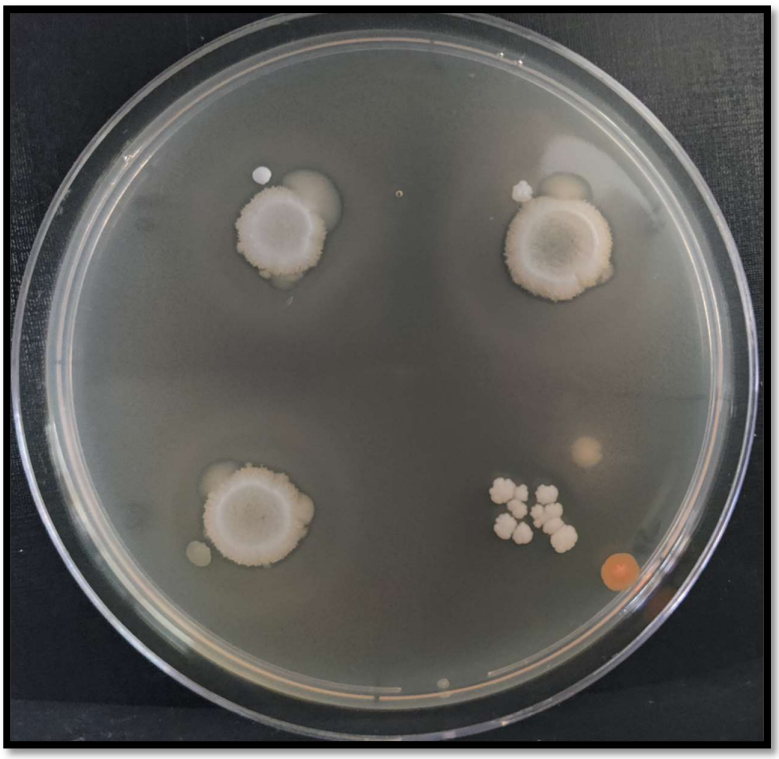


PIC 1 : Biofilm production & its grading by Candida isolates

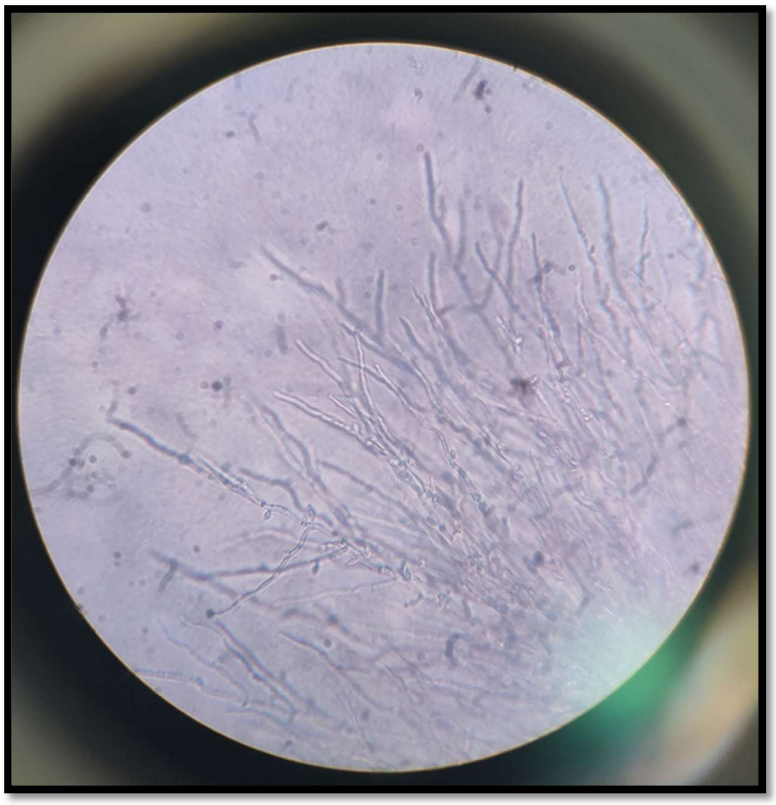


Pic 2: Hemolytic activity

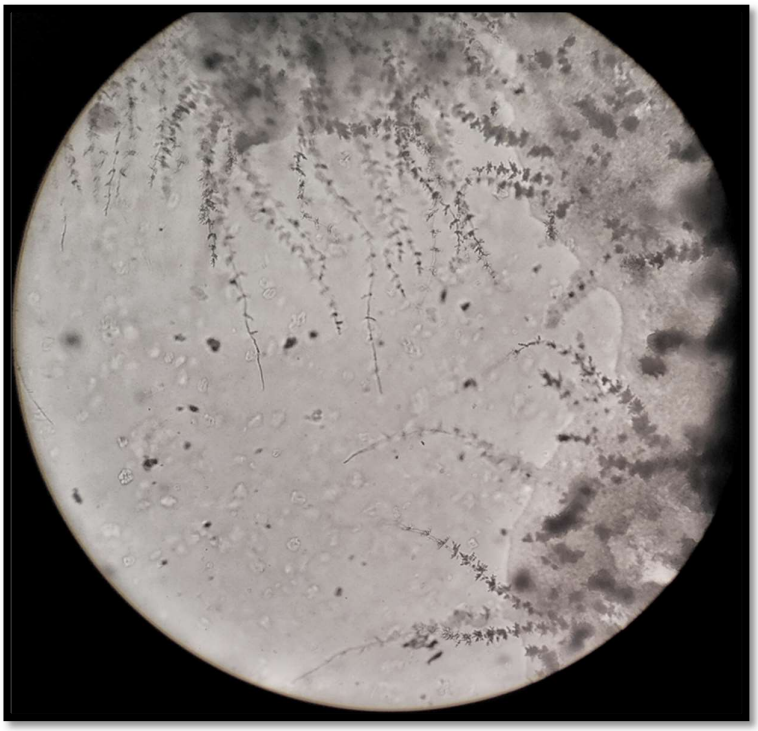
Pic 3 : Phospholipase activity



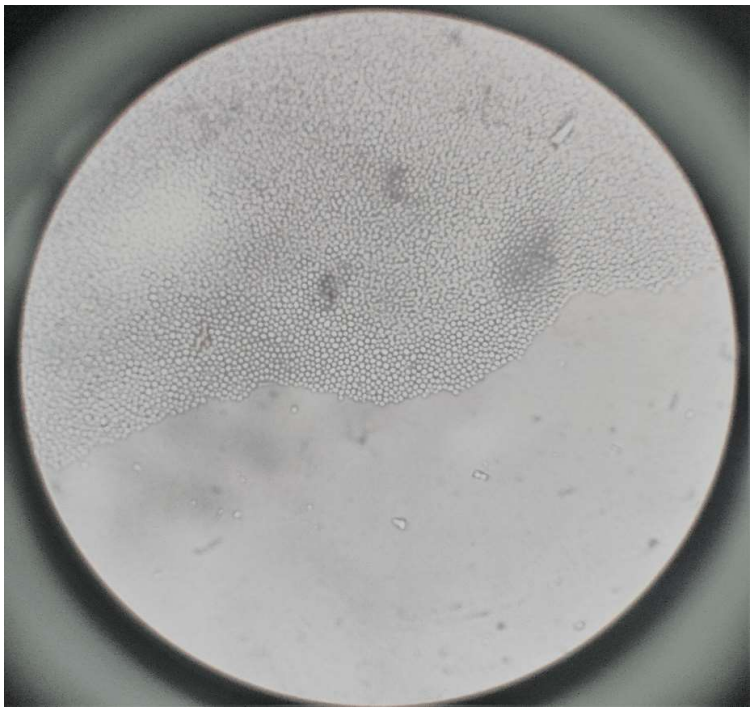
Pic 4: Esterase activity



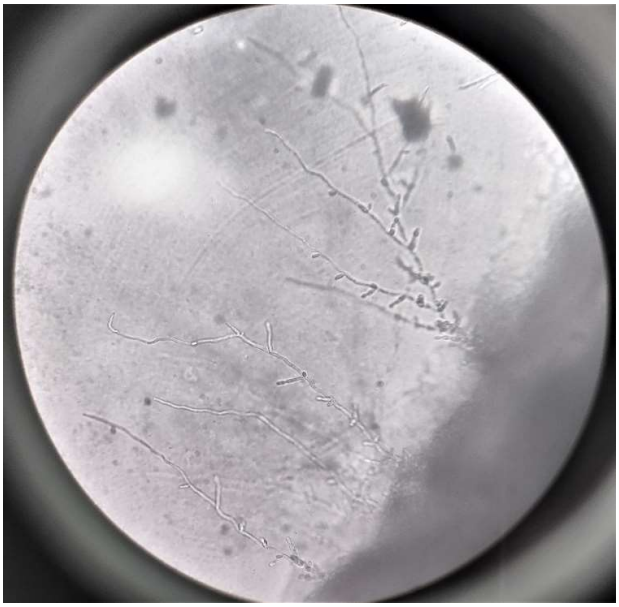
Pic 6: *Candida parapsilosis* on Corn-meal agar



Pic 7: *Candida lusitaniae* on cornmeal agar



Pic 8: *Candida glabrata* on corn meal agar



Pic 9: *Candida tropicalis* on cornmeal agar

RESULTS

Table no 1: Age wise distribution of isolates n=69

AGE GROUP IN YEARS	NO	PERCENTAGE
18-32	60	87%
33-45	09	13%

The table shows highest no of isolates in the age group 18-32 years.

