A STUDY ON PREVALENCE OF CANDIDA IN VULVOVAGINAL CANDIDIASIS AND THEIR PHENOTYPIC CHARACTERIZATION WITH ANTIFUNGAL SUSCEPTABILITY PATTERN IN A TERTIARY CARE HOSPITAL

DISSERTATION SUBMITTED FOR

BRANCH – IV – M.D. DEGREE

(MICROBIOLOGY)

REG.NO: 201714101

THE TAMILNADU

DR. M.G.R MEDICAL UNIVERSITY

CHENNAI, TAMIL NADU

MAY 2020

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This is to certify that the dissertation entitled **"A STUDY ON PREVALENCE OF CANDIDA IN VULVOVAGINAL CANDIDIASIS AND THEIR PHENOTYPIC CHARACTERIZATION WITH ANTIFUNGAL SUSCEPTABILITY PATTERN IN A TERTIARY CARE HOSPITAL"** submitted by **Dr.D.S.KALAIVANI,** to the Tamil Nadu Dr.M.G.R Medical University, Chennai in partial fulfillment of the requirement for the award of M.D Degree Branch – IV (Microbiology) is a bonafide research work carried out by her under direct supervision & guidance.

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I, **Dr.D.S.KALAIVANI,** solemnly declare that this dissertation titled **"A STUDY ON PREVALENCE OF CANDIDA IN VULVOVAGINAL CANDIDIASIS AND THEIR PHENOTYPIC CHARACTERIZATION WITH ANTIFUNGAL SUSCEPTABILITY PATTERN IN A TERTIARY CARE HOSPITAL"** is a bonafide record work done by me at the Institute of Microbiology, Madurai medical college, Madurai. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree or diploma to any other University, Board, either in India or abroad. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulations for the award of M.D. degree Branch-IV (Microbiology) examination to be held in May 2020.

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Research Topic	:	A study on prevalence of candida in vulvovaginal candidiasis and their phenotypic characterization with antifungal susceptability pattern in a tertiary care hospital
Ethical Committee as on	:	29.10.2018

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ACKNOWLEDGEMENT

I would like to thank **Dr.K.VANITHA, M.D., D.CH.,** The Dean, Madurai medical college, Madurai for permitting me to use the hospital facilities for the dissertation.

I also extend my sincere thanks to **Dr. MANGALA ADISESH, M.D.,** Director and Professor, Institute of Microbiology, Madurai medical college for her constant support during the study.

I express my sincere thanks to the Vice-Principal **Dr.V.DHANALAKSHMI, M.D.,** Professor of Microbiology for her encouragement and support given to me during the study.

I would like to express my deep sense of gratitude and thanks to my guide **Dr.S.RADHAKUMARI, M.D.,** Professor, Institute of Microbiology, for her valuable suggestions and guidance throughout this study.

I wholeheartedly thank **Dr.S.LALITHA, M.D.,** Associate Professor of Microbiology for her encouragement.

I also sincerely thank **Dr.C.SUGUMARI, M.D.,** Associate Professor of Microbiology for her guidance and support.

I also sincerely thank **Dr.RAMMURUGAN, M.D.,** Associate Professor of Microbiology for his constant support.

I am immensely grateful to **Dr.J.SURIAKUMAR, M.D.,** Assistant Professor of Microbiology for guiding me during this study.

My heartfelt thanks goes to **Dr.S.Mahesh Prabhu, M.D., Dr.N.Anuradha, M.D., Dr.D.Saradha, Dr.M.R.Vasanthapriyan, M.D.,**

M.D., Dr.S.Nalayini, M.D., Dr.G.Manjula, M.D., Dr.Bharathi, M.D., Dr.Lakshmanakumar, M.D., Assistant Professors for their encouragement.

I offer my special thanks to the Senior Assistant Professor **Dr.D.S.Kavitha, M.D., OG.,** Institute of Obstetics & Gynaecology, Government Rajaji Hospital, Madurai for her valuable guidance and support.

I am immensely grateful to **Dr.Kamala,** Post Graduate (Final Year), Institute of OG, Government Rajaji Hospital, Madurai for her guidance in colleting samples.

I am thankful to my Postgraduate colleagues **Dr.J.Vijayanand, Dr.S.Rajeswari, Dr.S.Thamizhselvi, Dr.K.R.Pandiaraj, Dr.K.Anusha, Dr.J.Abinaya, Dr.Manjari and Dr.Bindhu** for their Moral Support and Cooperation.

I extend my thanks to My Technicians **Mrs.Geetha, Mr.Balagurunathan, and Mr.Hariram** for giving full cooperation and timely help in carrying out the Laboratory work.

I thank the Technical and non-technical staff members of the Institute of Microbiology for their kind cooperation to carry out this study successfully.

I express my heartfelt thanks to all the patients who participated in this study and I pray the Almighty for their speedy recovery.

I affectionately thank my husband **Mr.B.Sivaramakrishnan,** my daughter, **S.V.Srivarsha** and my family members for their constant support and encouragement without which this work would not have been possible.

I express my gratitude to The Lord Almighty for giving me physical and mental strength in all my endeavors.

ABBREVIATIONS

NAC	-	Non albicans Candida
OC	-	Oral Candidiasis
DM	-	Diabetes Mellitus
PID	-	Pelvic Inflammatory Disease
AB	-	Antibiotics
IUCD	-	Intra Uterine Contraceptive Devices
VVC	-	Vulvovaginal Candidiasis
RVVC	-	Recurrent Vulvovaginal Candidiasis
S	-	Sensitive
R	-	Resistant
SDA	-	Sabouraud dextrose agar

CONTENTS

Introduction

INTRODUCTION

Fungal infections have become a prominent infection over the last decade mainly due to worldwide increase in the number of immunosuppresed patients, who are highly susceptible to opportunistic infections including mycoses.

Prevalence of these infections has increased particularly in pregnancy, diabetic women, those who are on OC pills and after antibiotics [9],[12],[93].

History: The first description for Candida infection is found in Hippocrates 'Epidemics' from 4th century BC. Rosen von Rosenstein identified Candida infection in pediatrics 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 and later it was also isolated from blood and brain. 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. Zopf in 1890 named it as Monilia albicans.

In 1923 Berkhout structured Candida genus. In 1978 Torulopsis glabrata was renamed as Candida glabrata. This was internationally accepted in 1954. The term candidiosis is used in U.K, France and Canada, whereas candidiasis is an American English.

Vulvo vaginal candidiasis [VVC] is clinically defined as inflammation of the vulva and vagina due to the presence of Candida and in the absence of

other infectious etiology. It is due to the pH alteration of vagina in candida infection [2].

Vaginal candidiasis is a common gynecological finding among women worldwide [6], [7]It has been found that up to 75% of the sexually active women have symptomatic vaginal candidiasis at least once [2].

Candidiasis is the most common vaginal infection in most countries affecting 50 to 72% of women and 40 to 50% are having recurrent episodes. Nowadays candida constitutes the 3rd or 4th most common cause of blood stream infections [8],[10].

Vaginal candidiasis if untreated can lead to chorioamnionitis and which can result in subsequent abortion and prematurity in pregnant women and pelvic inflammatory disease which results in infertility in non-pregnant women [31],[32].

Candida species mostly Candida albicans, can be isolated in the vaginal tracts of 20 to 30% of healthy asymptomatic non-pregnant women. If the balance between colonization and the host is temporarily disturbed, Candida can cause disease such as VVC, which is associated with clinical signs of inflammation.[9]

The commonest organism implicated is Candida albicans [7], and the predisposing factors are hormonal fluctuations in pregnancy, luteal phase of menstrual cycle, use of oral contraceptives and hormonal replacement therapy among others [5].[26]

Although close to 200 Candida species have been described, only a few of these are of medical significance.[4][5] The diversity of Candida are associated with human infections which provides new challenges in the diagnosis and treatment of candidiasis and in the study of their virulence. Vaginal infections are a common form of Candida overgrowth in women. An over growth of Candida disrupts the normal balance of the bacteria and yeast in the vagina. This imbalance may also be due to drugs, stress, hormones and food habits[8] [17]. The most common clinical manifestations of vulvovaginal candidiasis are pruritis, dysuria, leucorrhoea, vulval erythema, which causes problems in marital and sexual relations.[32].

Several studies documented the increased rates of Candida non-albicans.[6] [8] [16] [24].Only 20 Candida species are significant pathogens. Out of these, seven are well-known opportunistic pathogens for humans.

Some of the species are: Candida albicans, Candida tropicalis, Candida krusei, Candida glabrata, Candida auris, Candida parapsilosis, Candida kefr, Candida rugosa, and Candida dubliniensis.

Although Candida albicans remains the most common agent of both superficial and deep infections, an increasing incidence of non albicans candida species have also been documented in the recent years. These species include Candida tropicalis, Candida glabrata, Candida parapsilosis and known for their variable resistance to azoles. [16]

The lack of specificity of symptoms and signs in vulvovaginal candidiasis explains the need for laboratory confirmation by direct microscopy and culture. Speciation of candida isolates are essential in routine specimen processing.

The **CHROM agar media** is selective and differential type of chromogenic medium, which is useful for identification of various Candida species. Due to the chromogenic substrate in the medium the colony morphology and color have been well defined when it is used to isolate the yeasts directly from clinical material, including stool, urine, respiratory, vaginal, oropharyngeal and esophageal samples.

It is based on direct enzymatic activities by adding multiple chemical dyes i.e. substrates of flourochromes. This facilitates presumptive differentiation of clinically significant yeasts and also for isolation of various Candida species.

The benefits of using this medium are that it shortens the time for identification of organisms and allows for easier detection of multiple yeast species present in the clinical specimens with mixed infections by the specific different color of the species.

Azoles as a group are active against Candida species, Cryptococcus neoformans, Blastomyces dermatitidis, Histoplasma capsulatum, Coccidioides spp., Paracoccidioides brasiliensis and dermatophytes. Aspergillus spp., Scedosporium apiospermum (Pseudallescheria boydii) Fusarium, and

sporothrix schenckii are intermediate in susceptibility. Candida krusei and the agents of mucormycosis are more resistant. Posaconazole has slightly improved activity in vitro against the agents of mucormycosis.

There is now an increasing need for differentiating these yeasts and identifying them to the species level. Candida speciation in vulvo vaginal candidiasis is essential nowadays due to its intrinsic resistance.

Present study was undertaken to determine the prevalence of vaginal candidiasis in symptomatic women and to evaluate the advantages of CHROM agar over the other conventional methods in speciation of candida. Antifungal susceptibility testing was done by Kirby Bauer disk diffusion method as per CLSI guidelines. It will guide for the detection of common species causing this infection and proper antifungal agents to be used for the complete treatment for this infection without any complications.

Aims and Objectives

AIMS AND OBJECTIVES

1. To study the prevalence of Candida species in vulvo vaginal infections.
2. To do the speciation of Candida by phenotypic methods.
3. To detect the antifungal susceptibility pattern for the isolated candida species.

Review of Literature

REVIEW OF LITERATURE

Introduction

Vulvovaginal candidiasis is defined as infection of the vulva and vaginal mucosa caused by species of genus Candida. Candidal species are able to cause superficial lesions in both oral and vaginal mucosa when the equilibrium between fungi and the host change in favor of the fungus[1]. It has wide clinical manifestations.

Prevalence

Vulvovaginal candidiasis (VVC) is the second most common cause of vaginal infections next to bacterial vaginosis in Asia and U.S. This is the first common cause of vaginitis in Europe. It is estimated that 75% of women during the fertile period have at least one episode of vaginal Candidiasis. Point prevalence studies indicate that the Candida species are found in 10-50% of healthy asymptomatic females of reproductive age group[26]. Hermen Gardner said 'Vaginitis cause more inconvenience than others'. About 85-90% of VVC is caused by Candida albicans[4]. Other species are C.glabrata, C.parapisilosis, C.krusei, C.tropicalis, and C.guilliermondii.

Anatomy of female genital tract: [1],[32]

Female genital tract includes vulva, vagina, cervix, uterus tubes and ovaries. Vulva includes external genitalia and perineum. External genitalia include Mons pubis, labia majora, labia minora, vestibule, clitoris and vagina.

With elevated levels of either endogenous or exogenous estrogen, the vaginal epithelium thickens due to glycogen storage. With decreasing levels of estrogen, the lining becomes thin and atrophic.

Labia majora

It is a fold of skin which encloses fat. Labia majora is lined with squamous epithelium consisting of sebaceous glands, apocrine sweat glands and hair follicles.

Labia minora

It is a thin fold of skin situated inner to the labium majora. It contains veins and elastic tissues and devoid of sebaceous glands and hair follicles.

Clitoris

It is a rounded subcutaneous structure situated at the junction of two labia minora and attached to the under surface of the pubic symphysis by suspensary ligament.

Vestibule

It is a space situated between the anterior and inner aspect of labia minora. External urethral meatus lies just below the clitoris. Vaginal orifice lies below the meatal opening.

Vagina

Vagina extends from cervix to hymen. Its anterior wall is 9cm in length and posterior wall is 12 cm. vaginal portions of the cervix projects into the

upper portion of vagina forming anterior, posterior and two lateral fornices. Histologically it is lined by stratified squamous epithelium. Vagina is devoid of any glands. So the secretion from the vagina is derived from the transudation of epithelial cells.

Structure of vaginal epithelium

The vaginal squamous epithelium is divided into three layers as superficial, middle and deep layers.

- Superficial cell layer consists of precornified cells and cornified cells.
- Middle layer consists of large ellipsoidal cells which are intermediate between basal and cornified layer.
- Deep layer consists of basal cells and parabasal cells.
- These cells are under the influence of hormones like estrogen and progesterone.
- During early proliferative phase, the vaginal squamous cells mature and cornification index increases. In late proliferative phase, the vaginal cells become mature and the cornification index is highest. During the secretory phase, the squamous cells are folded and become crumbled. Cornification index also falls.
- During pregnancy, the cornification index is low in the first half of pregnancy and further reduction occurs in the late months of pregnancy. In the post menopausal phase, the vaginal epithelium becomes thinner and parchment like.

Physiology of normal vaginal secretions[2]

In the reproductive age group, normal vaginal secretion consists of approximately 1-4 ml in 24 hours, which is transparent and odorless. It is increased during pregnancy, at the time of ovulation and with oral contraceptive pills usage.

Normal vaginal discharge is formed by

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

Vaginal pH

A healthy adult woman of child bearing age harbors lactobacillus, also called Doderllein's bacilli which are Gram positive bacilli and sugar fermenting.[9] It converts the vaginal glycogen into lactic acid which is responsible for the acidic pH of the vagina.

- Newborn – 5.7
- Children – 6 to 8
- Reproductive age group, pregnancy – 3.8 to 4.5
- Menopause – 7

Epidemiology: [5]

❖ **Agent factors**

Candida is yeast like fungus. Fungi are eukaryotic organisms which possess rigid cell wall made up of chitin and it contains ergosterol. Chitin is a polysaccharide consisting of long chains of N acetyl glucosamine. In addition to chitin the cell wall contains mannans which are the principal antigen component, betaglucans, and other polysaccharides. A basic difference in the membrane sterols is the target for the selective action of antifungals. Candida species are unicellular organisms that are round to oval in shape and size ranges from 2 to 6 micrometer. They reproduce by asexual methods to form budding blastoconidia and sexual methods by producing ascospores and basidiospores. Then the budding forms elongate to form pseudohyphae. *Pseudohyphae* are elongated buds with cell wall constrictions. True hyphae are characterized by cells in cylindrical shape and are separated by perpendicular septal walls.

Candida species are found in animals, human and many food stuffs especially in packaged fruit juices. In humans Candida species is commonly found in GIT, oral cavity, genital area as commensals.So infection can occur by endogenous invasion and carriage is seen in 21-32% of healthy women. Higher rates are found in pregnant women associated with high Lactobacillus colonization, diabetes mellitus and post antibiotic treatment.[10] Candidiasis is the most common opportunistic fungal infection encountered in

immunosuppressed patients (spinillo et al). Recent studies have demonstrated that candida has become resistant for common antifungals.

Nomenclature of candida

Order	:	Cryptococcales
Class	:	Blastomyces
Genus	:	Candida

Species

- Candida albicans
- Candida glabrata
- Candida krusei
- Candida tropicalis
- Candida parapsilosis
- Candida guillermondii
- Candida kefyr
- Candida rugosa
- Candida dubliniensis
- Candida vishwanathii

About 200 species of Candida are discovered so far of which Candida albicans is the most common species. Recently non albicans candida is increasing as a pathogen. These species can be differentiated by microscopy, culture characteristics, biochemical reactions such as sugar fermentation &

sugar assimilation and growth in different media.[12] SDA, CHROMagar & Cornmeal agar.

Prevalence of Candida glabrata has increased. C.tropicalis is most commonly encountered in patients with hematological malignancies especially with neutropenia.[14] C.krusei is associated with resistance to azoles. C.parapisilosis is the primary cause of fungemia in neonatal intensive care units and in blood cultures and also grows in intravenous catheter.

Virulence factors of Candida species

1. Biofilm formation:

After initial adherence, the fungi grow in multiple colonies and can form biofilm. Biofilm is composed of proteins and carbohydrates which are responsible for the poorer response to antifungal drugs. Biofilm formation on catheters and prosthetic valves can cause systemic disease.[17]

2. Enzymic activity:

The hydrolytic activities in Candida species play major role in pathogenesis. They are aspartyl proteinases, serineproteinases, proteases, lipases, phospholipases, esterase and phosphatases. Hyphal tip with phospholipases are more invasive.

3. Toxin Production:

These are glycoprotein extracts of Candida cell walls are lethal, pyrogenic and induce anaphylactic shock.

4. Complement receptors-Candida albicans possess C3b which binds to complement as well as fibrinogen there by affecting the binding of neutrophils which in turn influence phagocytosis.

5. Phenotype-switching

It is the ability of candida to switch reversibly to different colony phenotypes ranging from unicellular budding yeast to pseudo hyphae and true hyphae. The colony morphology changes from smooth to rough. This phenomenon is first described by Slutsky et al in 1985, 1987. This is analogous to phase variation in bacteria. It expresses fungal plasticity makes the organism to adapt various anatomical sites in human body. This property helps the organisms to evade the host's defense system.