Graph no 1: Age wise distribution of isolates (in percentage)

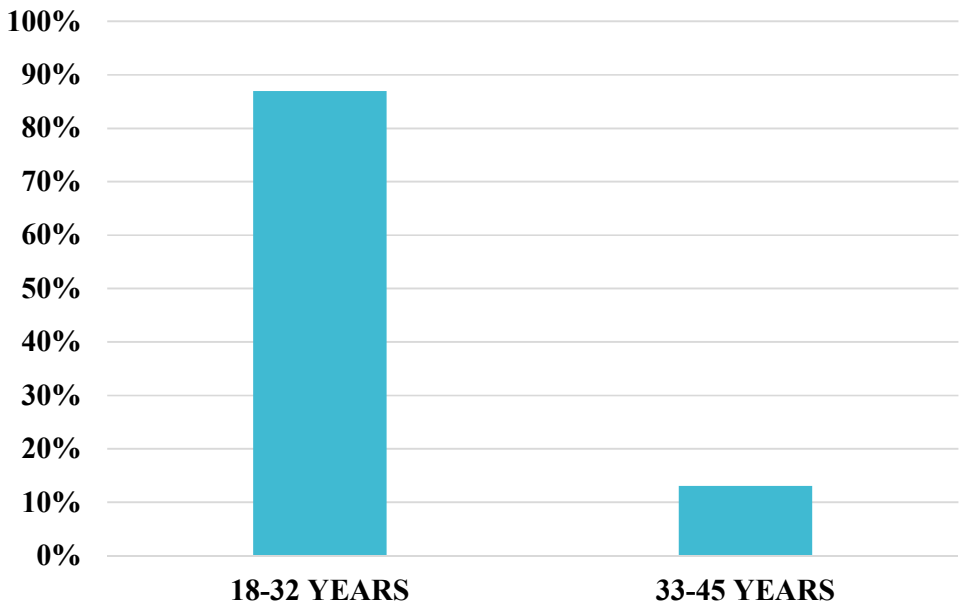


Table no 2:

Distribution of isolates - in relation to pregnancy.

PREGNANCY STATUS OF PATIENTS	NO	PERCENTAGE
PREGNANT	40	58%
NON-PREGNANT	29	42%

Graph no 2:

Distribution of isolates in relation to pregnancy

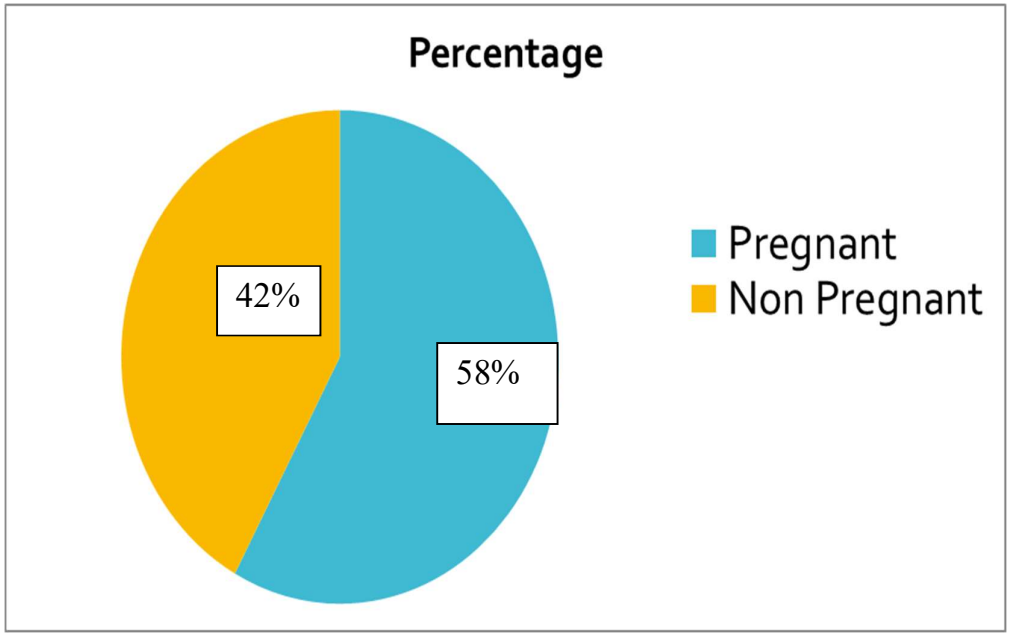


Table no 3: Distribution of isolates in relation to clinical symptoms

SYMPTOMS	NO
Only discharge	8
Discharge with Burning pain	7
Discharge with Dyspareunia	2
Discharge with Dysuria	5
Discharge with Pruritis	58
Total	69

Graph no 3: Distribution of isolates in relation to clinical symptoms

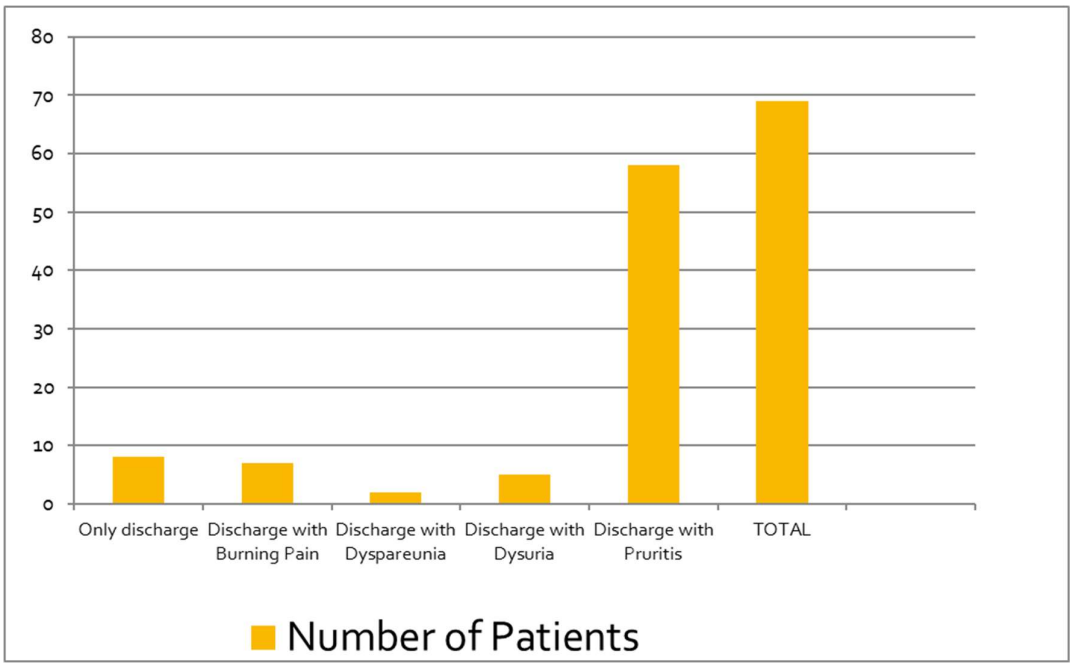


Table & Graph no 4: Distribution of isolates among *Candida albicans* & non albicans *Candida* (NAC)

Candida species isolated	No of isolates
Candida albicans	30
Candida non- albicans	39

Among the total 69 isolates *Candida albicans* were 30 & NAC were 39.

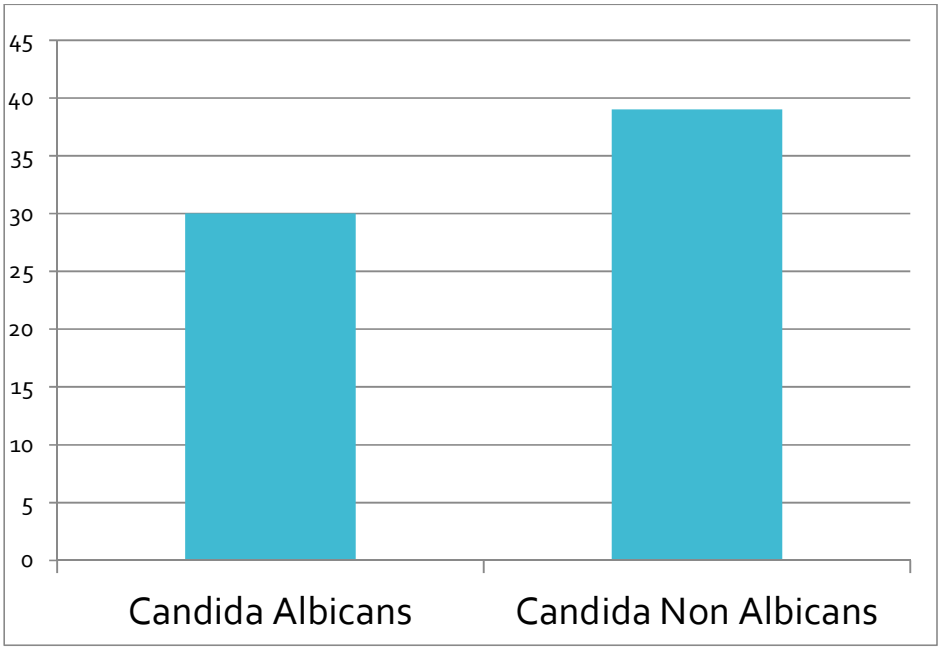


Table & graph no 5: Distribution among different species of non albicans *Candida.*

Among NAC highest no of isolates were *C. glabrata*

Candida species isolated	No. of isolates	Percentage
C. glabrata	23	60
C.tropicalis	11	28
C. Krusei	2	5
C. parasilosis	2	5
C.lusitaniae	1	3
Total	39	100

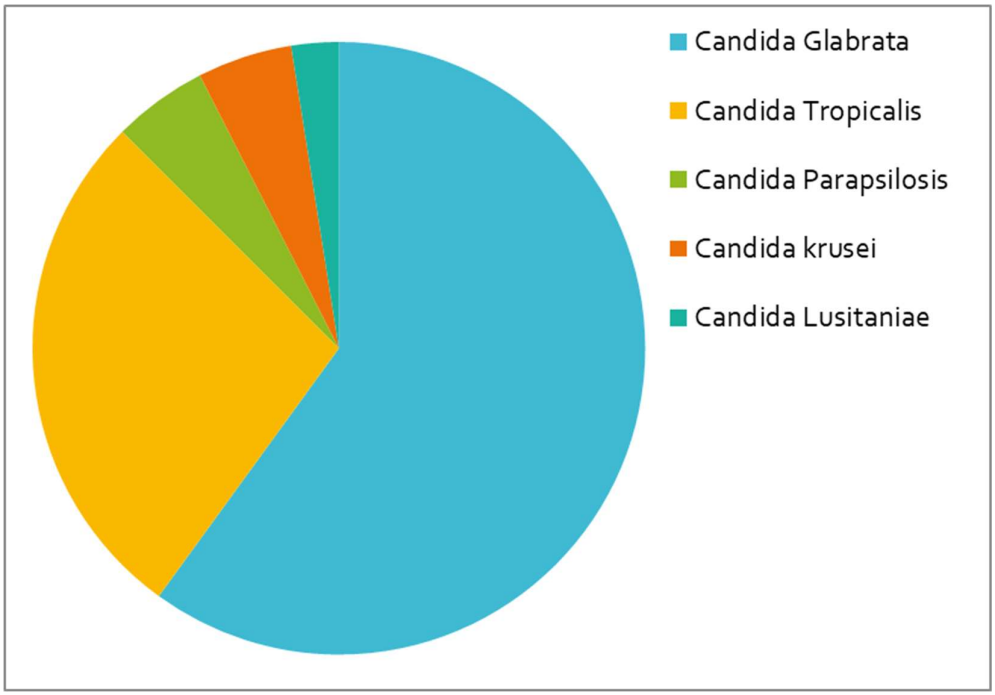


Table no 6: Distribution of virulence factors of *Candida* species.

Candida isolate (n=69)	Biofilm activity	Haemolytic activity	Phospholipase activity	Esterase activity
C.albicans(30)	9 (30%)	23(77%)	28(93%)	9(30%)
C.glabrata(23)	14 (61%)	19(83%)	06(26%)	8(27%)
C.tropicalis(11)	6(55%)	8(64%)	03(27%)	8(73%)
C.parapsilosis(2)	0	2(100%)	0	0
C.krusei(2)	2(100%)	1(50%)	1(50%)	2(100%)
C.lusitaniae(1)	1(100%)	0	0	0
Total	32 (46.37%)	53(76.81%)	38(55.07%)	27(39.13%)

Table no 7: Significant association of *Candida* spp. & virulence factors in VVC

Virulence factors	Candida species	Positive results by species in column 2	Positive results by species other than in column 2	P Value (By Chi-Square Test)
Biofilm production	C.glabrata	14/23	17/31	0.8182
Haemolytic activity	C.albicans	23/30	31/54	0.4175
Phospholipase activity	C.albicans	28/30	10/38	0.0034*
Esterase activity	C.tropicalis	8/11	19/27	0.9525

* Indicates statistical significance.

From Chi square test, it is observed that, there is significant difference in the distribution of phospholipase activity between C. Albicans species and other species.

Table no 8: Distribution of isolates according to grading of esterase & phospholipase activity

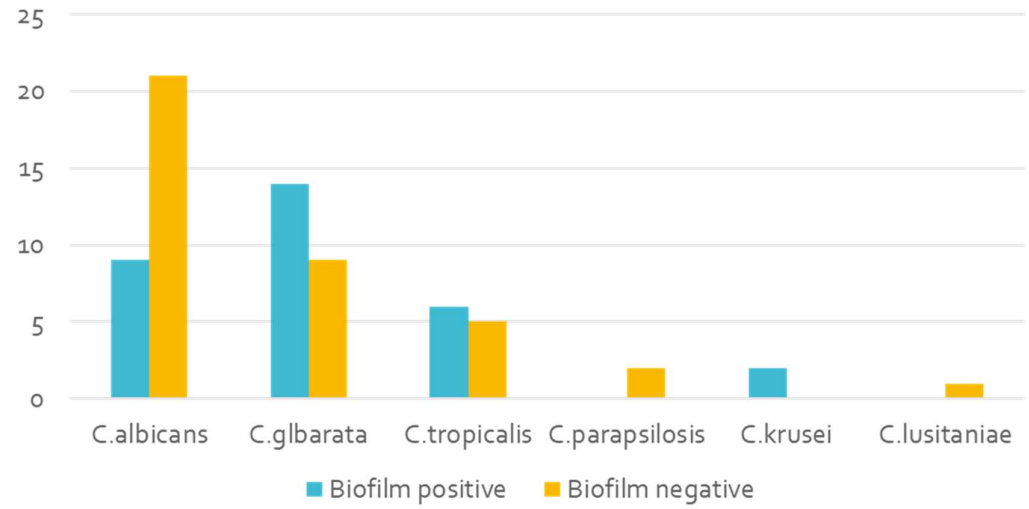
Pz/Ez value	No. of isolates Esterase activity	No of isolates Phospholipase activity
1 (negative)	42	31
0.64-0.99 (positive)	–	–
≤0.63 (strong positive)	27	38

$$Pz/Ez = \frac{\text{Colony diameter}}{\text{Zone diameter} + \text{Colony diameter}}$$

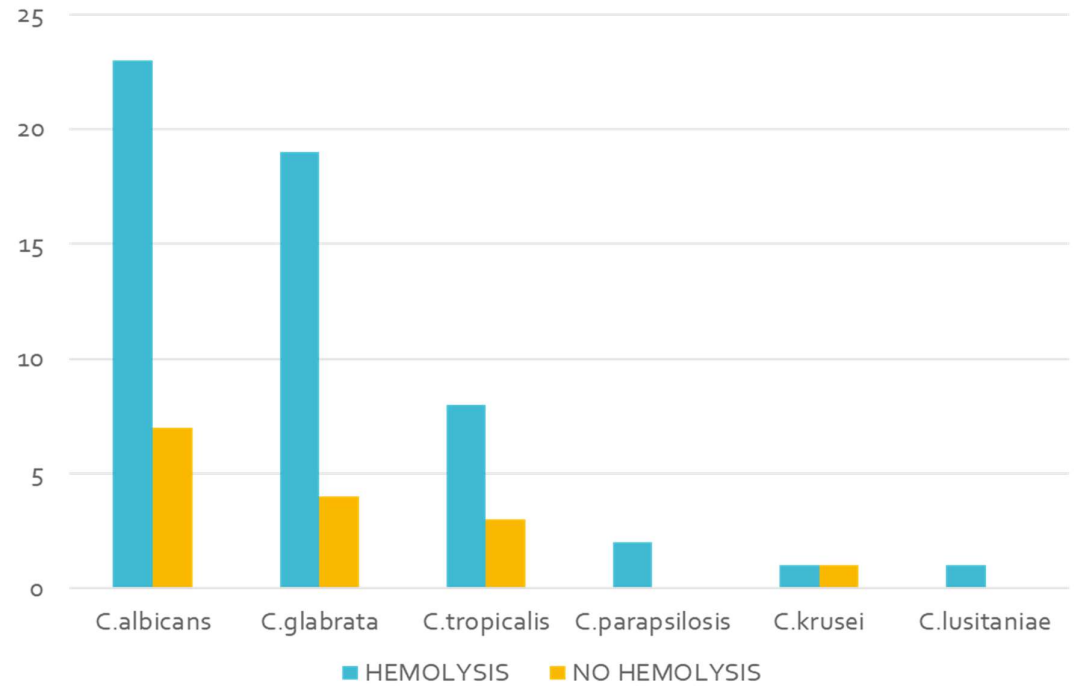
Table no 9: Distribution of isolates according to grade of biofilm production