Host factors

Predisposing factors:

1. Pregnancy have 30 – 40% of high risk especially in third trimester.[18] The acidic nature of the pregnant vagina suppress the growth of other microorganisms that are naturally inhibitory to candida. Although the high pH of 6-7 augments the initial attachment of the organism to the vaginal epithelia, the formation of germ tubes and the development of mycelia are favored by acidic vaginal ph of less than 5.
2. Diabetes mellitus is one of the predisposing factors for recurrent vulvovaginal candidiasis. An elevated level of blood glucose enhances the ability of C. albicans to bind to vaginal epithelial cells.

3. High estrogen content in oral contraceptive pills (>50μg) increases glycogen content in vaginal epithelial cells which acts as good source. Hormone also accelerates the formation of pseudohyphae. Progesterone and lactation are safe factors.

4. Drugs

   - Frequent antibiotics clear the normal protective vaginal flora there by facilitating candidal colonization.

   - Systemic steroids increase the susceptibility to candidiasis by diminishing the immune function.

   - Tamoxifen usage in post menopausal women also enhances candidal infection

5. IUCD, diaphragm, spermicide, douching, perfumes and use of feminine hygienic products will produce minor trauma and alter the vaginal bacterial flora. Douching is also a risk factor in females with recurrent vulvovaginal candidiasis caused by Candida glabrata.[22]
6. Endocrine diseases like, hypothyroidism, hypoparathyroidism, Cushing syndrome and polyendocrinopathy are associated with increased susceptibility to infection.
7. Immunosuppressive states like HIV infection, neutropenia, organ transplantation and congenital immunodeficiency express defective killing by neutrophils and macrophages thereby increasing the susceptibility to infection.
8. Maceration of skin due to tight dressings:

Perspiration associated with poorly ventilated and tightly fitted clothes increases moisture and local temperature.

9. Nutritional factors

   Nutritional deficiencies may alter host defense mechanisms or epithelial barrier integrity and allows increased adherence.

HIV and vaginal candidiasis

Acquired immunodeficiency syndrome (AIDS) is a chronic disease primarily affecting the human immune system caused by the human immunodeficiency virus (HIV). The CD4 cell count is the marker to determine the severity of the diseases. Patients with a CD4 cell level less than 200 cells per microlitre are prone for opportunistic infections. Vulvovaginal candidiasis (VVC) is one of the most common fungal infections which recur frequently in females with human immunodeficiency virus (HIV) infection. Candida dubliniensis is commonly occurring species in HIV positives.[36][53]

Refractory candidiasis

Refractory vaginal candidiasis is fairly less common. It is defined as the failure of response to antifungal drugs for a standard time duration that is for 14 days with suitable dose.

Immunology[5]

Candida species are commensal as well as opportunistic pathogens of mucosal tissues. Both innate and acquired immunity play a vital role in

keeping the organism in the commensal state and preventing it to enter in to the systemic circulation. Neutrophils plays important role in the protection against systemic infections, whereas cell-mediated immunity aids protection against mucosal infections. Studies indicate some protective role for the epithelial cells and suggesting that immunity to Candida is site-specific and compartmentalized.

The level of innate immunity to pathogenic fungi is high in most humans. The intact skin and mucosal surfaces are the primary barriers to infection. Epithelial cell turn over, fatty acid content of the skin, low pH of vagina are the important factors of host resistance. Alteration in bacterial flora and any break in the natural barrier facilitate infection.

The outcome depends on the virulence of organism and host defense. Cell mediated immunity provides protection against pathogenic candidal colonization. Phagocytosis by neutrophils is the primary mode of elimination of infection. Evidences showed that antibody along with complement play a role in eliminating fungi from the body.

Candida antigens

1. Cell wall antigens
2. Cytoplasmic antigens

These antigens are useful for serological applications. Mannan is the major antigenic component of cell wall.

Pathogenesis

Most of the candidal species produce virulence factors including protease factors. The ability of yeast form to adhere the host's epithelium is a crucial step in the hyphae formation and pathogenic tissue invasion. Elimination of the competing bacteria from the skin and mucosa results in reduced nutritional and environmental competition that favors the growth of candidal organism. In keratinocytes, C albicans phospholipomannan provokes an inflammatory response through toll-like receptor 2. Candida albicans reduces the expression of interferon-grama-inducible protein – 10 in human keratinocytes. These factors explain innate defenses against candidal organisms. Interleukin 12 receptor β1 deficiency predisposes to candidiasis, most commonly mucocutaneous candidiasis.

Steps in pathogenesis

- Adherence to epithelial endothelial surface
- Invasion
- Host immune response

1. Adherence

C. albicans have more affinity towards vaginal epithelial cells Order of affinity- C.albicans>C.tropicalis>C.parapisilosis

Factors influencing adherence

1. Local vaginal pH

Candida adheres to the vaginal mucosa even if the pH is acidic. In some areas like web spaces the bacteria especially gram negatives act as co pathogens.

-Hormones – Estrogen increases the glycogen content of the vaginal epithelial cells thereby increases the affinity.

-Presence of lg A interfere with adherence

-Cell surface hydrophobicity: germ tube formation is enhanced by hydrophobicity. An adherence result in colonization at the local site or it invades deeper tissue and cause symptomatic disease.

2. Invasion

During active infection, yeast form is converted in to hyphal form. Phospholipases at the tip of hyphae is related to greater invasiveness compared to yeast form. Hyphae which are longer than 200 micrometer are more resistant to phagocytosis. Invasion is aided by various enzymes like proteinases which cleave the peptide bonds. Phospholipases enhance invasion by proteolysis in keratinocytes. Another study revealed elevated levels of hyaluronan in patient with RVVC.

3. Host immune response

Candidal infections stimulate both Cell mediated [CMI] and humoral immune responses. But CMI is the first line of defense. CMI includes activation of neutrophils, mononuclear leucocytes, keratinocytes and dendritic

cells. Candida albicans interact with the major antigen presenting cells i.e., dendritic cells. The dendrite cells stimulate Candida albicans specific lymphocyte proliferation which recognizes them via mannose receptors. T cell activation via toll like receptors leads to cytokine release and inflammatory cascade proceeds.

In addition to cell mediated immunity, humoral immunity also acts against Candida. IgM and IgG antibodies are found in mucocutaneous candidiasis. Witkin et al 1989 demonstrated IgE antibodies in women with vulvovaginitis indicating a possibility of type 1 hypersensitivity in the pathogenesis of vulvovaginitis. Local immunity also plays an important role in defense against Candida. IgA antibodies have been demonstrated in vaginal fluid.[46]

Clinical features

Clinical features are ranging from asymptomatic to severe symptoms like itching and vaginal white discharge.

Clinical classification

Centers for disease control and prevention classified VVC into two groups.[48]

Uncomplicated VVC

- Single episode or less than 4 episodes in a year.
- Mild or moderate symptoms
- Causative organism is Candida albicans.

Complicated VVC

- Recurrent episodes – 4 or more episodes per year
- Severe symptoms of vulvovaginitis
- Associated with pregnancy, poorly controlled diabetes and HIV infection
- Caused by the non Candida albicans species

Acute vulvovaginal Candidiasis

- Vaginal pruritus
- Burning pain
- Vaginal discharge
- Dyspareunia
- Dysuria

Signs

- ❖ Erythema
- ❖ Fissuring
- ❖ Edema of vestibule, labia
- ❖ Thick white curd like or ‘cottage cheese like’ vaginal discharge adherent to the walls of vagina.
- ❖ Maceration in the adjacent skin

Chronic VVC

Symptoms

- Severe pruritus
- Burning sensation
- Pain

Signs

- Lichenification of Vulva

Differential diagnosis

1. Infectious conditions

- **Recurrent bacterial vaginosis**

  It presents as profuse grayish white thin homogenous discharge lining the vagina and smear positive for clue cells.

- **Trichomonas vaginalis**

  Trichomonas vaginalis is distinguished from VVC by the presence of copious greenish frothy discharge with fishy odour associated with intense pruritus. Wet mount of vaginal smear show motile Trichomonas.

- Recurrent genital herpes present with either vesiculation or painful superficial ulcers with polycyclic borders. Tzanck smear show multinucleated giant cells.

- Genital scabies: Nocturnal pruritus, family h/o itching, excoriated papules over the genital region, around the umbilicus, medical thighs and around the areola.

2. Non infections conditions

-Allergic

-Chemical

The diagnosis is based on the patient's detailed history and physical examination. Pruritus is the general symptom. An acute reaction may develop as a result of exposure to a potent irritant that involves the mucosa, leading to burning sensation, pain followed by oozing, which may lead to secondary infection.

Laboratory diagnosis

The diagnosis of VVC is often made clinically.

The following diagnostic tests are done from high vaginal swab

1. Direct microscopy

Direct microscopical examination of clinical specimens – high vaginal swab reveals budding yeast cells called blastoconidia, 2-4 µm in diameter with pseudohyphae showing regular points of constrictions. True septate hyphae may also be produced by C.albicans and C.dubilianensis. It is done by following six methods

1) 10%KOH mount-vaginal smear is made on a glass slide and one drop of 10%KOH is added. Gentle warming of the slide or addition of dimethyl sulfoxide causes rapid clearance of the keratin and other cellular debris, leaving the fungal element intact. Microscopic visualization reveals budding yeasts and pseudo hyphae. It has 70% sensitivity.

2) Calcofluor white stain fluoresces as bright under filtered UV light because of its binding to polysaccharide in the chitin of the fungal cell wall.

3) Gram stain-Thin vaginal smear was made and Gram staining was done. It is seen under microscopy. Yeasts and pseudo hyphae appear as Gram positive elements. It has 65-68% sensitivity.

4) Simple stain: Vaginal swab smear stained by methylene blue & examined microscopically shows presence of regular blastospores as well as pseudo hyphae in resource constraint setting.

5) H & E stain and Gomori's methenamine silver stains demonstrate fungal elements in tissues.

2. **Culture**

Sabouraud's dextrose agar [SDA] is the common medium used for culture. It contains glucose, neopeptone, poly peptone agar and supplemented with chlorheximide, chloramphenicol and gentamycin to prevent bacterial contamination. Glucose provides carbon and peptone agar provides nitrogen. The pH of the media is slightly acidic [5.6]. Vaginal swabs are inoculated in SDA agar and incubated at 25˚c and 37˚c. The colonies appear in 3-4 days.

Species	colony morphology
Candida albicans	creamy white, pasty colonies
Candida glabrata	creamy smooth soft colonies
Candida parapsilosis	creamy, colonies
Candida krusei	smooth colonies
Candida tropicalis	creamy and wrinkled colonies

3. Germ tube test

This test proves yeast germination. Suspected colonies are incubated in sheep or normal human serum at 37˚c for 2-3 hours. A drop of suspension is spread on a slide and examined under the microscope. Germ tubes are seen as long tube like projections extending from the yeast cells. There is no constriction at the point of attachment of pseudohyphae. This test is known as REYNOLDS BRAUDE phenomenon. This test speciates candida into albicans and Non albicans .Germ tube formation is seen in candida albicans and candida dubliniensis.

4. Chlamydospore formation

Yeast produces chlamydospores on cornmeal polysorbate 80 Agar at 22˚c to 25˚c in 48-72 hrs by Dalmau plate technique. Chlamydospores are refractile thick walled cells.

5. Carbohydrate assimilation test

It represents safest identification of non-candida albicans species. Agar plates with Candida on which paper discs impregnated with different carbohydrates [10%] are placed. Candidal growth around a particular disc is an indication for assimilation of that particular carbohydrate. Sugars used for this test are glucose, lactose, sucrose, maltose, galactose, xylose and dulcitol. Candida albicans, Candida tropicalis and Candida parapsilosis assimilate all sugars except dulcitol. Candida glabrata assimilates only three sugars [glucose, xylose and dulcitol].Candida krusei is biochemically inert which assimilates only xylose.

6. Fermentation reaction

It is done in Liquid media supplemented with different carbohydrates [2%] and colour indicator. Durhams tube is used to indicate gas production in the fermentation reaction.[50] Acid production and pH changes are indicated by yellow colour changes. Sugars used for this test are glucose, maltose, sucrose and lactose. Lactose is not fermented by any of the candida species. Glucose is fermented with gas by candida glabrata, candida parapsilosis and candida krusei. Glucose and maltose is fermented by candida albicans, apart from this two sugars sucrose is also fermented with gas by candida tropicalis.

7. HI CHROMagar CANDIDA- differential agar (HIMEDIA)

CHROMagar candida is a rapid plate based test for isolation and identification of various candida species simultaneously.[51]It contains

chromogenic material which is acted upon by distinct enzymes secreted by different candida species to yield a characteristic colony colour. It contains glucose, peptone, agar, chromogenic mix with the pH adjustment of 7-7.2. It should be stored in 2-8 degree C away from direct light. Sample was inoculated and incubated at 37˚ c for 24hrs and are identified by the different colors produced by the candida species which are observed by the naked eye.

Characteristics of various Candida species

C.albicans: grows as a medium sized, smooth, green to dark metallic green colored wet colonies at 48 hours.

C.krusei: grows as a large, flat, spreading, rough to crenated, and pink coloured dry colonies.

C.tropicalis: grows as medium sized smooth medium to dark metallic blue coloured colonies in 24 hours.

C.glabrata: grows as medium sized pink coloured wet colonies with darker mauve center.

C.parapsilosis: grows as white to pink coloured colonies.

Non culture methods

Differentiation of diverse species of candida in laboratories is done by time consuming unreliable methods. Discovery of PCR overcome this problem.

Serological tests

- Antibodies to Mannan proteins
- Gel immune diffusion, immune electrophoresis, ELISA detects antibodies against enolase and heat shock proteins.
- Detection of Arabinitol in the body fluids of infected persons.

Polymerase chain reaction [PCR]

Polymerase chain reaction is a method by which an area of DNA molecule is chosen and amplified. This method is very expensive but much more specific than conventional methods. It can detect <10 yeast in the vaginal swab. So it is highly sensitive but not suitable for differentiating the asymptomatic candidiasis from others.[52]

Modifications of PCR

- Direct PCR
- Multiplex PCR-this test is much faster and more sensitive.
- PCR with dot blot hybridization.
- Restricted fragment length polymorphism is also used for the speciation of candida.

TREATMENT

A wide variety of drugs are effective for the treatment of candidiasis. The mainstay of treatment of vulvovaginal candidiasis is the antifungals. An antifungal agent is a drug that selectively eliminates fungal pathogens from a

host. Besides antifungal agent clinical response is determined by the severity of disease and patient adherence to the treatment and the pharmacological properties of the drug. Treatment of vulvo vaginal candidiasis is very simple and most of the strains respond to therapy.

The discovery of antifungal agents has lagged behind that of antibacterial agents. This is due to the cellular structure of the organisms involved. Fungi are eukaryotes, and consequently most agents that are toxic to fungi are also toxic to the host. Furthermore, as growth of the fungi is slow and often exhibit various cellular forms, they are more difficult to quantify than bacteria.

Major developments in the research of the azole antifungal agents during the 1990s gave expanded options for the treatment of many opportunistic and systemic fungal infections. Fluconazole and Itraconazole have proved to be safer. Inspite of these advances, systemic fungal infections remain difficult to treat and drug resistance to the available drugs is up coming. Use of the newer azole group of drugs in combination with other antifungal agents with different mechanisms of action is likely to provide enhanced efficacy. Second generation triazoles developed to provide extended coverage of opportunistic and emerging fungal organisms, as well as to overcome resistance to older drugs.

Classification of antifungals

Polyenes

1. Nystatin
2. Amphotericin B

Azoles

1. **Imidazoles**
    - Clotrimazole
    - Ketoconazole
    - Miconazole
    - Oxiconazole
2. **Triazoles**
    - Fluconazole
    - Itraconazole
    - Voriconazole
3. **Allyl amines**
    - Naftifine
    - Terbinafine
4. Benzyl amines : Butenafine
5. Echinocandins:
    - Caspofungin
    - Micafungin
    - Anidulafungin

6. Hydroxypyridone : Cyclosporine
7. Heterocyclic benzofurans : Griseofulvin

Polyene Antifungal Drugs

Nystatin, amphotericin, and pimaricin (Natamycin) belongs to this group. They interact with sterols in the fungal cell membrane to induce osmotic instability. Ergosterol in fungi and cholesterol in humans form channels through which small molecules leak from inside to the outside of the fungal cell.

Amphotericin B

It is derived from bacteria streptomyces nodosus. It has broad spectrum of action against Candida and other deep fungal infections. It is fungicidal at high serum concentrations. It is not absorbed orally. It has poor CSF penetration. It is metabolized in the liver and excreted through both urine and bile. It enhances the antifungal action of Flucytosine.

The adverse effects are very high which includes

- ❖ Nephrotoxicity
- ❖ Bone marrow suppression and anemia
- ❖ CNS toxicity

Azole Antifungal Drugs

Ketoconazole, fluconazole and Itraconazole belongs to this group. They inhibit cytochrome P_{450}-dependent lanosterol 14α-demethylase involved in the

biosynthesis of ergosterol, which is necessary for the integrity of the cell membrane structure and function of fungus. Inhibition of this enzyme causes abnormal sterol accumulation inside the cell and leads to fungal cell death.

Echinocandins and cyclo peptides

They are the newly discovered class of fungicidal agents. They noncompetitively inhibit the fungal cell wall β-D-glucan synthesis via inhibition of the enzyme 1,3-β glucan synthase. Examples of Echinocandins include Anidulafungin, Caspofungin and Micafungin. Adverse events are fever, nausea, vomiting and infused-vein complications. They are less toxic drug. This drug causes embryo toxicity hence contraindicated in pregnancy. It can be used in azole resistant Candida infection

AZOLES

The azole antifungals include two classes, Imidazoles and triazoles, which share the same mechanism of action and antifungal spectrum. The systemic triazoles are metabolized very slowly and have less effect on human sterol synthesis than the Imidazoles. Because of these advantages, new drugs under development are mostly triazoles. Of the drugs now on the market, ketoconazole, Clotrimazole, miconazole, ecnazloe, Oxiconazole, sertaconazole, butoconazole and sulconazole are imidazoles. Fluconazole, terconazole, itraconazole, voricoazole, posaconazole and isavuconazole are triazoles. Ketoconazole and miconazole have more effect on mammalian cytochromes

than triazoles and tend to have severe adverse effects. Hepatotoxicity is common to all of them, and the risk of endocrine dysfunctions also exists.

Mechanism of action

The major effect of Imidazoles and triazoles on fungi is inhibition of a microsomal enzyme 14-alpha-sterol demethylase. Imidazoles and triazoles impair the biosynthesis of ergosterol and directly increases the permeability of the cytoplasmic membrane.

Ketoconazole

It is the first orally available imidazole. The absorption is pH dependent and variable with food. It reaches its peak plasma concentration at 1-2 hours of single dose. The drug does not penetrate CSF. So it is not used in fungal meningitis. It is metabolized in the liver by the enzyme cytochrome 3A4. It is excreted in urine and feces.

Adverse drug reactions

Hepatotoxicity

Gynecomastia,

Impotence, loss of libido and oligospermia

Fluconazole

- Triazoles are fungistatic. The imidazole group is substituted by nitrogen group which shows improved antifungal activity and resistance to metabolic degradation. Excreted unchanged in urine. As the drug crosses blood brain barrier, it is useful for the treatment of fungal meningitis.

Adverse reactions

- ❖ Gastro intestinal symptoms like nausea, abdominal pain.
- ❖ Hepatotoxic

Itraconazole

-It is a broad spectrum fungistatic

-Food and gastric acidity enhances the absorption.

-It undergoes hepatic metabolism via cytochrome 3A4 and excreted in feces and urine. It is measurable in stratum corneum 3-4 wks after discontinuation of drug. It does not penetrate CSF.

Use with caution

- ✸ Hepatic disease
- ✸ Renal disease
- ✸ COPD
- ✸ Cardiac diseases

Voriconazole

- ✸ It is 2nd generation broad spectrum triazoles active against severe fungal infections oral candidiasis, chronic muco cutaneous candidiasis, resistant candida and Fusarium infection.

Clotrimazole

- ❖ It is available as 1-2% cream, lotion lozenges, troches, powder, spray and solution. It causes leakage of intra cellular proteins break down of nucleic acid, potassium efflux and finally cell death.

Adverse reaction

- Contact dermatitis especially to the ingredients and preservatives

-Utriicarial rashes

Nystatin

Mechanism of action

Like amphotericin B and natamycin, nystatin binds to ergosterol, which is a major component of the fungal cell membrane. It forms pores in the membrane that lead to K^+ leakage, acidification, and death of the fungi.

***Use*s:** Candidiasis involving skin, vaginal mucosa and esophagus usually respond well to treatment with nystatin. It is available in many forms. Oral nystatin tablets is often used as a preventative treatment in risky individuals for fungal infections, such as HIV positive individuals with a low $CD4^+$ count and patients receiving immunosuppressive drugs.

- An oral suspension form is used for the prophylaxis or treatment of oropharyngeal thrush. A tablet form is preferred for candidal infections of GIT
- It is also available as a topical cream and can be used for superficial candidal skin infections.

- A liposomal formulation of nystatin was investigated in the 1980s and in the early 21st century and was intended to resolve problems arising from the poor solubility of the parent molecule and the associated systemic toxicity of the free drug.

Adverse effects

The oral suspension produces a number of adverse effects including

- Abdominal pain
- Diarrhea
- Rarely, tachycardia, facial swelling, bronchospasm and myalgia.

Spectrum of Antifungal action

Azoles are effective against Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata, Cryptococcus neoformans, Balstomyces dermatitidis, Histoplasma capsulatum, Coccidioides spp., Paracoccidioides brasiliensis, and dermatophytes. Aspergillus spp., Scedosporium apiospermum (Pseudallescheria boydii) Fusarium, and sporothrix schenckii are intermediate in susceptibility. Candida krusei and the agents of mucormycosis are more resistant. Posaconazole has slightly improved activity in vitro against the agents of mucormycosis.

Resistance

Widespread use of azoles has lead to the emergence of both primary and secondary resistance, causing clinical failure. The main mechanism of

resistance in Candida albicans is the accumulation of mutations in ERG11, the gene which codes for the 14-alpha-sterol demethylase. The above mutations protect heme in the enzyme pocket from binding to the azole but allow access of the natural substrate for the enzyme lanosterol.

Cross-resistance is seen in all azoles. The azole efflux by both ATP binding cassette (ABC) and major facilitator transporters can enhance fluconazole resistance in candida albicans and candida glabrata. Excess production of C14-alpha-sterol demethylase is another potential factor of resistance. C5, 6 sterol reductase gene ERG3 mutations also can increase azole resistance in some species.

Antifungal susceptibility testing:

Methods available are

1. Conventional methods - a) Broth dilution method
   b) Disk Diffusion method
2. Other methods - a) Epsilometer test
   b) Neo Sensitabs
   c) Colorimetric methods like Fungistat and yeast-one sensitive assay.
   d) Spectro photometric method
   e) Flow cytometry
   f) VITEK 2 Yeast susceptibility test
   g) Bioluminescence Assay

Among this testing Disk diffusion method by Kirby Bauer method was chosen as it was easy to perform and its sensitivity was also high and also report can be obtained quickly. CLSI guidelines M44-A2 was followed in this study.

Materials and Methods

MATERIALS AND METHODS

After getting the ethical clearance from the institutional ethical committee, the specimens were collected from the patient with vulvovaginal infections and also the consent of the patient was obtained.

Study centre	:	This study was conducted in patients attending the gynecology OP at Govt.Rajaji Hospital, and Institute of Microbiology, Madurai Medical College, Madurai.
Study period	:	This study was conducted from May 2018 to April 2019.
Sample size	:	The study population consists of 250 patients attending the gynecology out patient at Govt.Rajaji hospital.
Study type	:	Prospective study

INCLUSION CRITERIA

All married females with H/O leucorrhoea attending the gynecology OP block.

EXCLUSION CRITERIA

1) Extremely ill female patients

2) Female patients without white discharge

3) Patients taking antifungal treatment

4) Unmarried female patients.

A total of 258 women in the age group of 18 to 50 years with clinically suspected vulvovaginal candidiasis referred from gynecology out-patient block from May 2018 to April 2019 formed the study group.

Consent from patients was also obtained after explaining the procedure for sample collection and also regarding study.

A questionnaire was completed with information covering complaints, nature of vaginal discharge, personal history, marital history, predisposing factors, per vaginal examination and collection of high vaginal swabs.

Data collected included name, age, complaints like vaginal discharge, pain and itching, duration of illness whether acute or chronic, any past history of presenting episode, any systemic diseases such as diabetes, hypertension, tuberculosis etc.

SPECIMEN: High Vaginal Swab: is a technique used to obtain a sample of discharge from the vagina.

PROCEDURE

1. The procedure was explained to the patient and consent taken for the procedure.
2. Patient was asked to void urine.
3. After ensuring privacy, patient was placed in dorsal position on the examination table and draped properly.
4. Hands of the sample collector were washed thoroughly and with gloved hands, the vaginal speculum .was lubricated with jelly.
5. Speculum was passed into the vagina and cervix located.
6. Specimen was collected from the posterior fornix of vagina using sterile swab stick and placed in a bottle.
7. Samples were labeled and microbiology request form was filled.
8. The specimen and request form were send to microbiology lab.

After receiving the specimens the following tests were performed:

1. Direct microscopy – by KOH and Grams staining
2. Culture in Sabourauds dextrose agar
3. Germ tube test
4. CHROM agar for Candida
5. Corn meal agar
6. Carbohydrate fermentation test
7. Carbohydrate assimilation test
8. Anti fungal susceptibility test.

Two high vaginal swabs were collected from each patient and processed in the Department of Microbiology. One swab was used to determine the presence of yeast by direct wet mount microscopy using a drop of 10% potassium hydroxide solution and Gram staining. The other swab was used to inoculate Sabourauds dextrose agar (SDA) and CHROM agar plates. The inoculated plates were incubated at 37 C and 35 C, respectively for 48 hours. Isolates on SDA plates were identified and speciated using conventional methods i.e. Germ tube test, Sugar assimilation test and morphology on Corn meal agar as per standard methods.[8,9].The isolates on CHROM agar were identified by color of the colonies. Antifungal susceptibility testing was performed by Disc diffusion method using control strains as per CLSI guidelines[10].

Laboratory diagnosis

The following diagnostic methods are done:

1. Direct microscopy

Direct microscopical examination of clinical specimens - high vaginal swab was done by two methods:

a) 10%KOH mount-vaginal smear is made on a glass slide and one drop of 10%KOH is added. Apply cover slip over the smear. Gentle warming of the slide or addition of dimethyl sulfoxide causes rapid clearance of the keratin and other cellular debris, leaving the fungal element intact.

Microscopic visualization reveals budding yeasts and pseudo hyphae. It has 70% sensitivity.

b) Gram stain-Thin vaginal smear is made. Methyl violet is added and washed after I minute. Then Gram's iodine is added and washed after 1 minute. Then acetone is added to decolorize for less than a second. Finally carbol fuschin 1:20 dilution is added as a counter stain. After washing and drying, it is seen under microscopy. Yeasts appear as Gram positive budding yeast cells. It has 65-68% sensitivity.

2. Culture

Sabouraud's dextrose agar [SDA] is the most common medium used for cultivation of fungi, moulds and yeasts. It contains glucose, neopeptone, poly peptone agar and supplemented with gentamycin. Vaginal swabs were inoculated in SDA agar and incubated at 25˚c and 37˚c. The colonies appear in 2-4 days.

Morphology of the candida species

- Candida albicans produces cream coloured smooth pasty colonies
- Candida tropicalis produces cream coloured to white, glistening to dull, smooth or wrinkled colonies with mycelia fringes.
- Candida glabrata produces smooth cream, colored, and glistening, small colonies.
- Candida parapsilosis produces creamy colonies in lacy pattern.
- Candida kefyr produces smooth soft creamy colonies.

- Candida guillermondii produces thin flat, glossy, cream to pink glistening smooth wrinkled colonies.
- Candida krusei produces flat dull dry smooth or wrinkled colonies with lateral fringe.

3. Germ tube test

This test proves yeast germination. Suspected colonies are incubated in sheep or normal human serum at 37˚c for 2-3 hours. A drop of suspension is spread on a slide and examined under microscope. Germ tubes are seen as long tube like projections extending from the yeast cells. There is no constriction at the point of attachment of pseudohyphae.

Report: Germ tube test positive- Candida albicans

Germ tube test negative-Non albicans candida

4. Chlamydospore formation

Yeast produces chlamydospores on cornmeal polysorbate 80 Agar at 22˚c to 25˚c in 48-72 hrs by Dalmau plate technique and Chlamydospores are observed under microscopy. Chlamydospores are refractile thick walled cells produced under nutrition.

*Candida albicans – formation of large thick walled terminal chlamydospores.

*Candida tropicalis - shows fir tree appearance, blastospores are formed in single or in small groups.

*Candida krusei – elongated blastospores which showed cross match stick appearance.

*Candida parapsilosis – blastospores in clusters along the pseudohyphae seen.

*Candida glabrata – small blastospores in clusters was seen.

5. Carbohydrate assimilation test

On Agar plate, colony from 24hrs growth was streaked and on which paper discs impregnated with different carbohydrates like glucose, lactose, sucrose, xylose, dulcitol, galactose and mannose in 10% concentration are placed. Candidal growth around a particular disc is an indication for assimilation of that particular carbohydrate.

6. Fermentation reaction

It is done in Liquid media supplemented with different carbohydrates in 2% concentration and colour indicator. Durhams tube is used to indicate gas production in the fermentation reaction.[50] Acid production and pH changes are indicated by colour changes. Sugars used were glucose, lactose, sucrose and maltose.

7. HI CHROM agar Candida - differential agar (HIMEDIA)

CHROM agar is rapid plate based test for isolation and identification of various candida species simultaneously.[51] It contains chromogenic material which is acted upon by distinct enzymes secreted by different candida species to yield a characteristic colony colour. It contains glucose, peptone, agar, chromogenic mix with the pH adjustment of 6.1. It should be stored in 2-8

degree C away from direct light. Sample has to be inoculated and incubated at 37˚ c for 48 hrs.

Characteristics of various Candida species

C.albicans: grows as a medium sized, smooth, green to dark metallic green colored wet colonies at 48 hours.

C.krusei: grows as a large, flat, spreading, rough to crenated, and pink coloured dry colonies.

C.tropicalis: grows as medium sized smooth medium to dark metallic blue coloured colonies in 24 hours.

C.glabrata: grows as medium sized pink coloured wet colonies with darker mauve center.

C.parapisilosis: grows as white to pink coloured colonies.

8. ANTIFUNGAL SUSCEPTIBILITY TESTING:

By KIRBY BAUER DISK DIFFUSION METHOD

1. The CLSI reference document for performing this method is M44-A.

2. CLSI MAA-A2 method uses Mueller-Hinton agar with 2% glucose and 0.5microgram methylene blue/ml.

Procedure:

The following media and other materials are required in this method.

The increased glucose helps in improving yeast growth and methylene blue provides sharper delineating zones surrounding the disc.

1. Mueller – Hinton agar was be prepared from a commercially available dehydrated Mueller-Hinton agar base according to the manufacturer's instructions.

2. Methylene blue dye (0.1 gm) was dissolved in 20ml of distilled water and warmed gently to dissolve. Should not be over-heated. 100 micro liter of this solution was added to the per liter of agar suspension.

3. To 1 litre of agar suspension, 20gm of glucose per liter was added.

4. Autoclave was done as per manufacturer's instructions.

5. Immediately after autoclaving, the agar solution was allowed to cool in a 45 to 50 degree water bath.

6. Cooled medium was poured into the petri dishes on a level, horizontal surface to give a uniform depth of approximately 4mm. This corresponds to 67 to 70 ml of medium for plates with diameters of 150mm and 28 to 30 ml for plates with a diameter of 100mm.

7. The medium was allowed to settle and cool to room temperature. It was stored at 2 to 8 degree Celsius. The agar medium should have a pH between 7.2

and 7.4 at room temperature. Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar.

INOCULUM PREPARATION: The candidal colonies were inoculated into 1ml of dimethyl sulfoxide solution and the turbidity was compared with 0.5 Mac Far land standard to get 10^8 cells, if needed dilution was done with peptone water.

ANTIFUNGAL DISK: The readymade antifungal disks [HIMEDIA] were used for testing the antifungal susceptability pattern of the isolated candida species. Disk used are fluconazole [25μg] voriconazole [1μg] itraconazole [10μg] and nystatin [50μg].

SUSCEPTIBILITY TESTING PROCEDURE

Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the suspension. The swab should be rotated several times and pressed firmly against the inside wall of the tube above the fluid level. This will remove excess fluid from the swab. The dried surface of a sterile Mueller – Hinton agar plate was inoculated by evenly streaking the swab over the entire agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed. With the help of forceps, antifungal disks are placed onto the surface of the inoculated agar plate. Each disk must be pressed down

to ensure its complete contact with the agar surface. Five disks were placed on a 100mm plate. Because the drug diffuses almost instantaneously, a disk should not be moved once it has come into contact with the agar surface.

The plates were incubated at 35°c for 24 hrs and measurement of diameter of zone of inhibition was done with AST scale.

The same procedure of antifungal susceptability test was done for the control strain candida albicans ATCC 90028.

RESULTS AND INTERPRETATION

The zone diameters were measured to the nearest point at which there is prominent reduction in growth. Pinpoint colonies at the zone edge or larger colonies within a zone are frequently encountered and should be ignored. After measurement of zone of inhibition, results of antifungal susceptibility testing are interpreted according to following criteria:

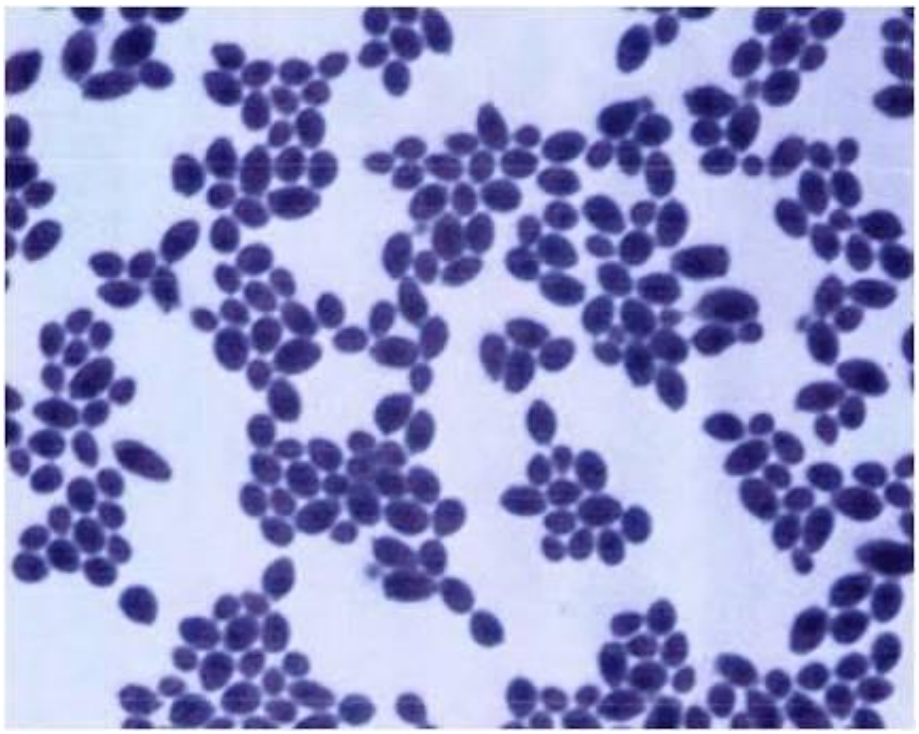
Sensitive: The zone diameter of test strain is more than 80% than that of control strain.

Intermediate: The zonal diameter of test strain is less than 80% than that of the control strain.

Resistant: There is no zone of inhibition.