Grade	Distribution among isolates
Negative	19
+1	7 (5 C. albicans, 2 C.tropicalis, 1 C.glabrata)
+2	22(11 C.glabrata, 4 C.albicans, 3 C.tropicalis, 1 krusei, 1 C.lusitaniae, 2 C,krusei)
+3	3 (2 C.glabrata, 1 C.tropicalis)

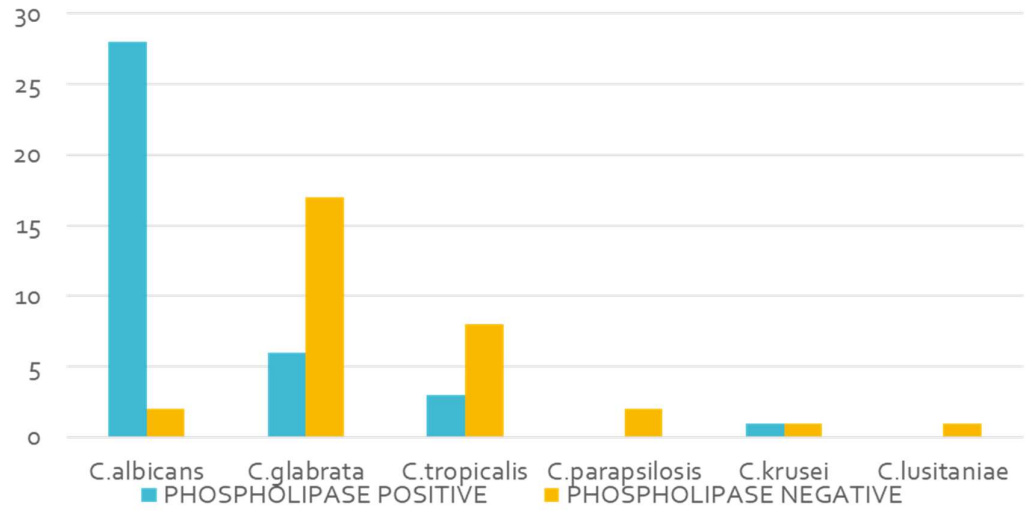
Graph no 6: Biofilm production by different *Candida* species



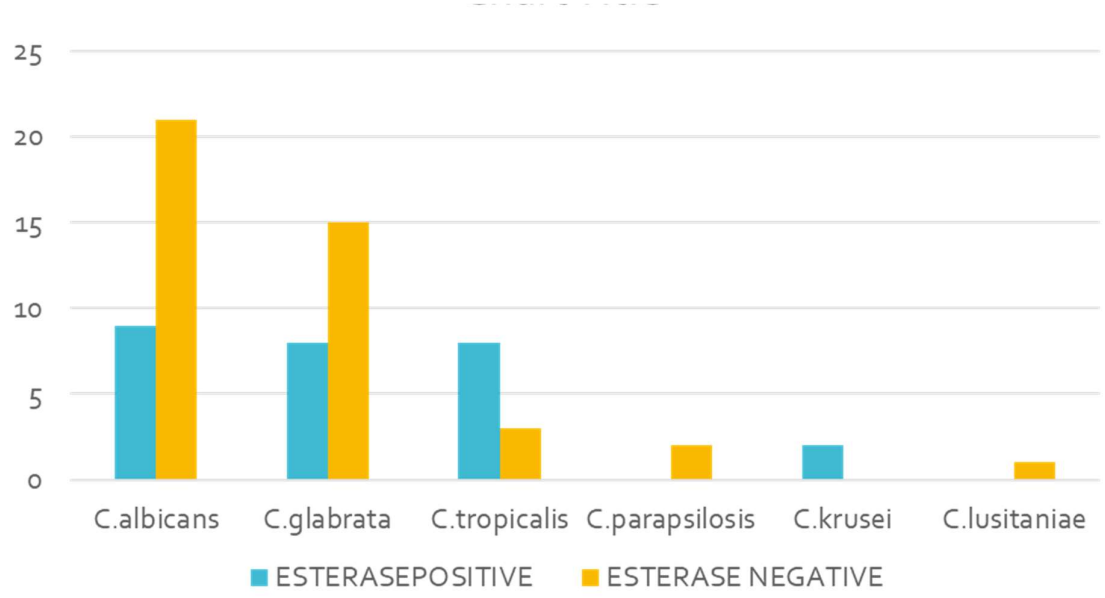
Graph no 7: Hemolytic activity by different *Candida* species



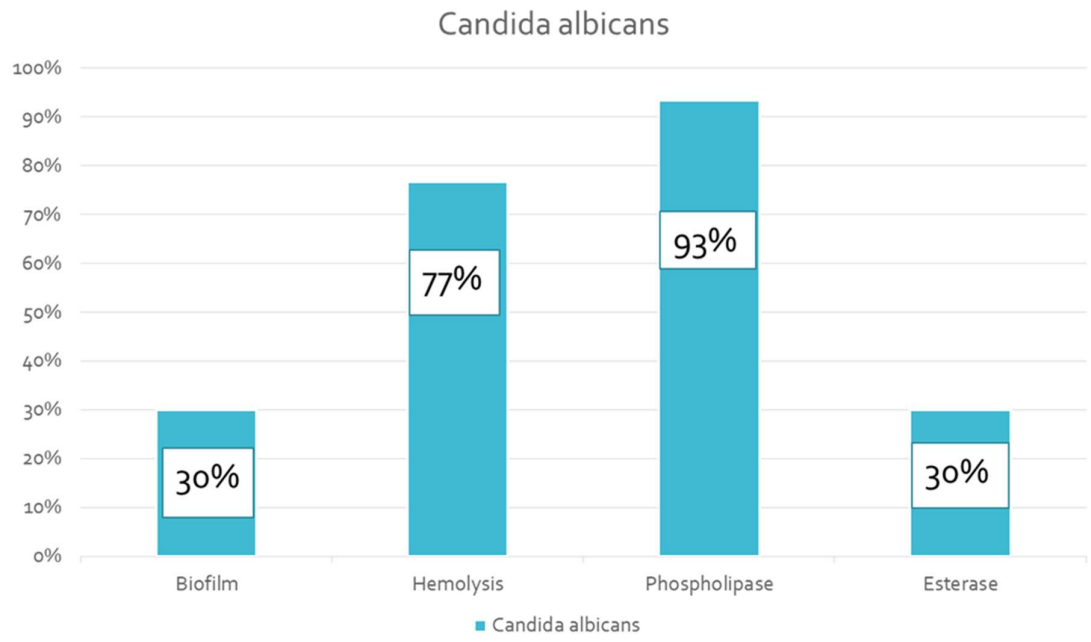
Graph no 8: Phospholipase activity by different *Candida* species



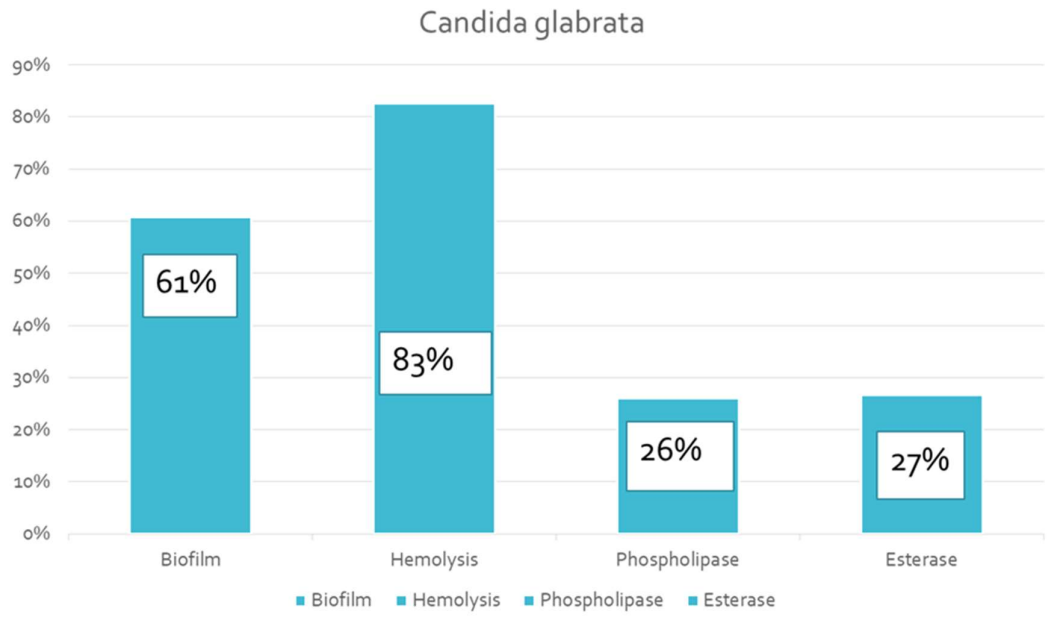
Graph no 9: Esterase activity by different *Candida* species