Z- THICK CURDY WHITE DISCHARGE WITH INFLAMED VAGINAL MUCOSA IN SPECULAM EXAMINATION

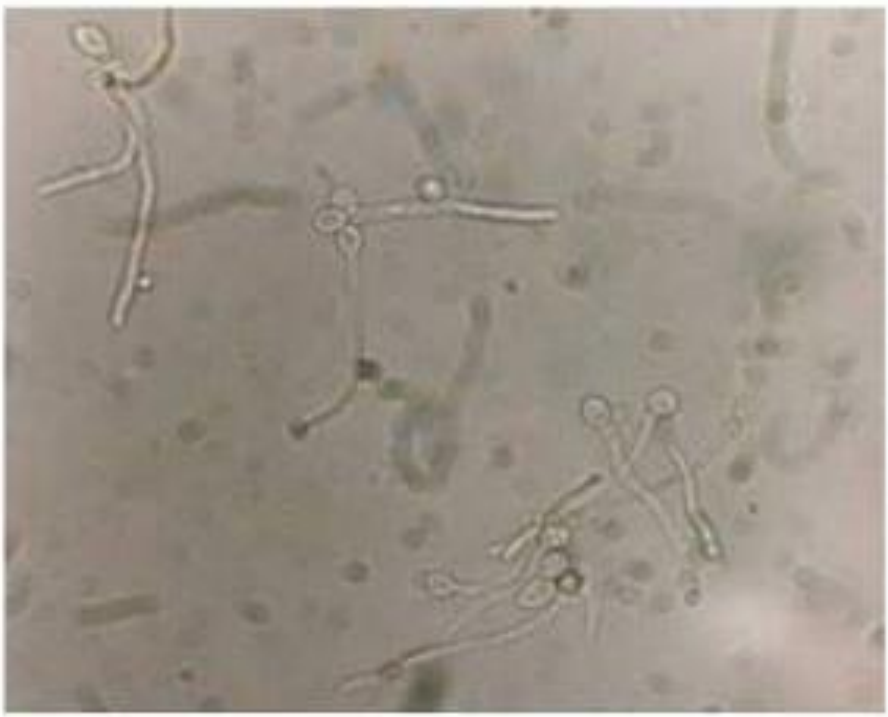
GRAM STAINING



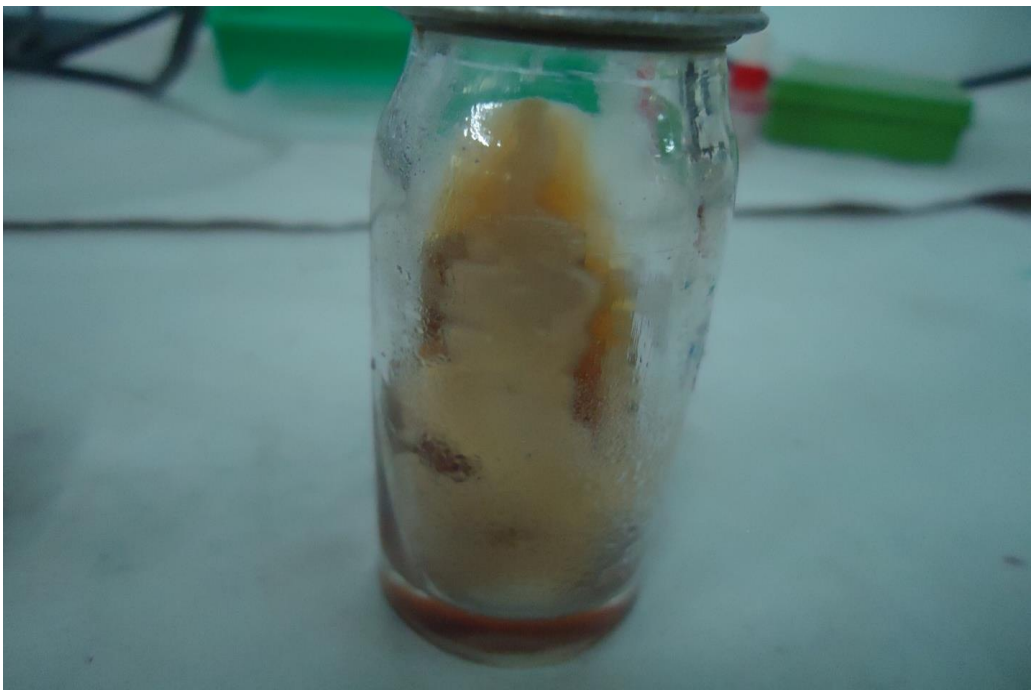
GRAM TUBE TEST

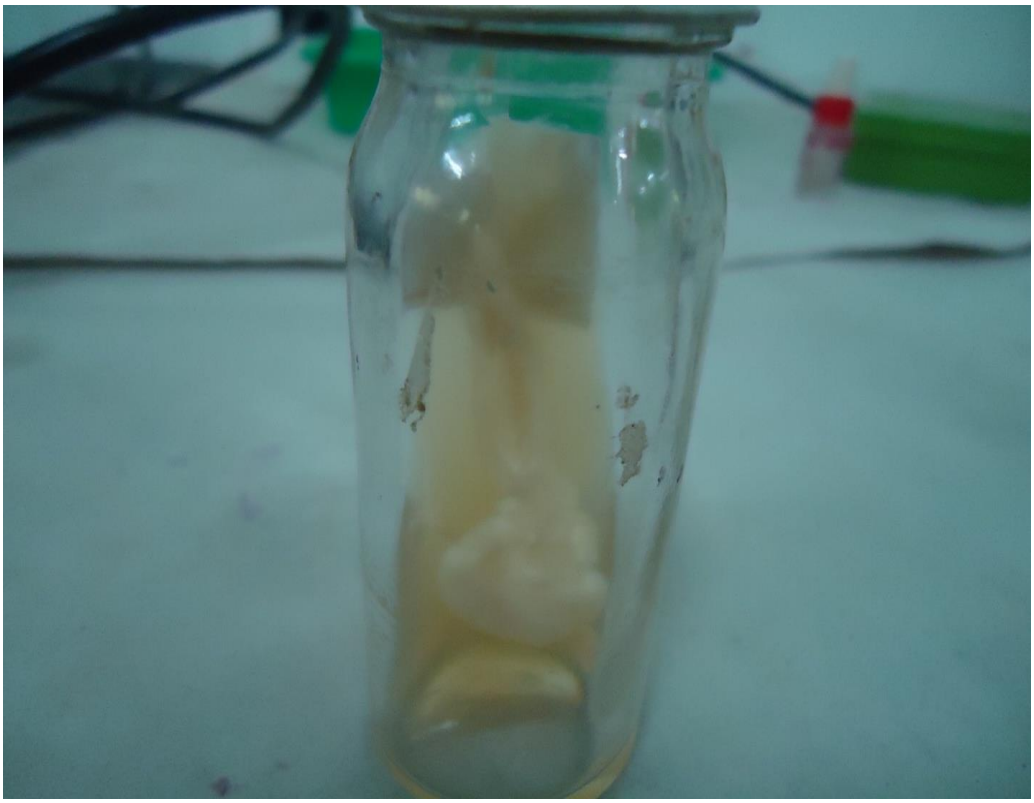
C.albicans

(Positive Germ Tube test)

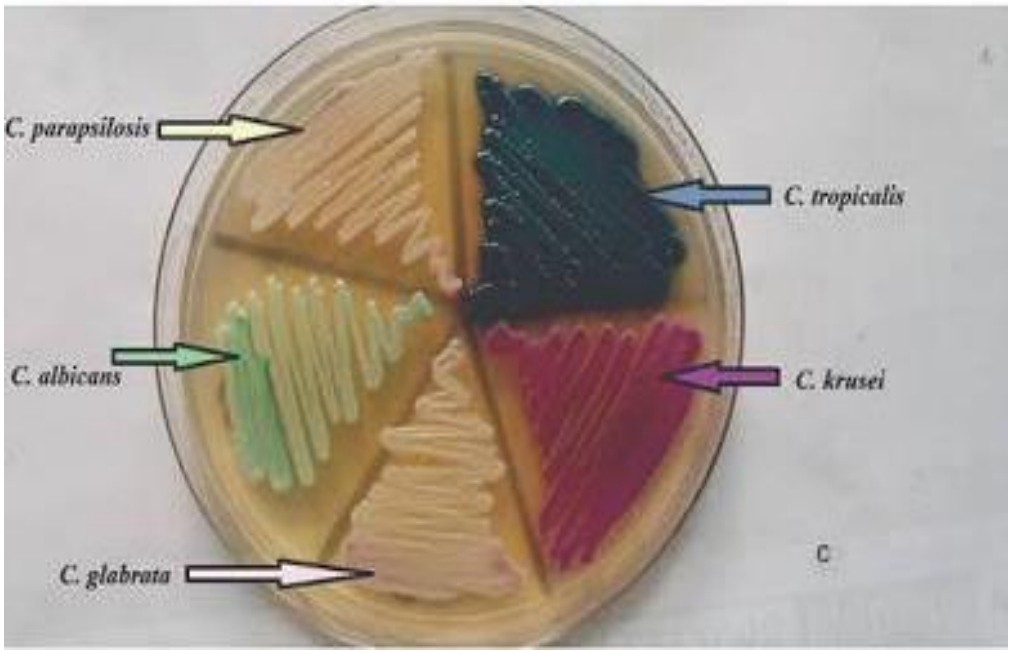


CULTURE – SDA



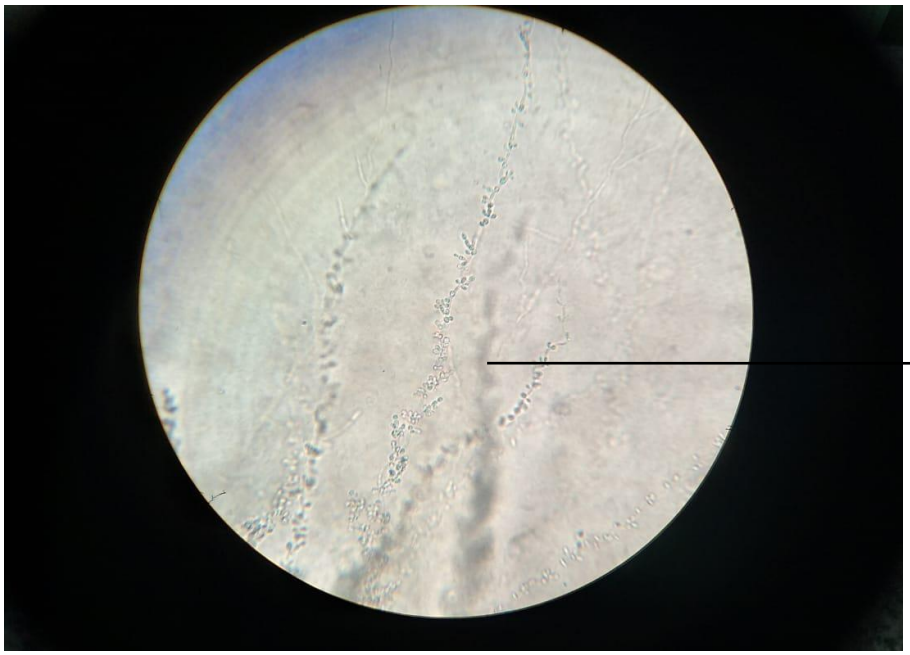


CULTURE – CHROMOAGAR



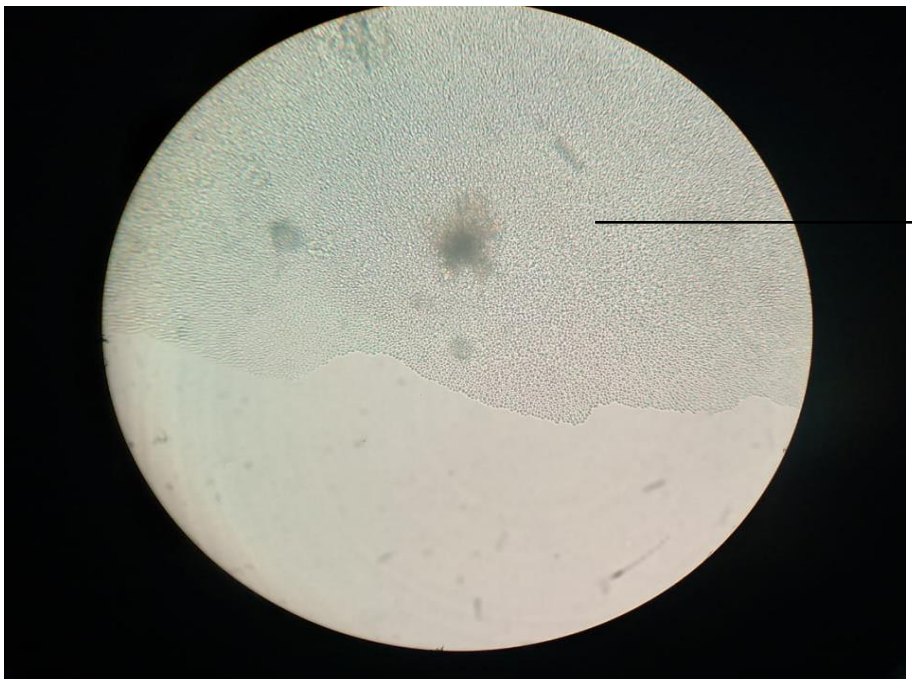
CULTURE CORNMEAL AGAR – DALMAU PLATE TECHNIQUE

1. C.tropicalis



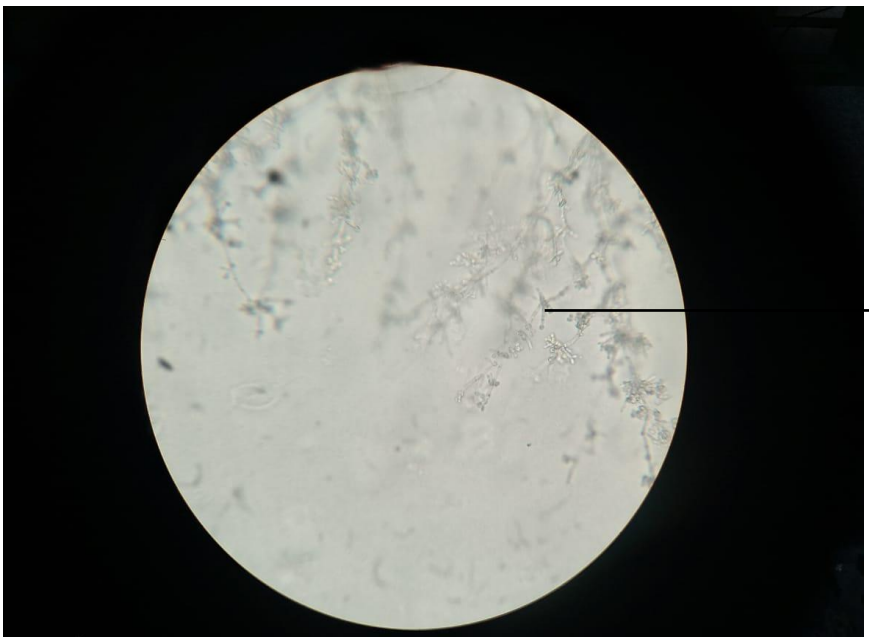
Fir tree appearance, blastospores are in small groups

2. C.glabrata



Small blastopores in clusters

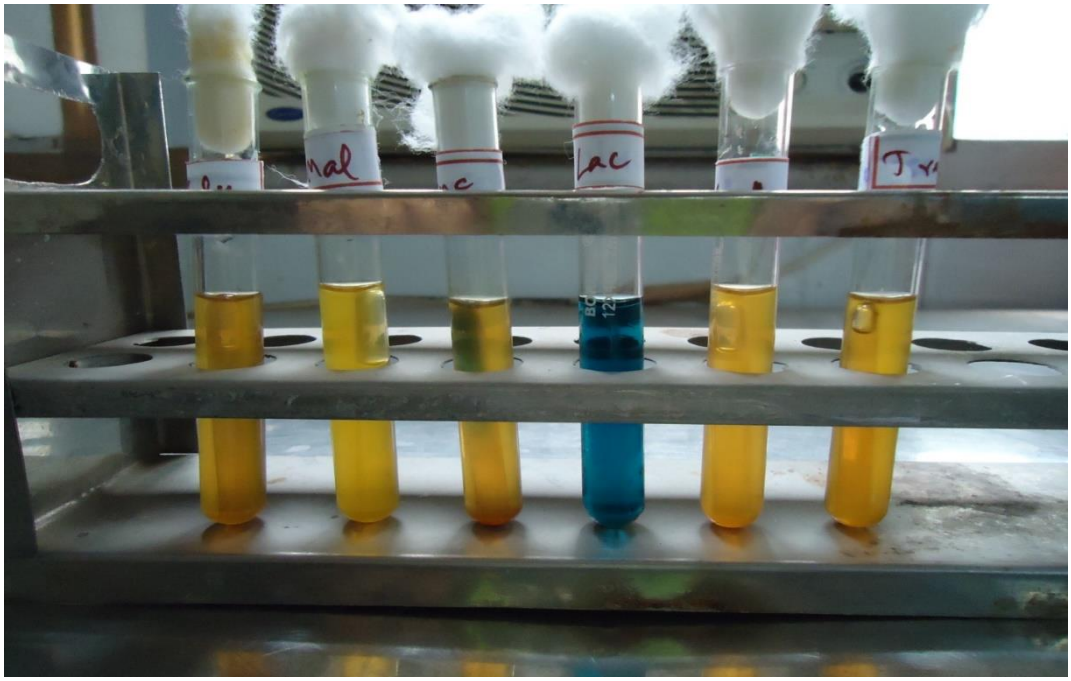
3. C.krusei



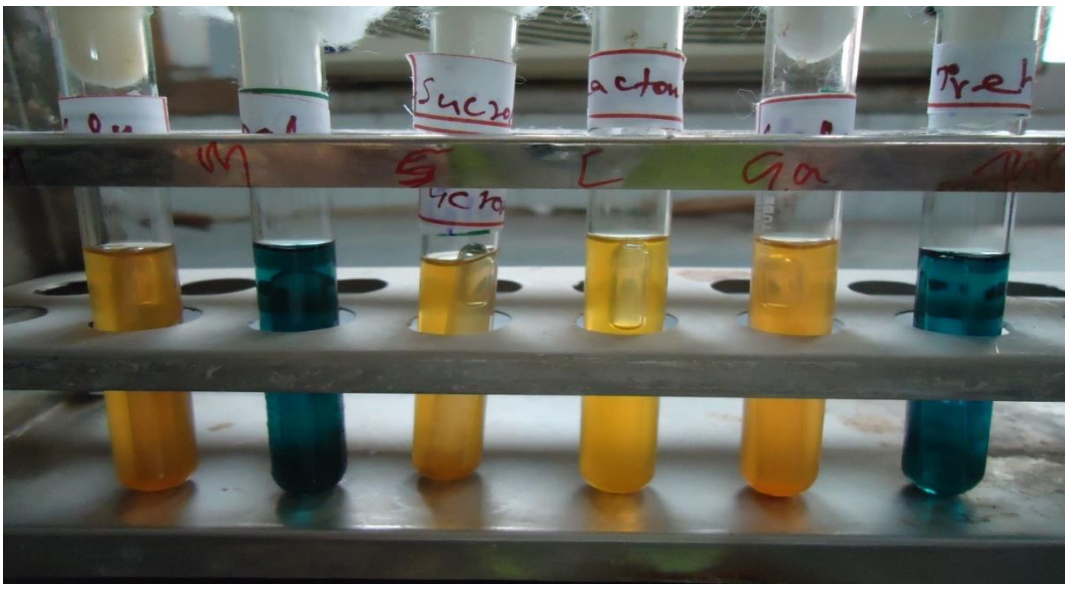
Elongated blastopores, cross match stick appearance