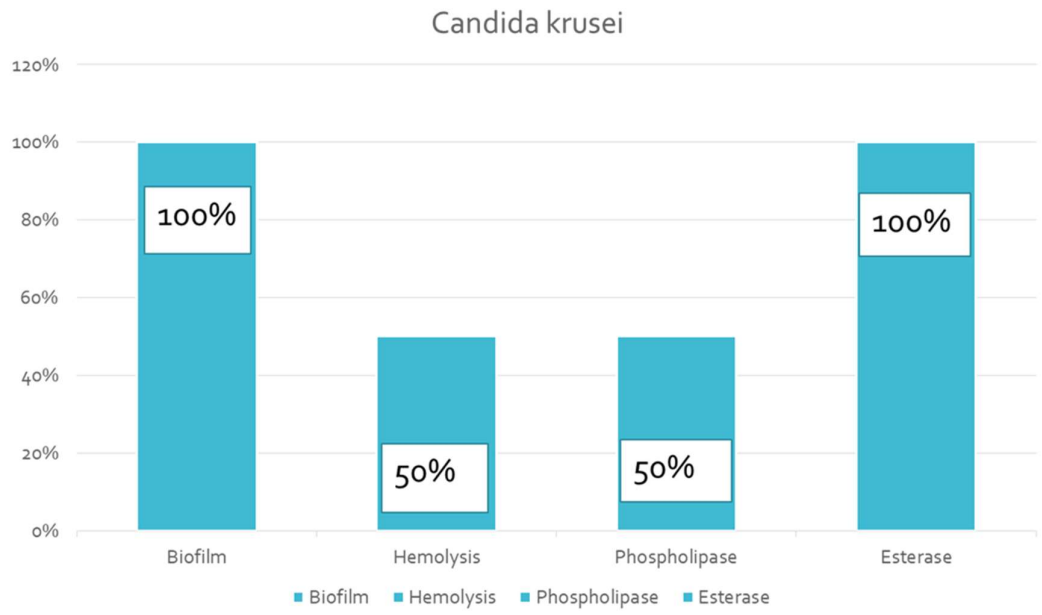
Graph no 10: Distribution of virulence factors among *Candida albicans*



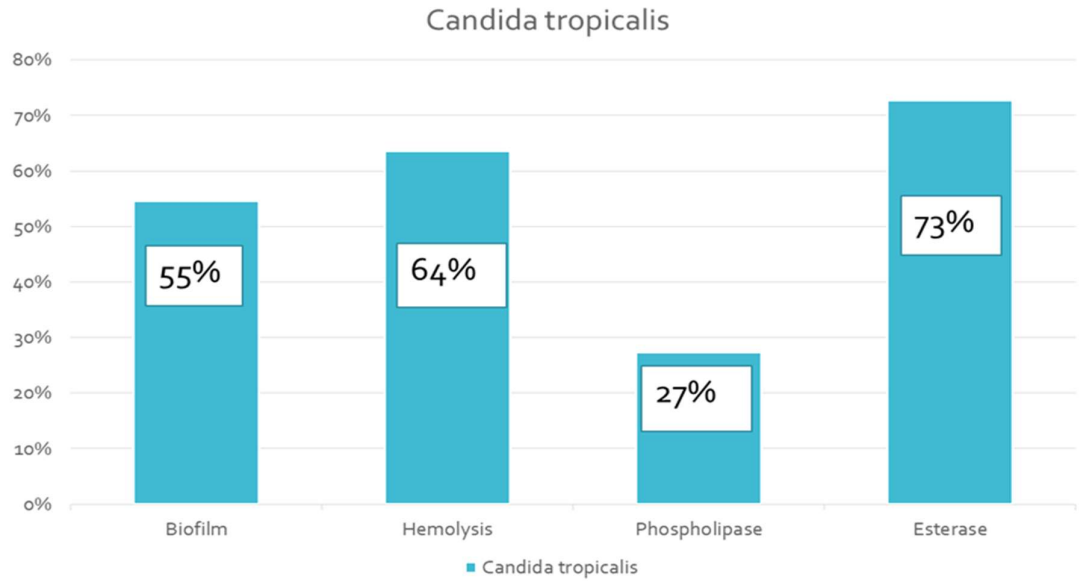
Graph no 11: Distribution of virulence factors among *Candida glabrata*



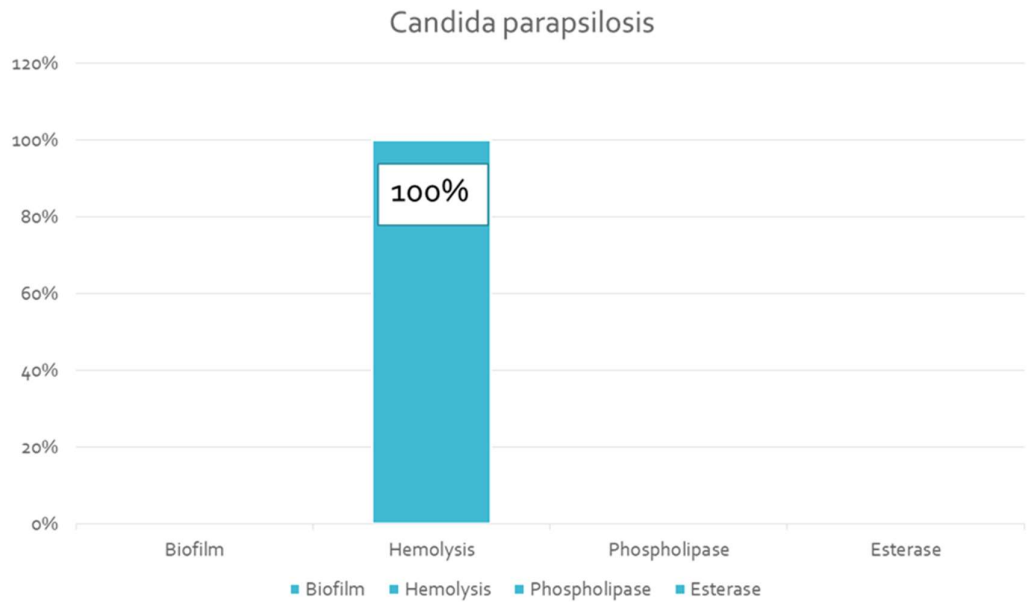
Graph no 12: Distribution of virulence factors among *Candida krusei*