CARBOHYDRATE FERMENTATION TEST

1. C.albicans and C.glabrata

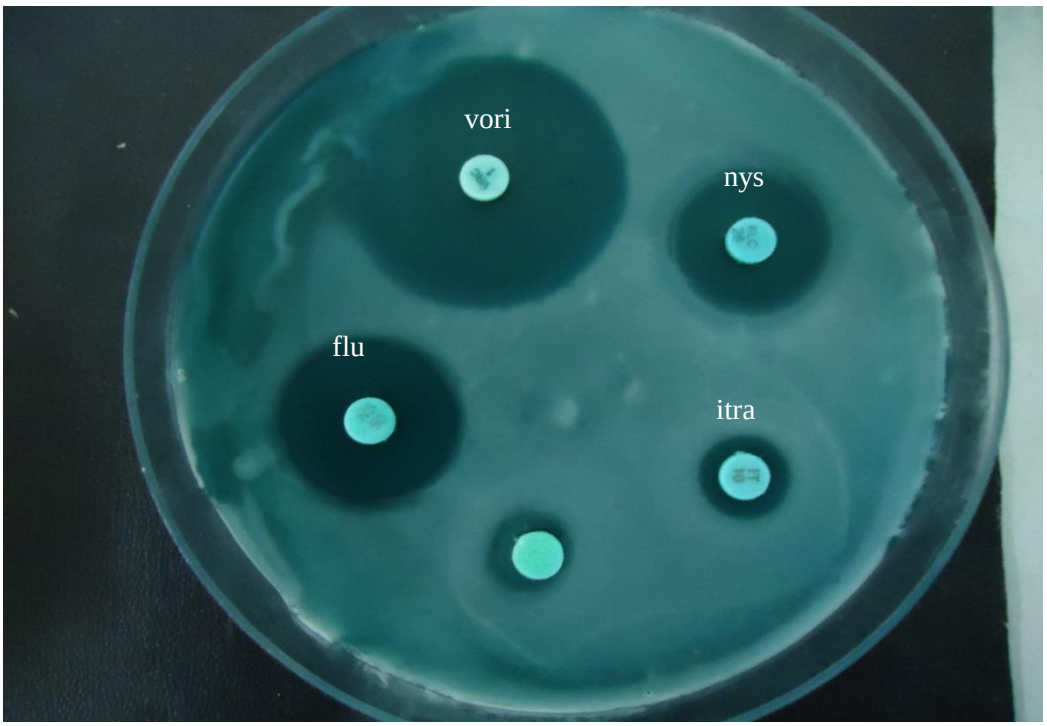


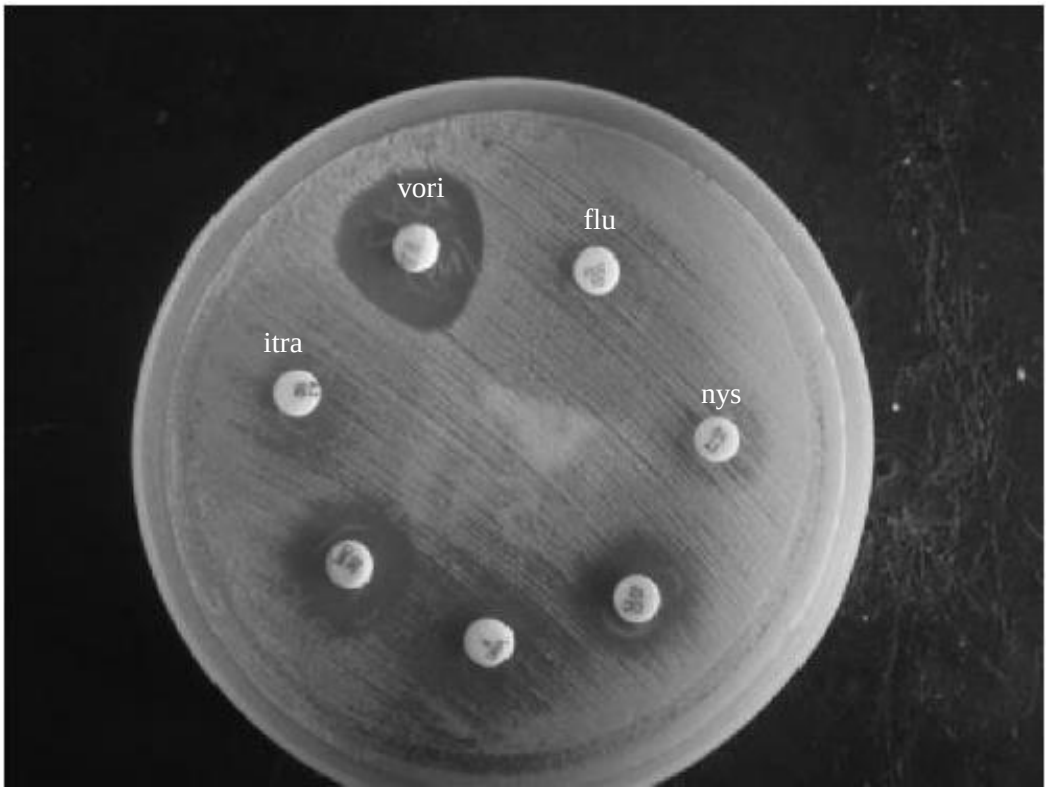
2. C.tropicalis and C.krusei and C.parapsilosis



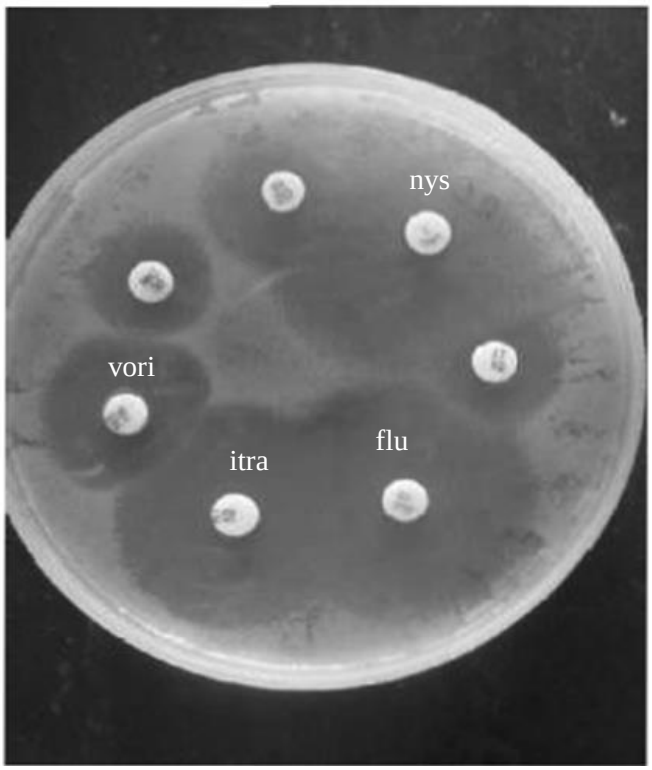
CARBOHYDRATE ASSIMILATION TEST

ANTIFUNGAL SUSCEPTABILITY





ANTIFUNGAL SUSCEPTABILITY



Observation and Results

OBSERVATION AND RESULTS

A total of 250 samples from symptomatic female patients above 18 years who fulfilled the clinical inclusion criteria were screened by microscopy and culture on SDA. Diagnosis of vulvovaginal candidiasis was confirmed in 180 cases and the 70 cases were negative for candida. All the results obtained were analysed statistically for their completeness.

TABLE 1

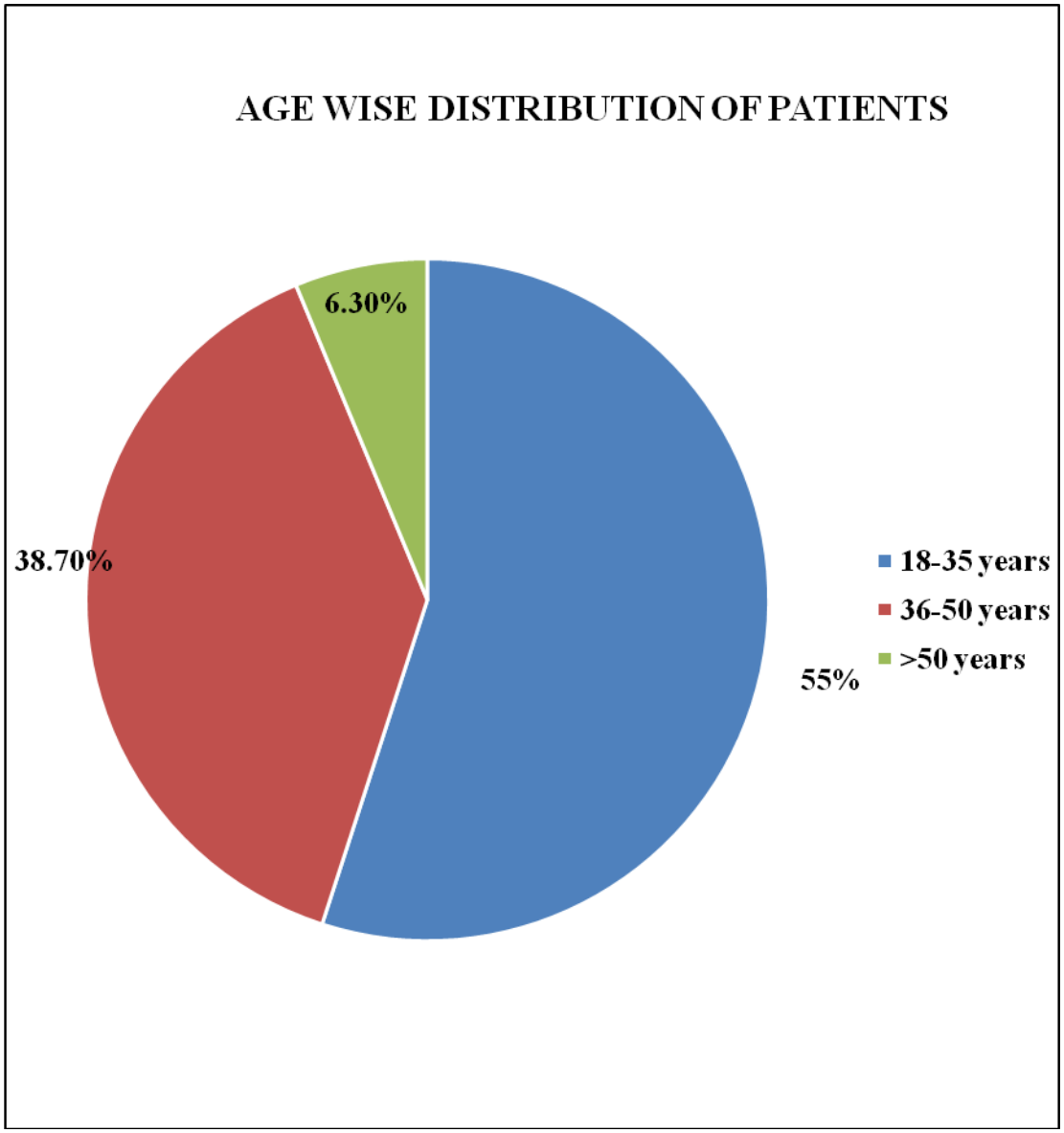
AGE WISE DISTRIBUTION OF VVC

[n =180]

Age	Number of Patients	Percentage
18-35years	106	55%
36-50 years	64	38.7%
>50years	10	6.3%

The 180 study subjects were analyzed based on age and tabulated (table l). Of the 180 patients, 106(55%) belonged to l8-35 years; 64 patients (38.7%) belonged to the age group of 36- 50 years and only 10 patients (6.3%) were in the age group of 50 and above. So majority of the patients fall in the sexually active group (18-35years) ,were vulnerable to get VVC.

CHART – 1



All the study group patients were symptomatic and presented with profuse vaginal curdy white discharge.

TABLE 2

SPECIES PREVALENCE BASED ON GERM TUBE TEST

[n= 180]

Positive	%	Negative	%
94	52%	86	48%

Total 180 isolated candida species were subjected to germ tube test. It was positive in 94 specimens and they were considered as Candida albicans and 86 specimens were negative for germ tube test and were considered as Non albicans Candida.

All the isolated candidas were tested for carbohydrate fermentation and carbohydrate assimilation test for the sugars glucose, sucrose, lactose, mannose, mannitol, xylose and dulcitol. Based on the fermentation and assimilation of different sugars, speciation of candida was done.

Table 3: Carbohydrate fermentation test result [n = 180]

Species	Glucose	Maltose	Sucrose	Lactose
Candida albicans	AG	AG	NF	NF
Candida glabrata	AG	NF	NF	NF
Candida parapsilosis	AG	NF	NF	NF
Candida tropicalis	AG	AG	AG	NF
Candida krusei	AG	NF	NF	NF

AG = Acid and Gas production; NF = Non Fermented

Table 4: Carbohydrate Assimilation test result [n = 180]

Species	Glucose	Maltose	Sucrose	Lactose	Galactose	Xylose	Dulcitol
Candida albicans	+	+	+	+	+	+	-
Candida glabrata	+	-	-	-	-	+	+
Candida parapsilosis	+	+	+	+	+	+	-
Candida tropicalis	+	+	+	+	+	+	-
Candida krusei	-	-	-	-	-	+	-

TABLE: 5

Identification of species prevalence based upon culture on Candida CHROMagar and Corn meal agar

[n=180]

Species	Number of patients	Percentage
Candida albicans	94	52%
Candida glabrata	56	32%
Candida tropicalis	17	9%
Candida parapsilosis	11	6%
Candida krusei	02	1%

Among the 180 culture positive cases, 94 (52%) were Candida albicans; 56 cases (32%) were C.glabrata; 17 cases (9%) were C.tropicalis and 11 cases were C.parapsilosis (6%) and 2 cases (1%) were C.krusei.

CHART 2

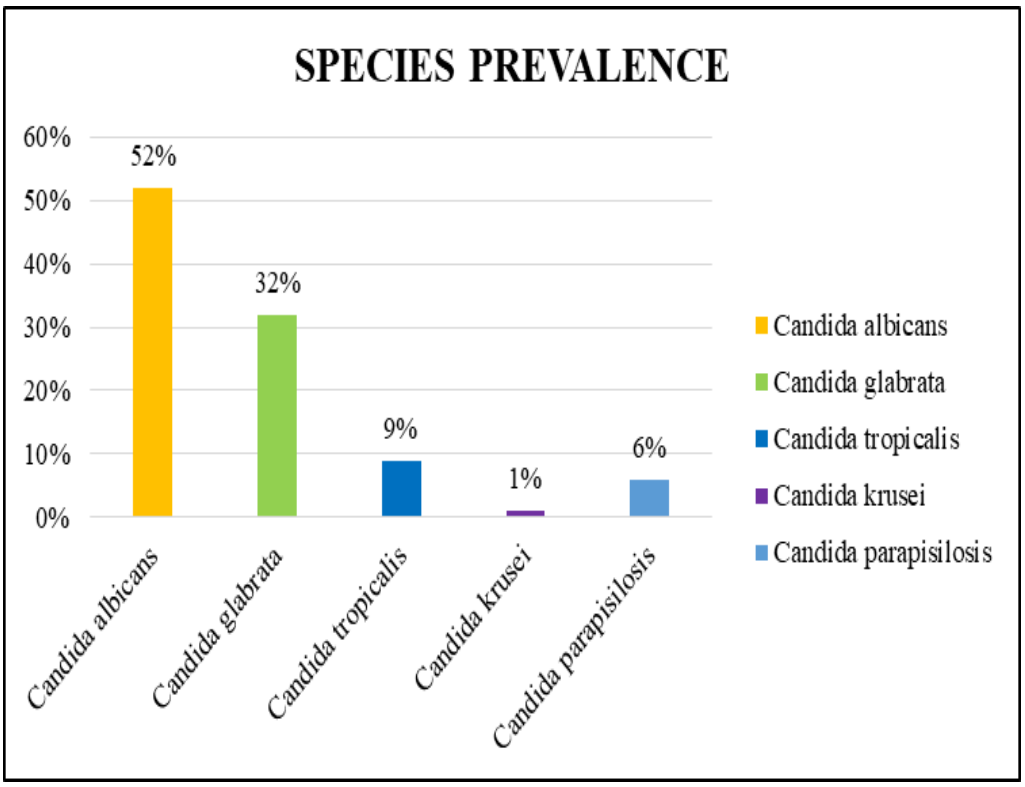

SPECIES PREVALENCE
60%
50%
40%
30%
20%
10%
0%
52%
32%
9%
1%
6%
Candida albicans
Candida glabrata
Candida tropicalis
Candida krusei
Candida parapisilosis
Candida albicans
Candida glabrata
Candida tropicalis
Candida krusei
Candida parapisilosis

TABLE 6

Analysis 0f antifungal drug sensitivity pattern of Candida Species

[n = 180]

Drugs	Sensitive	Percentage	Resistant	Percentage
Fluconazole	103	57%	77	43%
Itraconazole	144	80%	36	20%
Nystatin	162	90%	18	10%
Voriconazole	126	70%	54	30%

Of the 180 culture positive cases, the isolates were subjected to drug sensitivity by Kirby Bauer disc diffusion method.162 cases (90%) were sensitive to Nystatin; 103 cases (57%) were sensitive to fluconazole;144 cases(80%) were sensitive to Itraconazole; 126 cases (70%) were voriconazole sensitive.

CHART 3

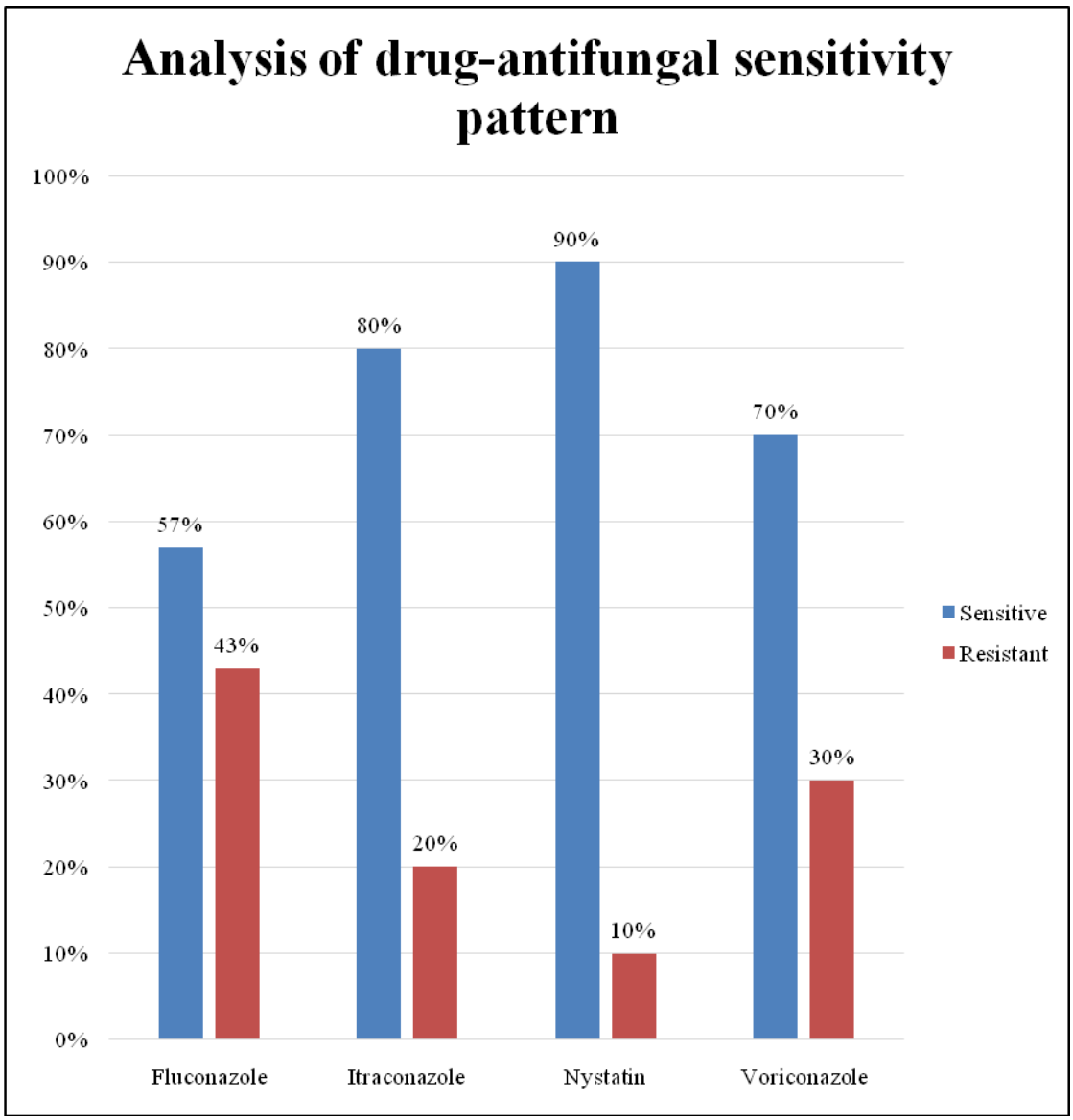

Analysis of drug-antifungal sensitivity pattern
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
57%
43%
80%
20%
90%
10%
70%
30%
Fluconazole
Itraconazole
Nystatin
Voriconazole
Sensitive
Resistant

TABLE 7

Analysis of drug sensitivity pattern in Candida albicans

[n= 94]

Drugs	Sensitive	Percentage	Resistance	Percentage
Fluconazole	74	78%	20	22%
Itraconazole	65	69%	29	31%
Nystatin	82	87%	12	13%
Voriconazole	55	58%	39	42%

Among the 94 cases of Candida albicans, Fluconazole, Itraconazole, Nystatin were highly sensitive drugs. 82[87%] cases were sensitive to Nystatin; Itraconazole was sensitive in 65[69%] patients; Fluconazole was sensitive in 74[78%] patients and voriconazole was sensitive in 55[58%] patients.

CHART 4

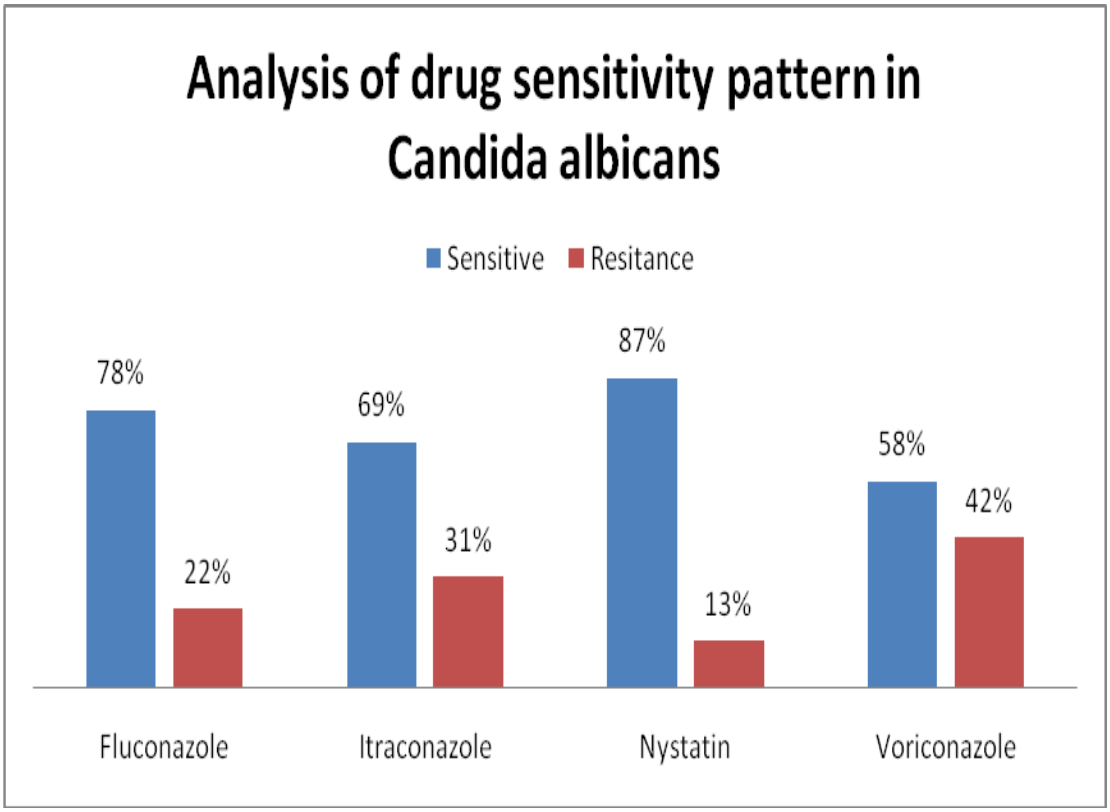

Analysis of drug sensitivity pattern in Candida albicans
Sensitive
Resitance
78%
22%
69%
31%
87%
13%
58%
42%
Fluconazole
Itraconazole
Nystatin
Voriconazole

TABLE 8

Drug sensitivity pattern in Non Candida albicans patients

[n= 86]

Drugs	Sensitivity (No of patients patients)	Percentage	Resistance (No of patients)	Percentage
Fluconazole	29	34.9%	57	65.1%
Itraconazole	79	91%	7	09%
Nystatin	80	93%	6	07%
Voriconazole	71	82%	15	18%

Among the 86 Non albicans Candida group 80 were sensitive to Nystatin (93%). Fluconazole, voriconazole showed sensitivity in 29(34.9%) and 71(82%) patients respectively and 79 patients (91%) were sensitive to Itraconazole.

CHART 5

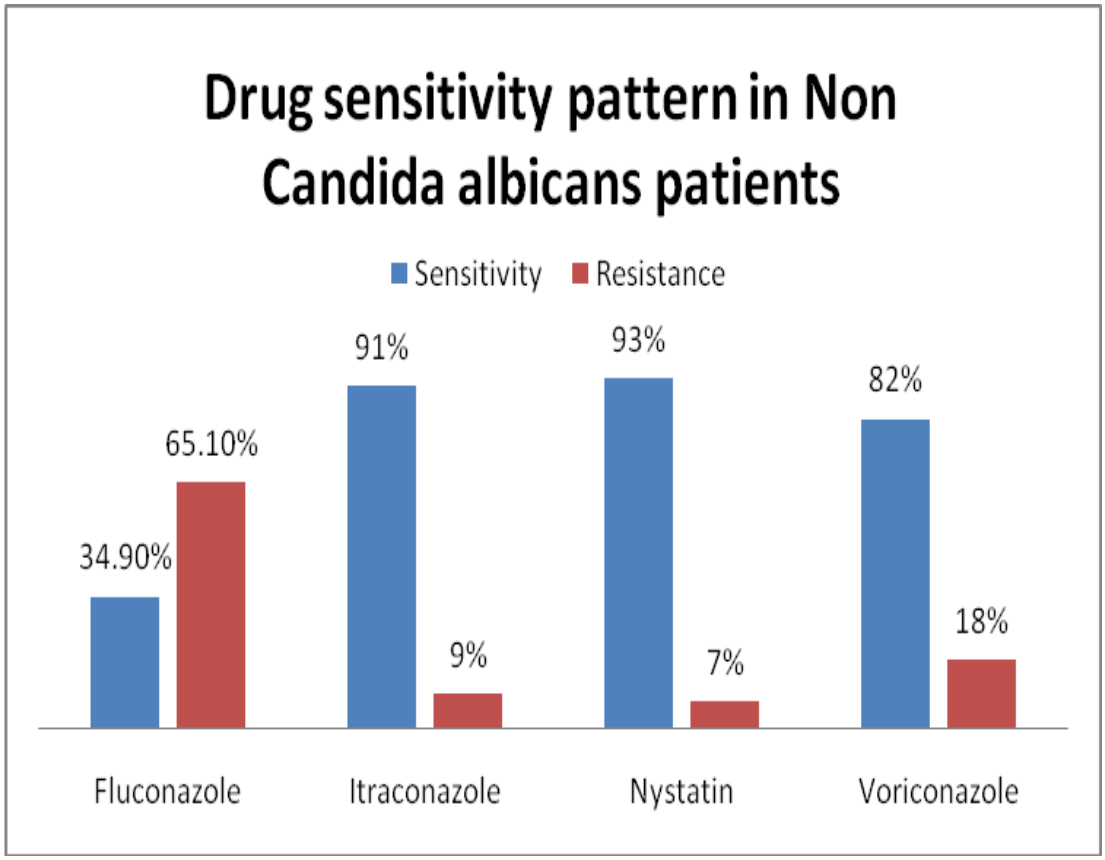

Drug sensitivity pattern in Non Candida albicans patients
Sensitivity
Resistance
34.90%
65.10%
91%
9%
93%
7%
82%
18%
Fluconazole
Itraconazole
Nystatin
Voriconazole

TABLE 9

Sensitivity pattern of C. glabrata [Total = 56]

Drugs	Sensitive	Resistance
Fluconazole	0	56(100%)
Itraconazole	55(98%)	1(2%)
Nystatin	55(98%)	1(2%)
Voriconazole	54(96%)	2(4%)

Among 56 cases of candida glabrata, all the cases [100%] were resistant to fluconazole, 2[1%] cases to voriconazole, 1[2%] case were resistant to itraconazole and nystatin.

TABLE 10

Drug sensitivity in Candida tropicalis

[n= 17]

Drugs	Sensitive	Resistance
Fluconazole	17(100%)	0
Itraconazole	11(64%)	6(35%)
Nystatin	16(94%)	1(6%)
Voriconazole	11(64%)	6(41%)

Among the C.tropicalis6 cases were resistant to Itraconazole and 6[35%] cases were resistant to voriconazole and 01 [6%] case were resistant to nystatin and no case to fluconazole.

TABLE 11

Sensitivity pattern of C. parapsilosis

[n = 11]

Drugs	Sensitive	Resistance
Fluconazole	11(100%)	0
Itraconazole	11(100%)	0
Nystatin	8(72%)	3(28%)
Voriconazole	5(45%)	6(55%)

Among 11 cases of candida parapsilosis, 6[55%] cases showed resistance to voriconazole, and 3[28%] cases to nystatin and no resistant to itraconazole and fluconazole.

TABLE 12

Drug sensitivity in Candida krusei

[n = 2]

Drugs	Sensitive	Resistance
Fluconazole	1[50%]	1[50%]
Itraconazole	2[100%]	0
Nystatin	1[50%]	1[50%]
Voriconazole	1[50%]	1[50%]

Out of the 2 cases of candida krusei, 1[50%] case was resistant to Fluconazole, nystatin and voriconazole and no resistant to itraconazole.

Discussion

DISCUSSION

Vaginal candidiasis is a common infection in 60 to 70% of females of child bearing age. This study was done among the females attending the Gynaecology OP with complaints of vaginal discharge, which revealed highest frequency of vaginal candidiasis in the age group of 18 to 35 years (55%), followed by 36-50 years of age group (38.7%). Least frequency of around 6.3% is observed in menopausal age group. The studies done by A.K. Ako-nai, O.O.Kasim et al, Deepababin et a1[55] and Shegal et al[56] also showed the higher frequency of vulvovaginal candidiasis in the reproductive age group was 52%, 51%, 62% respectively which was similar to our observation.

The number of colonies of yeast in Chrom agar was also greater than on SDA as found in the study of Baumgartner et al [9] which also demonstrated a detection rate of candida on chrom agar was 20% higher than on SDA. CHROMagar candida provides a simple and precise means for rapid identification of candida isolates in the routine laboratory [11][22]

The most prevalent species among the culture positive cases was observed that 52% were C.albicans and 48% were non albicans candida. 86 cases of non albicans candida species was isolated, of which 56 (32%) belongs to C.glabrata, 17(9%) belongs to C.tropicalis, 11 cases belongs to C.parapisilosis (6%) and 2(1%) belongs to C.krusei. Reports from Varsha kumari et al [8],Uma et al, Raginitilak et al, Deepababin et al, Mahmud et al, Fan et al,[94] have also documented similar results and has shown that non

albicans has increased in prevalence with 32.39% candida albicans, 22.5% candida glabrata, 45% candida parapsilosis and 39% candida tropicalis and also non albicans candida has increased in the Tumker et al study[6] where as in Novikova et al study result was 87% candida albicans, 9% candida krusei, 4% candida glabrata and one case candida parapsilosis and one case of mixed infection with candida albicans and candida krusei which was concordance with this study[11]. In this study C.glabrata was most common followed by C.tropicalis and C.parapisilosis. In Jawed ahmed et al, In Maria et al in Peshawar, Pakistan shown the result as similar with this study as 41.7% candida albicans, 16.7% candida tropicalis, 16.7% candida krusei and 14.8% candida glabrata. This emphasizes Nonalbicans candida speciation is very essential and also because of drug resistance. Various study reports done by sobel et a1,[77], Redondo et al,[92] were similar to our results which has shown 32% increased resistance to azoles in non albicans candida. In this present study Nonalbicans candida has shown 39.3% resistance to azoles. Hence speciation by reliable culture methods is beneficial in the diagnostic aspect.

In vitro drug sensitivity testing has been improved over the recent years because of the acquired resistance to azoles. Some studies like Hawser, S Costa et al have documented that the invitro resistance to antifungal drugs has increased. The rate of fluconazole resistance varies from 5-25% as shown in study. Fluconazole resistance in candida species: current perspective by Berkow et al at Atlanta[18] and 62% resistant to azoles by Maria et al and Jawed et al in a Peshawar, Pakistan study[9].

Also comparison was done for the drug sensitivity pattern of the albicans and Nonalbicans. For candida albicans, sensitivity to nystatin (87%) was high followed by Itraconazole (69%), fluconazole (78%) and voriconazole (58%). Similar results were noted in the study of emergence of non albicans candida species and antifungal resistance from North India by Varsha kumara et al, Pankajkumar et al at Uttarpradesh.[8] and also in the study of Fluconazole resistance in candida species: a current perspective by Berkow et al at Atlanta.

Regarding non albicans candida, 56[100%] cases of candida glabrata showed resistant to fluconazole and 2[4%] cases were resistant to voriconazole. Itraconazole was resistant in 01[2%] case and also nystatin 01[2%] cases. Similar results were observed in the following study by Tumker et al, Maria et al,[6][9] as 2.5% resistant to fluconazole and 3.5% resistant to voriconazole.

Drug sensitivity in Candida tropicalis showed 17[100%] cases were sensitive to fluconazole, 11[64%] cases to itraconazole, 11[64%] to voriconazole and all 16[94%] were sensitive to nystatin similar to the result in Tumker et al, Maria et al, Jawed et al, Peshawar, Pakistan study.[6] [9] [24] as 8.9% and 6.9% to fluconazole and voriconazole respectively.

Drug sensitivity in candida parapsilosis shows sensitive to 100% to flucanozole and itraconazole which is in correlation with the study by Latha et al[7] who showed 22.5% sensitive to flucanozole. In a Karnataka study by Tumker et al, C.parapsilosis was 100% resistant to flucanozole and voricanozole. It is in concordance with the present study.

Drug sensitivity in candida krusei, showed fluconazole, nystatin and voriconazole were only 50% sensitive and 100% sensitive for itraconazole. Similar results were observed in the study amina et al[11]

While comparing the results obtained in the studies of changing trends of vulvovaginal candidiasis byTumkar et al [6], all non albicans candida species C.glabrata, C.tropicalis and C.parapsilosis has shown increase in the rate of resistant to fluconazole from 4% to 12% and has also shown candida glabrata and candida parapsilosis sensitive only to voricanozole. In this present study, C.glabrata showed 100% resistant to flucanozole, followed by C.krusei.

Out of 86 cases of non albicans candida 93% were sensitive to nystatin. 34.9%, 82%, and 91% were sensitive to fluconazole, voriconazole and Itraconazole respectively. Further analysis of drug sensitivity pattern in large study group is necessary. This study showed same results with a study done by Panchaletal [95] in western India as 32%, 46% and 86% flucanozole, voricanozole and itraconazole respectively.