Graph no 13: Distribution of virulence factors among *Candida tropicalis*



Graph no 14: Distribution of virulence factors among *Candida parapsilosis*



Graph no 15: Distribution of virulence factors among Candida lusitaniae

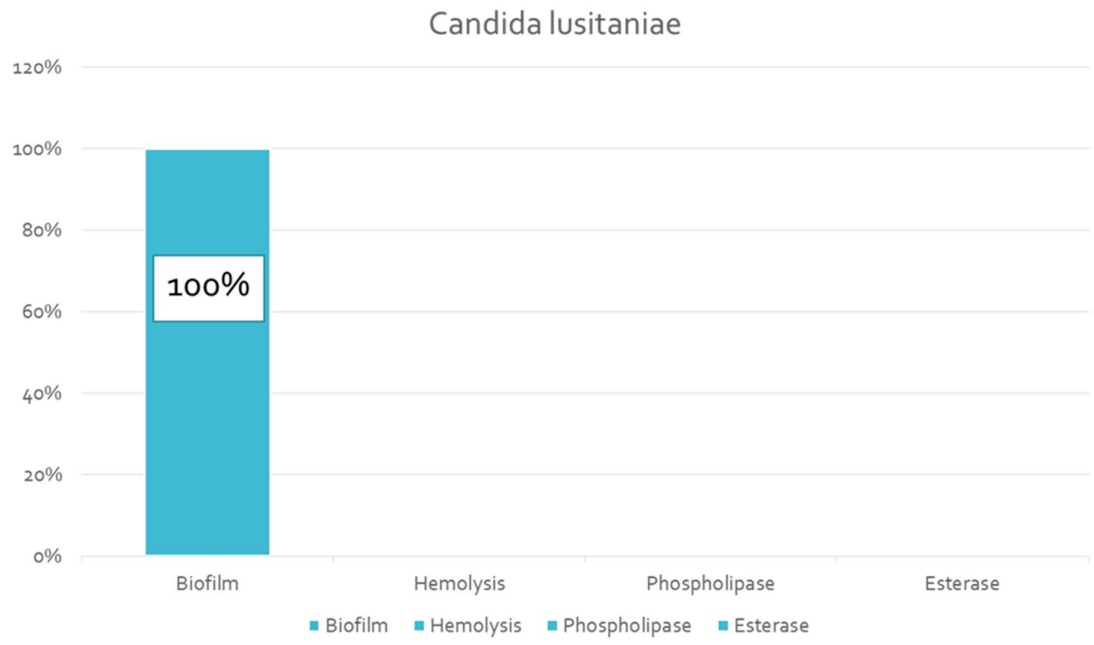
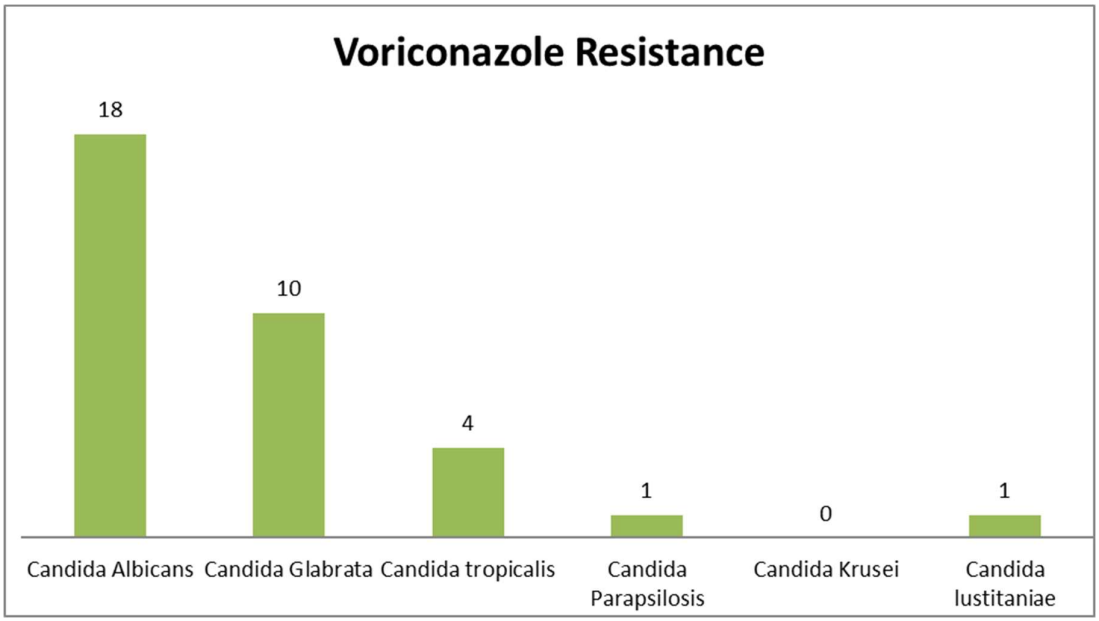


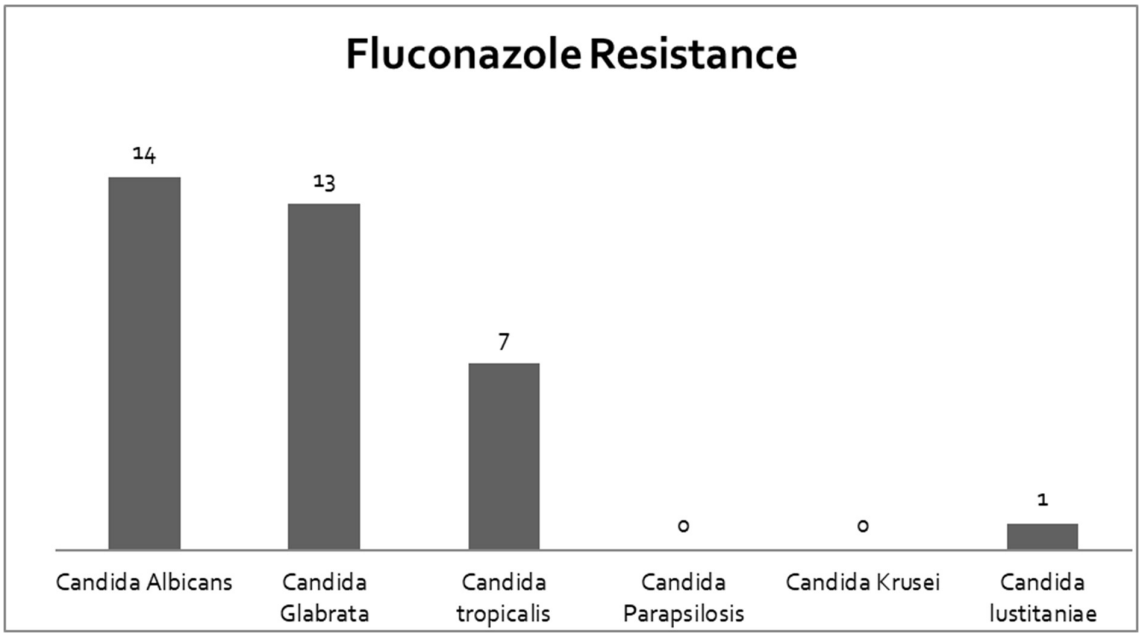
Table no 10: Antifungal resistance pattern among isolates

SPECIES	FLUCONAZOLE	VORICONAZOLE
C. Albicans	14	18
C.Glabrata	13	10
C.Tropicalis	07	4
C.prapsilosis	0	1
C.Krusei	0	0
C.lusitaniae	1	1

Graph no 16 : Voriconazole resistance among *Candida* isolates



Graph no 17 : Fluconazole resistance among *Candida* isolates.



DISCUSSION

In this study a total of 69 *Candida* species were isolated, from women of reproductive age group (18-45 years) who complained of curdy white vaginal discharge; among which 40(58%) were isolated from pregnant women. Similar to our study Vijaya et al [46] reported that 60% of the *Candida* isolates were from Pregnant women. In the study Fule et al, 79.66% women were pregnant who showed evidence of VVC.[47]

Most of the cases belonged to age group 18-32 years, suggesting higher prevalence of VVC in younger age group. Estrogen governs susceptibility to VVC infections, pregnant women because of high estrogen levels do have a high risk for VVC. Increased level of estrogen can elevate glycogen at vaginal mucosa as well as reduce leukocyte infiltration, and also reduces antifungal activity of epithelial cells - together promoting infection.

During the present study the most common clinical presentations were curdy white discharge, pruritis, burning pain during micturition and dysuria.

In this present study out of 69 isolates 30 (43.47%) were *C. albicans* & 39(56.52%) non albicans *Candida*. Other studies that showed similar results are Lavanya et al[7]. Compared to our study in Noori M et al [3] showed 66.6% *C. albicans* & 33.4% non albicans *Candida*; Fule et al [47] they found 62.59% were *C. albicans* & 37.28% were non albicans *Candida*. Another study Kalaiarasan et al [2] they have isolated 15.25% C. albicans & 82.35% non albicans *Candida* from a total 51 sample. Thus, from the current study we can conclude that there is an increase in non albicans *Candida* compared to *C. albicans.*

In present study 61% of *Candida glabrata* has shown biofilm production followed by *C. tropicalis* (55%) & *C. albicans* (30%). A single isolate of *C. lusitaniae* has shown biofilm production. In Kalairasan et al [2] has reported 22.2% strains of *C. albicans*, 58.3% of *C. tropicalis* & 30.4% of *C. glabrata* showed for biofilm production. In Tulasidas et al, 74% *Candida* sp. were biofilm producers; among *C. albicans* 20.6% & 17.6% of *C. tropicalis* were biofilm producers[48]. Also, biofilm is considered to be one of the most capable pathogenic factors that has contribution towards treatment failure and recurrent infections. Biofilm production was more among Non albicans *Candida* in present study compared to *C. albicans*.

In present study 83% of C. glabrata has shown hemolytic activity followed by *C. albicans* (77%) & *C. tropicalis* (64%). In contrast to present study Sachin et al [49] reported that 94.8% *Candida albicans* followed by 48% of *C. tropicalis* & 21.4% *C. glabrata* has shown hemolytic activity. Similar to our study Kalairasan et al [2] has reported 88.3% strains of *C. tropicalis*, 77.8 % of *C. albicans* & 87% of *C. glabrata* showed positivity for hemolytic activity. Udayalaxmi et al [50] detected all strains of *C. krusei* & *C. glabrata* were hemolytic along with 97.5% *C. albicans*, 94.7% of *C. tropicalis* were hemolytic. Hemolysins secreted by *Candida* species degrade hemoglobin, facilitate recovery of elemental iron from host cells and enable survival of *Candida* in host cell and our study shows that 76.81% of the isolate were Hemolysin producer.