Comparison of the drug susceptibility pattern in Candida albicans and non albicans candida in this study was done which showed similar pattern of sensitivity and resistance in both groups for nystatin. Nystatin scored the highest sensitivity followed by fluconazole, Itraconazole and voriconazole. Fluconazole resistance has increased in C.glabrata. This result was supported by the studies like candida and candidaemia: susceptability and epidemiology [10], by Arendrup and also in the study of Poongothai et al, Latharagunathan et al in Puducherry, candida glabrata showed 2.5% resistant to flucanozole.

Summary

SUMMARY

The following are the implications derived from this prospective of vulvovaginal candidiasis among the patients more than 18 years of age over a period of one and a half years.

- The most common age group affected was 18 to 35 years which constitutes 55% of the study group
- The most common species isolated was Candida albicans which constituted 94 [52%] and 86 [48%] were non albicans candida species.
- Non albicans candida species prevalence has increased.
- Clinical presentation of VVC was severe in non albicans candida
- Candida glabrata 56 [32%] was the most prevalent species among the Non albicans Candida.
- Candida CHROM agar-differential media is the rapid diagnostic test when compared to other conventional tests.
- Within 24hours results were obtained and the species was identified by the specific colour produced by the each candida species without the use of any instrument.
- In resource limiting setups this media can be used to identify candida up to species level.
- Antifungal susceptibility testing by Kirby Bauer disk diffusion method as per CLSI guidelines was easy to perform when compared to other methods.

- Drug sensitivity pattern of Candida albicans showed highest sensitivity to Nystatin [87%] followed by azoles [58-78%].
- Drug sensitivity pattern of non albicans candida showed [93%] sensitivity to nystatin followed by azoles [35-82%].
- Among the non albicans candida, Candida glabrata was totally resistant to fluconazole due to its intrinsic resistance. This indicates to avoid empirical treatment with fluconazole in vulvovaginal candidiasis.

Conclusion

CONCLUSION

Vulvovaginal candidiasis is common infection among women in the reproductive age group. Species identification by culture methods showed Candida albicans to be the most prevalent species. Emergence of Non-albicans Candida species and recurrent vulvovaginal candidiasis necessitates the species identification and antifungal susceptibility to be done as a part of laboratory evaluation of vaginal candidiasis. Fluconazole, Itraconazole and voriconazole were sensitive in both Candida albicans and non Candida albicans infections. Nystatin was found to be the highly sensitive drug followed by azoles. As C.glabrata is intrinsically resistant to fluconazole, it should not be used empirically in all VVC as non albicans species has increased in prevalence. According to this study results, the identification of causative fungal species and treatment according to the drug sensitivity pattern will reduce the incidence of vulvovaginal Candidiasis and also to prevent the complication of azoles and the development of emerging resistance in antifungals drugs. Earlier microbiological diagnosis for vulvovaginal infection is warranted to prevent the complications and to direct the clinicians in appropriate way of management.

Annexures

CULTURE MEDIA PREPARATION

1. **SABOURAUD DEXTROSE AGAR:** it is a peptone containing medium supplemented with 4% dextrose and has an acidic pH, to support the growth of fungi and is inhibitory to contaminating bacteria.

   Ingredients:

Peptone	- 10 gm
Dextrose	- 40 gm
Agar	- 20 gm
Distilled water	- 1000ml
Gentamycin	- 50mg/litre

   After mixing the above ingredients adjust the pH at 5.6.Sterilize the medium at 121 degree Celsius for 15min.Dissolve gentamycin in10ml of 95% ethanoland add to the boiling medium after removing the heat. Aftercooling, the medium is poured into sterile test tubes. These are cooled at an angle to form an agar slant. When the medium has solidified and cooled to room temperature, it is ready for inoculation and stored in the refrigerator for future use.

2. **CORN MEAL AGAR:** The Dalmau plate culture on corn meal agar is commonly used to observe the chlamydospores production in yeasts.

   Ingredients:

Corn meal	- 8gm
Agar	- 4gm
Distilled water	- 200ml
Tween 80(1%)	- 2ml

Heat corn meal agar and water at 60 C for 1 hour and pass it through the filter paper. Add distilled water to make it 200ml and then add agar. Autoclave at 121 C for 15min and pour in the plates and allowed to settle.

3. **CHROMagar CANDIDA MEDIUM:** This is a selective and differential type of chromogenic medium, which is useful for rapid identification of various Candida species from mixed infections and also Prototheca.

   Ingredients:

Peptone	- 5gm/litre
Malt extract	- 3gm/litre
Yeast extract	- 3gm/litre
Glucose	- 10gm/litre
Chloramphenicol	- 0.050gm/litre
Chromogenic mixture	- 3gm/litre
Agar	- 18gm/litre

   Suspend 21gms in 500ml distilled water. Heat to boiling, to dissolve the medium completely. Do not autoclave. Cool it and aseptically add rehydrated contents of 1 vial of HiChrome candida selective supplement (FD192).Mix well and pour into sterile Petri dishes.

BIBLIOGRAPHY

1. Grays Anatomy book 3rd edition
2. Ganongs physiology 26th edition
3. Fundamental of Diagnostic Mycology - Fran Fisher & Cook.
4. Medically important fungi – A guide to identification, 4th Edition. – DaviseH.Larone
5. Text book of Medical Mycology– Jagdishchander
6. Changing Trends of Vulvovaginal Candidiasis – Tumker et.al
7. Phenotypic Characterization and Antifungal Suseeptibility pattern to Fluconazole in Candida species isolated from Vulvovaginal Candidiasis in a Teritiary care hospital – LathaRagunathan, Poongothai et.al.
8. Emergence of non-albicans Candida among candida vulvovaginitis cases and study of their potential virulence factors from a tertiary care center, North India – VarshaKumari, Pankajkumar et.al.
9. Antifungal susceptibility testing of vulvovaginal Candida species among women attending antenatal clinic in tertiary care hospitals of Peshawar – Maria Khan, Jawad Ahmed et.al.
10. Candida and candidaemia. Susceptibility and epidemiology – ArendrupPubmed.
11. Can the diagnosis of recurrent vulvovaginalcandidosis be improved by use of vaginal lavage samples and cultures on chromogenic agar – Novikova, Rodrigues et.al.

12. Pattern of Candida species isolated from patients with diabetes mellitus and vulvovaginal candidiasis and their response to single dose oral fluconazole therapy – DeeptiGoswami, RavinderGoswam et.al.
13. Egypt Acad. J.biolog. sci 1(1): 37-47(2009).
14. Species distribution and susceptibility of candida isolates from patients in vulvovaginal candidiasis. PUBMED 24746725.
15. Phenotypic characteristics of vulvovaginal candidiasis and antifungal susceptibility pattern of candida species. PUBMED 18937710.
16. Evolving pathogens in vulvovaginal candidiasis. PUBMED 1564129.
17. Fungal profile of vulvovaginal candidiasis in a tertiary care hospital. PUBMED 28511380.
18. Flucanozole resistance in candida species PMC 5546770.
19. Role of non albicans candida species and biofilm in vulvovaginal candidiasis. PUBMED 27538491.
20. Management of persistent vulvovaginal candidiasis due to azole resistant candida. PUBMED 8389793
21. Emergence of non albicans candida species and anti fungal resistance Article/pii/3222116116301940/science.
22. Use of CHROM agar Candida medium for presumptive identification of Candida yeast species and detection of mixed fungal infections. science/article/S1198743X1465143.
23. Evaluation of antifungal susceptibility testing in Candida isolates by Candifast and Disk diffusion method. Ijpmonline.org/article.

24. Emergence of non-albicans candida among candidialvulvovaginitis cases and study of their potential virulence factors from a tertiary care hospital. www.ijpmonline.org/article.0377.
25. Vulvovaginal candidiasis: Epidemiology, microbiology and risk factors.
26. SobelJD pathophysiology of vulvovaginal candidiasis. J. Reprod. Med. 1989;34(suppl);542-80
27. HurleyR, De louvois J. Candida vaginitis Post grad medj 1979;55:645-7.
28. Morton RS, Rashid. S, candidial vaginitis; Natural history, predisposing factors and prevention. Proc R soc med 1977; 70:3-6
29. Nyirjesy P, Weitz MY, Grody MH, Lorber B. Over the counter and alternative medicines in the treatment of chronic vaginal symptoms. Obstet gynecol1997;90-50-3.
30. Richter Galask RP, Messer SA et al. Epidemiology of current Candida & Antifungal susceptibility of Candida species causing vulvovaginitis. J. Clin. Microbiol2005; 43(5); 2155-62
31. Text Book of Sexually transmitted diseases- II Edition Someshgupta and Bushan Kumar.
32. 9 Shaws text book of gynecology by Howkins& Brown 12th Edition.
33. East African medical journal vol; 87 Noll April2010 Species distribution and antifungal sensitivity pattern.
34. Text book of sexually transmitted infections- II Edition Somesh Gupta & Bushan Kumar.

35. Rodgers CA, Beardel AJ, Recurrent Vulvovaginal Infections candidiasis Int J Std AIDS 1999;10:435-41
36. MirelaBabiEt AI Mycological Profile ofVulvo Vaginal Candidiasis. Int J of Micro Biol.
37. T. Lunstorm, J Sobel, Nosocomial Candiduria a review CD, 32(2001) 1602- 1607.
38. A. Ahmad, A. Ukkan "Prevalence of Candida species and potential risk factors for VVC. European J. of obstetric, Gynecology and reproductive biology 144(2009) 68-71.
39. Deepti Goswami et al. Pattern of candida isolated from patients with DM & VVC Journal of infection Vol 52 issue 2 February 2017
40. SchumAn Pet. AI, sobet m et al. lnfection disease 2003; 188: 118-27.
41. Spinillo A, Capuzzo 220 E, Egbe to, et al. Torulopsisglabrata vaginitis Obstet gynecol1995;85: 993-8.
42. Chaim W, Foxman B et al association of RVVC and secretory ABO and Lewis phenotype J infect dis 1997; 176: 828-30.
43. Giraldo Pc, Babula et al-Mannose binding lectin gene polymorphism, obstet Gyneco12007; 109(5): 1123-8.
44. Fiedl PI JR, Barousse Metal J.lnfectlmmun 2004; 72(5): 2939-46
45. Barousse MM, Steele C, Dunlap et al. Growth inhibition of candida albicans By Human Vaginal Epithelial Cells J. Infect Dis 2001;184:1489-93.

46. Witkin SS, Jeremia's J Et AI A Localised Vaginal Allergic Response In Women WithRVVC .J. Allergy Clin Immuno11988;81:412-6 (Pub Med).
47. Geiger Am, Foxman B et al. Risk Factors for VVC- a case control study among University Students. Epidemiology 1996; 7: 182-7.
48. Text Book of Sexually Transmitted Infections Edition:2-Bushankumar And SomeshGuptha.
49. Duerr A, Sierra MF et al. Prevalance of VVC in IllYobstet and Gynecol 1997 Aug 90(2) 252-6.
50. Powderly WG et al mucosal candidiasis in IllY infected individuals AIDS 1992;6:81-4.
51. Tintelnot K, Hasse G, Seibold M, Bergmann F, et al. Evaluation of phenotypic markers for selection and identification of candida dubiliniensis J Clin Microbiol2000;38: 1599-608.
52. Schorling SR et al crit Rev Microbiol2000;26:59-68
53. Poruatta M WG et al candida albicans biotypes in IllY type 1 infected persons before and after antifungal therapy. J ClinMicrobiol 1991; 29:726-30
54. MC earthy GM et al factors associated with increased frequency of mucosal candidiasis J Oral Pathol Med 1991;20:
55. Gottfredson M, Cox Gm et al association of plasma level of HIV type 1 RNA in Mucosal candidiasis.

56. Arilla MC, Carbonero JL et al vulvovaginal candidiasis refractory to fluconazole Eur J ObstetGynecolReprod. Biol1992;44:77-80
57. Fichtenbaum CJ KoletarS et al- clin infect dis 200;30:749-56
58. Zingman BS et al. Resolution of refractory AIDS - related mucosal candidiasis after initiation of didanosine plus saquinavir N Engl J Med 1996;334:1674-5.
59. Bertagnolio S, Degaetanoclonati et al J Chemother 2004;16:172-8.
60. Cauda R, Tacconelli E et al Journal Acquired immWiedeficsyndr 199; 21:20-5.
61. Witkin SS et al. Vaginal eosinophillia and IgE antibodies to candida albicans in women with recurrent vaginitis J Med Vet Mycol, 27, 57-8
62. Schonheyder H, Johansen, J.A et al1983 clin. Infect. Dis 21 223-31
63. Centres for Disease control and prevention - Sexually transmitted diseases treatment guidelines MM WR 59RR-1:61-3.
64. Kwon, ChWig KJ, Bennet JE, Medical Mycology Lea Febiger 1992:280-336.
65. De Hoog GS, Guareo Jet al. Atlas of Clinical FWigi the Netherlands and UniversitatRovira I virgi Li, Reus, Spain, 200;184-225.
66. William G. Merz& Roderick J.Hay-Medical Mycology lOth edition.
67. Free SelbenHJ Sonntag HG et al. Vaginal Colonisation of candida in asymptomatic women. Obstet& gynecol200; 95:413-6.
68. SobelJD, wisenfield HC et al Maintenance of fluconazole therapy for RVVC N Eng J Med 2004; 357:876-83.

69. VanSlyke K.K. Michel VP, Rein MF Treatment of VVC with boric acid powder AM J Obstet Gynecol1981;141:145-8.
70. A.K Ako-nai, O.O.kassim, M.O.Adeniran, Study of urinary tract infections at ile- Ife, Nigeria, East African Medical Journal 70; (1993) 10-14.
71. Sehgal SC, Epidemiology of male urethritis in Nigeria J. Trop MedHyg 93.
72. Singh Sobel JD, WiesenField HC, Manten's et al. Maintenance of Fluconazole therapy for recurrent vulvovaginal candidiasis. N. Engl J Med 2004;351(9) :876-83.
73. E Shiffrin, Sha Benson C, Pottage J, et al. Six year longitudinal study In: IX international conference on AIDS; Berlin 1993 vol.1.
74. Spinillo A, capuzzo E, Accianos et al. Effect of antibiotic use on the prevalence of symptomatic vulvovaginal candidiasis, AMJ obstetrics & gynaecology 1999;180;4-7.
75. Pirotta M V, Garland SM, Genital candid species detected in samples from women in Australia, before & after treatment with antibiotics. Jclin Microbiological2006;44(9);3213-7.
76. De leon EM, Jacober SJ, Sober JD, et al. Prevalence and risk factors for vaginal candidia colonization in type I &type II Diabetes. BMC Inefc.dis 2002;2:1-1.
77. Donders G.G, Prenen H, Verbeke.Get al. Impaired tolerance for glucose in women with recurrent vulvovaginal candidiasis.AMJ obstetrics & gynecology 2002;187;989-93.

78. Duerr A', Sierra F, Feldman J, Clarke M, Erlich I et al, Immune compromise and prevalence of candida vulvo vaginitis in HIV infected women. AMJobstet & gynec 1997 aug 90(2):252-6.
79. A Spinillo, G michelone, CCavanna, L Colonna et al, clinical and microbiological characteristics of symptomatic vulvovaginal candidiasis in seropositive women.
80. Barbone F, Austin H, Louv Wet al. A follow up study of contraception, sexual activity, and rates of candidiasis, trichmoniasis and bacterial vaginosis; AMJ obstet & gynec 1990;163:510-4.
81. Hooten Tm, Roberts PL, Stamm WE. Effect of recent sexual activity and the use of diaphragm, run on vaginal flora. Clin infect dis 1994;19:274-8.
82. Fitchenhaem, Koletar S, yiannoutsosC, Holland F et al. On refractory mucosal candidiasisin advanced HIV infection. Clin infect dis 2000; 30:749-56.
83. White M.H. Is vulvovaginal candidiasis and AIDS related illness? Clin infect dis 1996;22 supp12:s124-7.
84. Rhoads JL, Wright DC, Redfield RR, Burke DS, Chronic vaginal candidiasis in women with HIV infection, JAMA 1987;257:3105-7.
85. MahmoudiRad,S Zafar Ghandi, B Abbasabadi. The epidemiology of candida species associated with vulvovaginal candidiasis in an Iranian patient population, European Journal Of obstetrics &Gynecology and reproductive biology 155(2011)199-203.

86. Spinillo A, Pizzoli G, Colonna L, Nicola S, DE Seta F, Guaschino S. Epidemiological characteristics of women with idiopathic recurrent vulvovaginal candidiasis, obstetric & gynecology 1993;81:721-7.
87. Redondolopez V, Lynch M, Schmitt, Cook R, SobelJD. Torulopsisglabrata vaginitis; clinical aspects and susceptibility to antifungal agents.
88. A.ahmed, A.U. Khan. Prevalence of candida species and potential risk factors for vulvovaginal candidiasis in Aligarh, India- Europeon journal of obstetrics and gynecology and reproductive biology 144(2009)68-71.
89. F.RFan X.P Liu J.W Li, "clinical characteristics of vulvovaginal candidiasis" and anti-fungal susceptibilities of candida species isolates among patients in southern china from 2003-2006.joumal of obstetrics and gynaecology research vol34 (2008) 561-566.
90. Panchal P.A, Katara RK, Mehta R. et al., Microbiological study of various candida species and its antifungal sensitivity testing from antenatal women in tertiary care hospital in western India -In international journal of microbiological research volume 5 issue 6 2013.
91. F Muhia,G Revathi and J.M. Machoki- Species distribution and anti-fungal sensitivity pattern of vaginal East in east African medical journal April 2010.

CLINICOMYCOLOGICAL STUDY OF VULVOVAGINAL CANDIDIASIS PROFORMA

Sl.No Date:

Patient Name :

Age : yrs

Sex : Female

OP/IP No :

Address :

Phone No :

Complaints :

Itching over genital - +/- duration

Discharge pervaginum - +/- duration

History of present illness:

Details of the

Discharge pervaganum - +/- duration Nature

Foul Smelling - +/-

Yeasty Odour - +/-

Itching over the perineum- +/-

H/o recurrent attacks - +/- No. of episodes

Relationship to menstrual cycles – premenstrual / Menstrual / Postmenstrual

H/o dyspareunia/dysuria - +/-

H/o similar lesions in any other parts of the body – Oral/Intertriginous

H/o spouse having similar complaints

Past History

H/o DM/HT/BA/TB

H/o previous episodes

H/o obstetric/gynaec problem

Drug History

Topical Application	+/-	details
Oral Medication	+/-	details
Other Medication	+/-	OHA
		Immuno suppressants
		OCP

Contact History if any

General Examination

Conscious □ Oriented □ Anemia □ Jaundice □ Lymph denopathy □

Pulse rate: /mt Blood Pressure: /mmHg

CVS RS P/A CNS

Local examination

Sites involved by curdy patches: Vulva/vagina/groins/Perianal

Macroscopic examination

Nature of discharge

Nature of Vulval Mucosa - Duskyred/White Soddened /excoriation/ulcer

Nature of Surrounding skin

Speculum Examination:

Nature of vagional mucosa

Erosions +/- Ulcers - +/-

Oral Mucosa –

Other cutaneous lesions – Nails/inter trigenous areas

Investigations

Blood	-	Urea
		Sugar
		Creatinine

Complete hemogram

Urine	-	Albumin
		Sugar
		Deposits
Vaginal Smear	-	KOH mount
		Wet mount
		Gram Staining

Culture Methods

Blood	-	VDRL
		HIV

Treatment given:

Topical

Oral

Follow up

ஒப்புதல் படிவம்

1. ஆராய்ச்சியின் தலைப்பு :

   படிவம் எண் :

   பங்குபெறுபவரின் பெயர் :

   வயதுபிறந்ததேதி :

   முகவரி :

1. எனக்கு (பங்குபெறுவோர்) இந்த ஆராய்ச்சியின் முழு விவரங்களும் தெரிவிக்கப்பட்டது.
2. என்னுடைய பங்களிப்பினை எந்தஒரு சூழலிலும், எவ்விதகாரணமுமின்றி விலக்கிக் கொள்ளவும் முழு உரிமை அளிக்கப்பட்டுள்ளது என்பதையும் அறிவேன்.
3. இந்த ஆராய்ச்சியின் முடிவையோ, என்னைப் பற்றி தகவல்களோவேறு எவருக்கும் தெரிவிக்கப்படமாட்டாது எனவும் உறுதி அளிக்கப்பட்டது என்பதையும் அறிவேன்.
4. நான் இந்த ஆராய்ச்சியின் தகவல்களை மேற்கூரிய ஆராய்ச்சி படிப்புக்கு பயன்படுத்திக் கொள்ளஎனது முழு சம்மதத்தை தெரிவிக்கிறேன்.
5. மேற்கண்ட அனைத்து ஒப்புதலுடன் என் முழு மனதுடன் இந்த ஆராய்ச்சியில் பங்கேற்கிறேன்.

பங்குபெறுவோரின் கையொப்பம்: தேதி:

பங்குபெறுவோரின் பெயர்: தேதி:

மருத்துவரின் கையொப்பம்: தேதி:

மருத்துவரின் பெயர்: தேதி:

சாட்சிகள்: தேதி:

INFORMED CONSENT FORM

Study/Title: _____

Study Number: _____

Subjects Full Name: _____

Date of Birth Age: _____

Address: _____

1. I confirm that I have read and understood the information sheer dated for the above study and have had the opportunity to ask questions.

OR I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.

2. I understand that my participation in the study is voluntary and that I am free to withdraw at any time. Without giving any reason and without any medical care or legal rights being affected.

3. I understand that the sponsor of the clinical trial/project. Others working on the Sponsor's behalf the Ethics committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conduced in relation to it, even if I withdraw from the trial. However, I understand that my identity will not be revealed any information released to third parties or published.

4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

5. I agree to take part in the above study

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative: _____

Signatory Name:_____ Date: _____

Signatory of the investigator: _____ Date: _____

Study Investigator's Name: _____

Signature of the witness: _____ Date: _____

Name of the Witness

MASTER CHART

All Candida albicans isolates are serialised from 1-94 and Non albicans candida isolates are serialised from 95-180 [c.glabrata (56), c.tropicalis (17), c.parapsilosis (11), c.krusei (2)]

S.No	Lab ID No	Age	Sex	Specimen	C.albicans				Non albicans candida			
					Flu	Itra	Vori	Nys	Flu	Itra	Vori	Nys
1.	235	36	F	HVS	S	S	S	S	-	-	-	-
2.	457	44	F	HVS	S	S	S	S	-	-	-	-
3.	153	22	F	HVS	S	S	S	S	-	-	-	-
4.	245	27	F	HVS	S	S	S	S	-	-	-	-
5.	685	39	F	HVS	S	S	S	S	-	-	-	-
6.	415	51	F	HVS	S	S	S	S	-	-	-	-
7.	236	54	F	HVS	S	S	S	S	-	-	-	-
8.	975	31	F	HVS	S	S	S	S	-	-	-	-
9.	862	42	F	HVS	S	S	S	S	-	-	-	-
10.	412	48	F	HVS	S	S	S	S	-	-	-	-
11.	215	36	F	HVS	S	S	S	S	-	-	-	-
12.	364	47	F	HVS	S	S	S	S	-	-	-	-
13.	741	51	F	HVS	S	S	S	S	-	-	-	-
14.	123	24	F	HVS	S	S	S	S	-	-	-	-
15.	258	46	F	HVS	S	S	S	S	-	-	-	-
16.	369	43	F	HVS	S	S	S	S	-	-	-	-
17.	456	32	F	HVS	S	S	S	S	-	-	-	-
18.	147	35	F	HVS	S	S	S	S	-	-	-	-
19.	158	37	F	HVS	S	S	S	S	-	-	-	-
20.	425	41	F	HVS	S	S	S	S	-	-	-	-

All Candida albicans isolates are serialised from 1-94 and Non albicans candida isolates are serialised from 95-180 [c.glabrata (56), c.tropicalis (17), c.parapsilosis (11), c.krusei (2)]

21.	427	44	F	HVS	S	S	S	S	-	-	-	-
22.	458	29	F	HVS	S	S	S	S	-	-	-	-
23.	697	27	F	HVS	S	S	S	S	-	-	-	-
24.	146	42	F	HVS	S	S	S	S	-	-	-	-
25.	327	35	F	HVS	S	S	S	S	-	-	-	-
26.	215	37	F	HVS	S	S	S	S	-	-	-	-
27.	345	31	F	HVS	S	S	S	S	-	-	-	-
28.	759	30	F	HVS	S	S	S	S	-	-	-	-
29.	143	29	F	HVS	S	S	S	S	-	-	-	-
30.	246	28	F	HVS	R	S	S	S	-	-	-	-
31.	378	24	F	HVS	R	S	S	S	-	-	-	-
32.	845	43	F	HVS	R	S	S	S	-	-	-	-
33.	941	48	F	HVS	R	S	S	S	-	-	-	-
34.	652	21	F	HVS	R	S	S	S	-	-	-	-
35.	148	33	F	HVS	R	S	S	S	-	-	-	-
36.	345	39	F	HVS	R	S	S	S	-	-	-	-
37.	657	40	F	HVS	R	S	S	S	-	-	-	-
38.	489	43	F	HVS	R	S	S	S	-	-	-	-
39.	574	46	F	HVS	R	S	S	S	-	-	-	-
40.	124	49	F	HVS	R	S	S	S	-	-	-	-
41.	138	39	F	HVS	R	S	S	S	-	-	-	-
42.	369	31	F	HVS	R	S	S	S	-	-	-	-
43.	147	30	F	HVS	R	S	S	S	-	-	-	-
44.	258	37	F	HVS	R	S	S	S	-	-	-	-
45.	369	42	F	HVS	R	S	S	S	-	-	-	-

All Candida albicans isolates are serialised from 1-94 and Non albicans candida isolates are serialised from 95-180 [c.glabrata (56), c.tropicalis (17), c.parapsilosis (11), c.krusei (2)]

46.	314	33	F	HVS	R	S	S	S	-	-	-	-
47.	214	38	F	HVS	R	S	S	S	-	-	-	-
48.	456	35	F	HVS	R	S	S	S	-	-	-	-
49.	854	41	F	HVS	R	S	S	S	-	-	-	-
50.	172	33	F	HVS	R	S	S	S	-	-	-	-
51.	174	42	F	HVS	R	S	S	S	-	-	-	-
52.	146	43	F	HVS	R	S	S	S	-	-	-	-
53.	1785	45	F	HVS	R	S	S	S	-	-	-	-
54.	785	32	F	HVS	R	S	S	S	-	-	-	-
55.	956	43	F	HVS	R	S	S	S	-	-	-	-
56.	786	44	F	HVS	R	S	S	S	-	-	-	-
57.	742	45	F	HVS	R	S	S	S	-	-	-	-
58.	451	45	F	HVS	R	S	S	S	-	-	-	-
59.	214	46	F	HVS	R	S	S	S	-	-	-	-
60.	369	30	F	HVS	R	S	S	S	-	-	-	-
61.	457	25	F	HVS	R	S	S	S	-	-	-	-
62.	246	48	F	HVS	R	S	S	S	-	-	-	-
63.	378	38	F	HVS	R	S	S	S	-	-	-	-
64.	845	44	F	HVS	R	S	S	S	-	-	-	-
65.	941	32	F	HVS	R	S	S	S	-	-	-	-
66.	652	37	F	HVS	R	S	S	S	-	-	-	-
67.	148	36	F	HVS	R	S	S	S	-	-	-	-
68.	345	35	F	HVS	R	S	S	S	-	-	-	-
69.	657	45	F	HVS	R	S	S	S	-	-	-	-
70.	489	46	F	HVS	R	S	S	S	-	-	-	-

All Candida albicans isolates are serialised from 1-94 and Non albicans candida isolates are serialised from 95-180 [c.glabrata (56), c.tropicalis (17), c.parapsilosis (11), c.krusei (2)]

71.	574	32	F	HVS	R	S	S	S	-	-	-	-
72.	124	34	F	HVS	R	S	R	S	-	-	-	-
73.	138	28	F	HVS	R	S	R	S	-	-	-	-
74.	369	29	F	HVS	R	S	R	S	-	-	-	-
75.	147	30	F	HVS	R	S	R	S	-	-	-	-
76.	258	34	F	HVS	R	S	R	S	-	-	-	-
77.	369	28	F	HVS	R	S	R	S	-	-	-	-
78.	314	43	F	HVS	R	S	R	S	-	-	-	-
79.	214	44	F	HVS	R	S	R	S	-	-	-	-
80.	456	43	F	HVS	R	R	R	S	-	-	-	-
81.	854	48	F	HVS	R	R	R	R	-	-	-	-
82.	172	38	F	HVS	R	R	R	R	-	-	-	-
83.	174	36	F	HVS	R	R	R	R	-	-	-	-
84.	146	34	F	HVS	R	R	R	R	-	-	-	-
85.	1785	33	F	HVS	R	R	R	R	-	-	-	-
86.	785	45	F	HVS	R	R	R	R	-	-	-	-
87.	956	44	F	HVS	R	R	R	R	-	-	-	-
88.	786	47	F	HVS	R	R	R	R	-	-	-	-
89.	742	46	F	HVS	R	R	R	R	-	-	-	-
90.	451	41	F	HVS	R	R	R	R	-	-	-	-
91.	214	49	F	HVS	R	R	R	R	-	-	-	-
92.	369	24	F	HVS	R	R	R	R	-	-	-	-
93.	457	37	F	HVS	R	R	R	R	-	-	-	-
94.	246	33	F	HVS	R	R	R	R	-	-	-	-
95.	378	31	F	HVS	-	-	-	-	R	S	S	S

All Candida albicans isolates are serialised from 1-94 and Non albicans candida isolates are serialised from 95-180 [c.glabrata (56), c.tropicalis (17), c.parapsilosis (11), c.krusei (2)]

96.	845	21	F	HVS	-	-	-	-	R	S	S	S
97.	941	35	F	HVS	-	-	-	-	R	S	S	S
98.	652	32	F	HVS	-	-	-	-	R	S	S	S
99.	148	33	F	HVS	-	-	-	-	R	S	S	S
100.	345	34	F	HVS	-	-	-	-	R	S	S	S
101.	657	43	F	HVS	-	-	-	-	R	S	S	S
102.	489	31	F	HVS	-	-	-	-	R	S	S	S
103.	574	42	F	HVS	-	-	-	-	R	S	S	S
104.	124	47	F	HVS	-	-	-	-	R	S	S	S
105.	138	48	F	HVS	-	-	-	-	R	S	S	S
106.	369	49	F	HVS	-	-	-	-	R	S	S	S
107.	147	44	F	HVS	-	-	-	-	R	S	S	S
108.	258	25	F	HVS	-	-	-	-	R	S	S	S
109.	369	44	F	HVS	-	-	-	-	R	S	S	S
110.	314	47	F	HVS	-	-	-	-	R	S	S	S
111.	214	34	F	HVS	-	-	-	-	R	S	S	S
112.	456	38	F	HVS	-	-	-	-	R	S	S	S
113.	854	44	F	HVS	-	-	-	-	R	S	S	S
114.	172	45	F	HVS	-	-	-	-	R	S	S	S
115.	174	43	F	HVS	-	-	-	-	R	S	S	S
116.	146	45	F	HVS	-	-	-	-	R	S	S	S
117.	1785	23	F	HVS	-	-	-	-	R	S	S	S
118.	785	44	F	HVS	-	-	-	-	R	S	S	S
119.	956	43	F	HVS	-	-	-	-	R	S	S	S
120.	786	38	F	HVS	-	-	-	-	R	S	S	S

All Candida albicans isolates are serialised from 1-94 and Non albicans candida isolates are serialised from 95-180 [c.glabrata (56), c.tropicalis (17), c.parapsilosis (11), c.krusei (2)]

121.	742	43	F	HVS	-	-	-	-	R	S	S	S
122.	451	36	F	HVS	-	-	-	-	R	S	S	S
123.	214	47	F	HVS	-	-	-	-	R	S	S	S
124.	369	41	F	HVS	-	-	-	-	R	S	S	S
125.	457	35	F	HVS	-	-	-	-	R	S	S	S
126.	246	37	F	HVS	-	-	-	-	R	S	S	S
127.	378	36	F	HVS	-	-	-	-	R	S	S	S
128.	845	44	F	HVS	-	-	-	-	R	S	S	S
129.	941	43	F	HVS	-	-	-	-	R	S	S	S
130.	652	45	F	HVS	-	-	-	-	R	S	S	S
131.	148	46	F	HVS	-	-	-	-	R	S	S	S
132.	345	43	F	HVS	-	-	-	-	R	S	S	S
133.	657	29	F	HVS	-	-	-	-	R	S	S	S
134.	489	31	F	HVS	-	-	-	-	R	S	S	S
135.	574	34	F	HVS	-	-	-	-	R	S	S	S
136.	124	32	F	HVS	-	-	-	-	R	S	S	S
137.	138	26	F	HVS	-	-	-	-	R	S	S	S
138.	369	28	F	HVS	-	-	-	-	R	S	S	S
139.	147	30	F	HVS	-	-	-	-	R	S	S	S
140.	258	32	F	HVS	-	-	-	-	R	S	S	S
141.	369	32	F	HVS	-	-	-	-	R	S	S	S
142.	314	34	F	HVS	-	-	-	-	R	S	S	S
143.	214	33	F	HVS	-	-	-	-	R	S	S	S
144.	456	42	F	HVS	-	-	-	-	R	S	S	S
145.	854	48	F	HVS	-	-	-	-	R	S	S	S

All Candida albicans isolates are serialised from 1-94 and Non albicans candida isolates are serialised from 95-180 [c.glabrata (56), c.tropicalis (17), c.parapsilosis (11), c.krusei (2)]

146.	172	26	F	HVS	-	-	-	-	R	S	S	S
147.	174	23	F	HVS	-	-	-	-	R	S	S	S
148.	146	36	F	HVS	-	-	-	-	R	S	S	S
149.	1785	33	F	HVS	-	-	-	-	R	S	S	R
150.	785	25	F	HVS	-	-	-	-	R	R	R	R
151.	956	21	F	HVS	-	-	-	-	S	S	S	S
152.	786	34	F	HVS	-	-	-	-	S	S	S	S
153.	742	45	F	HVS	-	-	-	-	S	S	S	S
154.	451	43	F	HVS	-	-	-	-	S	S	S	S
155.	214	44	F	HVS	-	-	-	-	S	S	S	S
156.	369	45	F	HVS	-	-	-	-	S	S	S	S
157.	457	46	F	HVS	-	-	-	-	S	S	S	S
158.	246	47	F	HVS	-	-	-	-	S	S	S	S
159.	378	45	F	HVS	-	-	-	-	S	S	S	S
160.	845	46	F	HVS	-	-	-	-	S	S	S	S
161.	941	37	F	HVS	-	-	-	-	S	S	S	S
162.	652	37	F	HVS	-	-	-	-	S	S	S	S
163.	148	36	F	HVS	-	-	-	-	S	R	R	S
164.	345	27	F	HVS	-	-	-	-	S	R	R	S
165.	657	39	F	HVS	-	-	-	-	S	R	R	S
166.	489	31	F	HVS	-	-	-	-	S	R	R	S

All Candida albicans isolates are serialised from 1-94 and Non albicans candida isolates are serialised from 95-180 [c.glabrata (56), c.tropicalis (17), c.parapsilosis (11), c.krusei (2)]

167.	574	34	F	HVS	-	-	-	-	S	R	R	S
168.	124	31	F	HVS	-	-	-	-	S	R	R	R
169.	138	42	F	HVS	-	-	-	-	S	S	S	S
170.	369	48	F	HVS	-	-	-	-	S	S	S	S
171.	147	36	F	HVS	-	-	-	-	S	S	S	S
172.	258	47	F	HVS	-	-	-	-	S	S	S	S
173.	369	31	F	HVS	-	-	-	-	S	S	R	S
174.	314	24	F	HVS	-	-	-	-	S	S	R	S
175.	214	46	F	HVS	-	-	-	-	S	S	R	S
176.	456	43	F	HVS	-	-	-	-	S	S	R	R
177.	854	32	F	HVS	-	-	-	-	S	S	R	R
178.	172	35	F	HVS	-	-	-	-	S	S	R	R
179.	174	37	F	HVS	-	-	-	-	S	S	R	R
180.	146	41	F	HVS	-	-	-	-	R	S	S	S

181-258 – No candida grown