In present study 93% of *C. albicans* has shown phospholipase activity followed by *C. tropicalis* (27%) & *C. glabrata* (26%); 38 (55%) has shown a Pz value of ≤0.63 i.e strong positivity for phospholipase activity. In Kalairasan et al [2] has reported all strains of *C. albicans* (100%) showed phospholipase activity which is correlating to present study & contrast to our study 4.3% of *C. glabrata* has shown

positivity for phospholipase activity. In Sachin et al [49] they have reported 92.3% of *C. albicans*, 76% *C. tropicalis*, 28.5% *C. glabrata* were positive for phospholipase activity. Fule et al [47] reported none of the non albicans *Candida* species showed phospholipase activity and 81.08% of *C. albicans* showed this enzymatic activity.

In present study 73% of *C. tropicalis* has shown esterase activity followed by *C. albicans* (30%) & *C. glabrata* (27%). All of the strains showed Ez score ≤ 0.63% of esterase activity i.e strongly positive. In contrast to present study Noori M et al [3] reported that 68.2% *Candida albicans* followed by 45.5% of both *C. glabrata* & *C. tropicalis* has shown esterase activity. Another study Kalairasan et al has [2] reported 66.7% strains of *C. tropicalis*, 33.3% of *C. albicans* & 30.4% of *C. glabrata* showed positivity for esterase activity.

Out of 69 *Candida* isolates 44 (63.76%) were susceptible to Fluconazole & 36.23% were resistant; 35 (50.72%) isolates were susceptible to Voriconazole where 34(49.27%) were resistant. *Candida tropicalis* has shown increased resistance to Fluconazole followed by *C. glabrata*. Whereas voriconazole resistance was more in *C. albicans* followed by *C. parapsilosis*. In Tulasidas et al.[48] 79.1% of the *Candida* isolates has shown susceptibility to Fluconazole & 20.5% were resistant; susceptibility to Voriconazole was seen in 83.3% isolates & resistance to it was shown by 16.6%.

CONCLUSION

From the results of the present study, we depict that high number of *C. glabrata* showing hemolytic activity & biofilm production. Extracellular hydrolytic enzymatic activity such as Phospholipase & Esterase activity was seen in *C. albicans* and NAC. Very few studies are done till date describing about esterase activity among non albicans *Candida*; in our study *C. tropicalis* has shown association with hemolytic & esterase activity in a greater percentage. Among non albicans *Candida* Fluconazole resistance was observed more compared to voriconazole. Our study also shows that drug resistance among *Candida* species has shown more association with biofilm production. The study indicates that the virulence factors which were restricted to *Candida albicans* are now being expressed in non albicans *Candida* & also that these factors may contribute to increased Antifungal resistance among *Candida* species.

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ANNEXURE – I –CONSENT FORM

WRITTEN INFORMED CONSENT

TITLE : PHENOTYPIC DETECTION OF SELECTED VIRULENCE FACTOR OF CANDIDA SPECIES ISOLATED FROM WOMEN OF REPRODUCTIVE AGE WITH VULVOVAGINAL CANDIDIASIS. A one year Cross- sectional study.

Purpose of the study: The purpose of the study is to detect the virulence factors and antifungal susceptibility of the isolated organism.

Procedure: You are requested to participate in this study in which a high vaginal sawb will be collected, it is a painless procedure and there are no side effects. The swab will be further processed for diagnostic and treatment purpose. During this study you will be asked about your disease and you are supposed to answer to the best of your knowledge.

Risks and benefits: There are no risks involed and benefit is to know about the species , the virulence factor and antifungal susceptibility of the same, so that appropriate treatment can be given.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and withdraw from the study. The study doctor or sponsor may terminate your participation in this study anytime.

Privacy and confidentiality: All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives for participation: You will not be paid / offered any gift /incentives for participating in this study.

Authorization to publish results: The results of this study would be forwarded to the KAHER, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

Questions: In case you have any questions related to the study in future you can contact:

1. Dr. ALAKANADA PANDIT, Department of Microbiology, J.N. Medical College, Ph No- 8414039658
2. Dr. SUMATI HOGADE, Department of Microbiology, J.N. Medical College, Ph No- 9448866944
3. If you have any queries about your rights as a study subject, you may call Dr. HARSHA HEGDE, Chairperson, JNMC, IEC & Scientist D, ICMR National Institute of Traditional Medicine, Belagavi, Ph. No: 9480422500

CONSENT STATEMENT

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form and have had all my questions answered.
In case of the queries during the study or in future you may contact following person.

Principal Investigator: Dr. ALAKANANDA PANDIT
Guide : Dr. SUMATI HOGADE

Name of the participant:
(signature/thumbprint)

Name of the witness : (signature)

Name of the investigator: (signature)

Date:

Address:

Phone no:

ANNEXURE – II – PROFORMA

QUESTIONNAIRE (PROFORMA) USED FOR COLLECTING DATA

Name :

Age :

IP no. :

Sample Id:

Sex :

Department:

Blood group :

DOA:

DOC:

Address :

Diagnosis:

Brief clinical history

Presenting complaints:

H/O presenting complains:

H/O any interventions : If yes than Type and course of therapy

Similar Past history : If yes; details of treatment ;

Any Associated illness :

INVESTIGATIONS

1.Gram stain

2. Culture methods:

- SDA
- CHROM agar
- CORN-MEAL AGAR

3. Antifungal Susceptibility pattern

4. Detection of virulence factors

- Biofilm formation
- Hemolytic activity
- Phospholipase activity
- Esterase activity

ANNEXURE – III – STAINING & CULTURE METHODS

A. STAINS AND REAGENTS

Gram staining:

- Methyl violet(2%)-10g of Methyl violet in 100 ml Absolute alcohol in 1litre of Distilled water.(primary stain)
- Grams Iodine-10g Iodine in 20 g KI (mordent)
- Acetone-Alcohol - Decolourizing agent.
- Saffranin(1%)-Secondary stain

B. MEDIA USED

1. SABOURAUD DEXTROSE AGAR

INGREDIENT	Gm/ltr
Peptone	10 gms
Dextrose	40 gms
Agar	20 gms
Distilled water	1000 gms

Final pH was adjusted to 5.6

The above ingredients were reconstituted in one litre of distilled water. Dissolve the powder water by boiling. The medium was then removed from heating ,mixed well and then dispersed in tubes and autoclaved at 121°C for 15 minutes and the final pH was adjusted to 5.6.The tubes were cooled in slanted position and later the slants were stored in refrigerator.

C. CHROM AGAR CANDIDA MEDIUM:

Ingredients	Gms/L
Peptone,	15 gms
Yeast extract	4 gms
Dipotassium hydrogen phosphate	1. gms
Chromogenic mixture	7.22 gms
Chloramphenicol	0.5 gms
Agar	15 gms

pH 6.3±0.2 at 25°C

Suspend 42.72 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

D.EGG YOLK MEDIUM

Ingredients:	Gms / Litre
SDA	65g
NaCl	58.4g
CaCl2	5.5 g

The egg yolk medium consisted of 65g SDA, 58.4g NaCl and 5.5 g CaCl2 dissolved in 980ml distilled water and sterilized at 121°C for 15 minutes. Egg yolk was centrifuged at 5000 g for 30 minutes and 2 ml of supernatant was added to medium cooled at 45-50°C.

ANNEXURE – IV– MASTER CHART

S. No.	IPD/OPD Number	DATE	Name	Age	Pregnant/non-pregnant	Symptoms	Isolates	PHENOTYPIC VIRULENCE FACTORS				ITIFUNGAL SUSCEPTIBILITY PATTE	
								BIOFILM	HEMOLYSIS	ESTERASE	PHOSPHOLIPASE	FLUCONAZOLE	VORICONAZOLE
1	5126273	15.03.21	NIRMALA	22	NP	PRURITIS	Candida albicans	neg	alpha	1	0.39	S	S
2	4968246	07.04.21	SUFIYA BANU ARIF	25	P	PRURITIS	Candida glabrata	G+2	gamma	0.6	1	R	S
3	6051449	07.04.21	ASHWINI BHOSAL	23	P	PRURITIS	Candida glabrata	G+2	alpha	1	1	R	S
4	1052233	13.05.21	JYOTI BALE	30	P	PRURITIS,BURNING PAIN	Candida albicans	neg	gamma	1	0.41	S	S
5	1054655	07.06.21	LAXMI SANJEEV	22	P	PRURITIS,BURNING PAIN	Candida albicans	G+1	gamma	1	0.5	S	R
6	6103325	09.06.21	SHILPA	27	NP	DYSPARIUNIA	Candida albicans	neg	beta	1	0.41	R	S
7	6112356	15.06.21	RADHA	23	P		Candida tropicalis	G+3	gamma	0.39	0.6	R	R
8	6120597	23.06.21	REKHA	34	P	PRURITIS	Candida glabrata	neg	alpha	0.43	1	R	S
9	6121904	24.06.21	MEENAXI K	23	P	PRURITIS	Candida glabrata	neg	beta	1	1	S	S
10	6112159	11.07.21	ANASUYA	27	NP	PRURITIS	Candida albicans	G+1	beta	1	0.39	R	R
11	6181399	13.08.21	POOJA	22	P		Candida glabrata	neg	gamma	0.41	0.6	S	S
12	6184291	19.08.21	KIRANTHI CHIKKAMATH	21	NP	PRURITIS	Candida tropicalis	G+2	gamma	0.39	1	R	R
13	1064823	19.08.21	MAHADEVI M	45	NP	PRURITIS	Candida parapsilosis	neg	beta	1	1	S	S
14	6151639	23.08.21	REETU	27	P		Candida albicans	neg	gamma	0.43	0.37	R	R
15	6126200	25.08.21	AFSANA M	21	NP	PRURITIS	Candida albicans	neg	beta	1	0.38	S	S
16	4755212	26.08.21	GOUTAMI	23	P	PRURITIS	Candida glabrata	G+3	gamma	1	1	R	S
17	6164152	01.10.21	PRANALI	26	P	PRURITIS	Candida glabrata	G+2	alpha	1	1	S	S
18	1071934	11.10.21	SHOBHA H	28	P	PRURITIS	Candida tropicalis	neg	alpha	0.6	1	S	S
19	4687518	15.10.21	SHRIDEVI EMMI	24	NP	PRURITIS	Candida albicans	G+1	beta	1	1	R	R
20	6277019	06.11.21	SANJANA	26	NP	PRURITIS	Candida albicans	neg	beta	0.39	0.4	S	S
21	6224347	15.11.21	PRIYANKA	23	P	PRURITIS	Candida tropicalis	neg	beta	0.4	1	S	S
22	6284746	15.11.21	KASHWA MALAPUR	25	P	PRURITIS	Candida glabrata	NEG	alpha	1	0.41	S	R
23	1080445	17.11.21	GOUQUIN SH.	27	P	PRURITIS	Candida krusei	NEG	beta	0.4	1	S	S
24	6278016	17.11.21	ARATI M.P.	24	P	PRURITIS,BURNING PAIN	Candida albicans	G+2	beta	1	0.37	S	R
25	6221591	17.11.21	POOJA MATAPATI	21	P	PRURITIS	Candida albicans	NEG	beta	0.63	0.5	R	R
26	6286749	17.11.21	PRIYANKA KATHKI	28	NP	PRURITIS	Candida tropicalis	NEG	gamma	0.41	1	R	R
27	1079851	19.11.21	VAISHNAVI MANJUNATH	25	P		Candida glabrata	NEG	alpha	0.47	1	S	S
28	1080859	19.11.21	KASTURI	29	P	PRURITIS	Candida tropicalis	G+2	gamma	1	1	R	R
29	6259226	19.11.21	LAXMI LOKESH	37	P	PRURITIS	Candida albicans	G+2	beta	1	0.47	R	R
30	1028456	30.11.21	VIJAYALAXMI	31	NP	PRURITIS	Candida glabrata	NEG	alpha	0.41	0.37	S	S
31	6250853	30.11.21	BHEEMAMBILA	20	P	PRURITIS, BURNING PAIN	Candida tropicalis	G+1	alpha	0.5	1	R	S
32	1082986	01.12.21	POONAM NAMDEN	21	NP	PRURITIS	Candida albicans	G+2	gamma	1	0.37	S	S
33	1083103	02.12.21	SILPA	29	P	PRURITIS,DYSURIA	Candida albicans	NEG	beta	1	0.39	S	S
34	1083480	03.12.21	PRERNA B	36	P	PRURITIS	Candida parapsilosis	neg	beta	1	1	S	S
35	1083740	06.12.21	KAVITA	25	NP	PRURITIS	Candida glabrata	NEG	alpha	1	0.4	S	S
36	6165042	06.12.21	BHAKTI	23	P		Candida glabrata	NEG	beta	0.39	1	S	S
37	6370541	06.12.21	LAXMI	27	P	PRURITIS	Candida tropicalis	NEG	alpha	1	1	S	S
38	6299150	06.12.21	RUKSHAR	45	NP	PRURITIS	Candida albicans	NEG	beta	0.5	0.35	S	R
39	6102142	06.12.21	POOJA	32	P	DYSURIA	Candida krusei	G+2	gamma	1	0.61	S	S
40	6237602	08.12.21	REKHA PRAKASH	29	NP	PRURITIS	Candida glabrata	G+2	alpha	1	1	R	R
41	6277021	10.12.21	RAJESHA	25	NP	PRURITIS	Candida glabrata	G+2	alpha	1	0.6	R	R
42	1084339	10.12.21	SHUKUBAI	45	NP	PRURITIS	Candida albicans	NEG	beta	0.47	0.31	S	S
43	1084313	10.12.21	SHRUTI SUNIL	27	P		Candida albicans	NEG	beta	0.41	0.5	S	S
44	6312387	11.12.21	MEGHA KUMARI	24	P	PRURITIS, DYSURIA	Candida albicans	G+2	gamma	1	0.33	R	R
45	6206902	15.12.21	MAHANJANA ABDUL	35	P	PRURITIS	Candida tropicalis	G+1	alpha	1	1	R	S
46	5102314	15.12.21	VIJAYA PATIL	23	NP	PRURITIS,BURNING PAIN	Candida glabrata	NEG	beta	0.39	1	R	S
47	1085808	16.12.21	NITA R	28	NP	PRURITIS,DYSURIA	Candida albicans	NEG	beta	0.6	0.43	R	R
48	6152422	17.12.21	ANURATA	23	P	PRURITIS,BURNING PAIN	Candida albicans	NEG	beta	0.5	0.41	S	S
49	6290885	20.12.21	LALITA MAHADEV	21	NP	PRURITIS	Candida glabrata	G+1	alpha	1	1	R	R
50	1089239	10.01.22	JYOTI M.	24	NP	PRURITIS	Candida tropicalis	NEG	alpha	0.49	0.5	S	S
51	5516700	31.01.22	SHRUTI PATIL	26	P	PRURITIS	Candida albicans	G+2	gamma	0.5	0.61	R	R
52	5516711	31.01.22	SHREYA PATIL	23	NP	DYSPARIUNIA	Candida albicans	NEG	beta	1	0.53	R	R

53	1093741	04.02.22	AKSHATA B	25	P	_	Candida albicans	NEG	beta	1	0.37	S	S
54	6377152	04.02.22	CHAITRA	24	P	PRURITIS, BURNING PAIN	Candida glabrata	G+2	alpha	1	1	R	S
55	6318876	05.02.22	KALASHRI ASHAH	20	NP	PRURITIS	Candida albicans	NEG	beta	1	0.39	R	R
56	1094331	07.02.22	ROMAN	28	P	PRURITIS	Candida glabrata	G+2	gamma	1	1	R	R
57	6220945	07.02.22	ROOPA RAJ	21	P	PRURITIS	Candida albicans	NEG	beta	1	0.41	S	S
58	6339944	09.02.22	SUJATA INGALE	20	NP	PRURITIS	Candida albicans	G+2	gamma	1	0.41	S	S
59	1100359	11.03.22	POONAM PATIL	29	NP	PRURITIS,	Candida glabrata	G+2	alpha	0.5	1	R	R
60	1100103	11.03.22	DEVAKI	31	P	PRURITIS,DYSURIA	Candida glabrata	G+3	gamma	1	1	R	R
61	6280796	13.03.22	KOMAL D CHACHADI	47	NP	PRURITIS	Candida albicans	NEG	alpha	1	0.37	R	R
62	1104533	14.03.22	NITA RAJU	27	P	PRURITIS	Candida albicans	NEG	beta	1	0.31	R	R
63	6336427	19.03.22	MEHEK	22	NP	PRURITIS	Candida albicans	NEG	beta	1	0.6	S	R
64	1105570	19.03.22	SUGANDH JAGATAP	45	NP	PRURITIS	Candida tropicalis	G+2	alpha	0.57	1	R	R
65	6493026	20.03.22	ROOPALI GOURAV WADDAR	34	P	PRURITIS	Candida glabrata	G+2	beta	1	0.4	R	R
66	6452454	22.03.22	SAVITRI	29	NP	PRURITIS	Candida lusitiniae	NEG	alpha	1	1	R	S
67	6449039	25.03.22	SAVITA	23	NP	PRURITIS	Candida albicans	NEG	beta	1	0.39	R	S
68	6327895	26.03.22	MANASHI AKASH	22	P	_	Candida glabrata	G+2	beta	1	1	R	R
69	6541280	26.03.22	JANIFER	27	P	PRURITIS	Candida glabrata	G+2	beta	1	1	R	R