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CANDIDA, A HUMAN PATHOGEN AND MAJOR TYPES OF CANDIDIASIS

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Abstract: The fungus candida with over 355 species is an anamorphic yeast. It consists of over 20 human pathogenic species which are the cause of candidiasis. Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systematic and potentially life-threatening diseases. Candida species are opportunistic human pathogens which despite treatment with antifungal drugs can cause fatal infections in immune-compromised and immune-deficient patients. *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. parapsilosis*, together account for about 95% of identifiable Candida infections. *C. albicans*, considered to be the most serious cause of candidiasis, is a diploid, polymorphic yeast producing three morphologic forms: yeast cells, pseudohyphae, and true hyphae. This phenotypic switching plays a vital role in virulence. Since invasive yeast infections are associated with high levels of morbidity and mortality so rapid and accurate identification can contribute to successful therapy, earlier resolution of infections and subsequent reduction of moribund outcomes. CHROM agar candida, a type of chromogenic medium, based on species-specific enzyme activity, has been found to be a medium of choice for isolation and identification of candida species from clinical samples. This review is mainly focused on the diversity of candida, its polymorphism and various identification techniques used for proper diagnosis and prevention of candida infection, thus improving the prognosis for a wide range of patients.

INTRODUCTION: Candida is the 6th most common nosocomial (hospital-related) pathogen in the United States and the fourth most frequent cause of bloodstream infections. Infections due to candida and other yeast have increased dramatically in recent years and are of particular importance because of the rising number of immuno-compromised individuals and due to the countless medical advances available in medical sciences for Example, transplant surgery, anticancer therapies, broad-spectrum antibacterial therapies, medical devices that transverse the protective skin barrier, that extended human life expectancy in addition to certain disease states (*e.g.* malignancy, HIV infection, diabetes mellitus) [5]. Species of candida that were considered to be saprophytic are becoming more commonly associated as opportunistic causing candidiasis in humans.

Under favorable conditions, it appears that over 20 candida species can cause human infections. *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. parapsilosis*, together account for about 95% of identifiable candida infections [26]. These candida species are carried by a large proportion of humans particularly on the epithelial surfaces of the mouth, gastrointestinal tract, vagina and skin.

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Candida cause infection of humans called candidiasis, which is an acute or chronic, superficial, or disseminated mycosis. It encompasses infections that range from superficial, such as oral thrush and vaginitis, to systematic and potentially life-threatening diseases. Candida infections of the latter category are also referred to as candidemia and are usually confined to severely immune-compromised persons, such as cancer, transplant, and AIDS patients, as well as non-trauma emergency surgery patients. Growth of candida is suppressed by the normal microbiota of the human body. When the normal microbiota is disturbed, candida multiplies and produces candidiasis endogenously. It may also spread from mother to child and transmitted sexually. Recently, candida species have become an important nosocomial infection and a cause of major concern in both the developed and developing countries.

The most common etiological agent is *candida albicans*, an endogenous commensal member of the mucosal microbiota of humans which may be isolated from 30 - 50% of healthy human beings. It is diploid, dimorphic yeast and it was first demonstrated as the etiologic agent of the thrush in 1839. Its genome is organized in eight diploid chromosomes having 6,655 genes. It causes infection of the skin, nail, and mucous membrane in healthy individuals and can lead to more severe infections of the vital organs in case of immune-compromised patients leading to death [6, 7].

Over the past 20 years, mycological literature has shown a rapid increase in the frequency of fungal invasion and the growing number of etiological agents of systematic fungal infections and organ damage. In order to ensure appropriate, timely and successful therapy and earlier resolution of infections, leading to reduction of the morbidity and mortality, patient hospitalization time and overall health costs, an updated awareness of new emerging pathogens, it is imperative that species of candida are identified rapidly and accurately to the species level. This review focuses on the biodiversity of candida species, the concept of dimorphism and the identification techniques that focuses on biochemical, phenotypic identification methods beginning with conventional approaches followed by a rapid screening on chromogenic media and automated molecular methods, for appropriate identification of the candidal pathogens leading to successful therapy.

Biodiversity: Charles Robin was the first person to name the fungus *Oidium albicans* as the causative agent of 'le vrai Muguet des enfants' in 1853. Seventy years later, in the year 1923, C.M. Berkhout transferred the species to a new taxon candida. The phylogeny of this taxon has been determined by multigene analysis. Currently, it is a large anamorphic genus with 355 species. It is classified in the phylum-Ascomycota, subphylum - saccharomycotina, class - saccharomycetes, subclass - saccharomycetidae, order - saccharomycetales, family-Saccharo-mycetaceae and genus - candida. Of the over 17 human pathogenic species of Candida, 5 species which are considered to be major human pathogens are described below:-

Candida Albicans: 100 synonyms have been applied to *Candida albicans*. It first was called Oidium Albicans by Charles-Philippe Robin (1821-1885) in 1853. After further studies, Zopf in 1890 changed its name to *Monilia albicans*. The currently accepted name, *C. albicans*, was introduced by Berkhoutin 1923. The names of several species of candida have been changed. *39*, *C. claussenii* and *C. langeronii* have been merged with *C. albicans* while C. dubliniensis was separated from *C. albicans* due to the different arrangement of the blastoconidia and the chlamydospores. It is diploid yeast with two pairs of 8 chromosomes. Its genome size is 16 Mb (haploid). The yeast cells multiply by budding and undergo morphogenesis; that is yeast germinates producing characteristic germ tubes that grow out as either pseudohyphae or true hyphae. On cornmeal agar, it produces characteristic terminal round resting cells called chlamydospores.

Detailed Description of *C. Albicans*: *C. albicans* is seldom isolated outside the bodies of animals and is known from 58 species including wild and domesticated animals and birds. Many of us harbor this pathogen as part of our normal intestinal and urogenital mycota without symptoms of candidiasis. Compromised immune system and hormone changes due to HIV infection, leukemia, diabetes, drug therapy, and pregnancy all may cause the yeast to infect us, hence referred to as an

opportunistic pathogen. The infections often caused are superficial causing skin, mouth, throat and genital lesions. *C. albicans* is the 4th most common hospital-acquired infection in the United States that show different levels of resistance to antifungal agents [9]. In India, *C. albicans* is considered to be the commonest and most virulent pathogenic species of the genus Candida. Virulence is possible in *C. albicans* strains that have the ability to grow with the full repertoire of vegetative morphologic forms: yeast cells, pseudohyphal and true hyphal cells. Being a polymorphic yeast, *C. albicans* genome displays a very high degree of plasticity.

Keeping in view the increase of incidence of candidiasis due to *C. albicans* around the globe, it is important to identify the causative organisms to the species level correctly. Morphological, biochemical, germ tube test and growth on different media are often used for identifying isolated yeasts which sometimes takes days to a week to develop in culture hence, rapid identification of Candida species in the clinical laboratory is becoming increasingly important. Molecular techniques utilizing amplification of target DNA or PCR amplification of conserved regions of the genome and sequencing of the resulting PCR product and restriction fragment length polymorphism (RFLP) analysis of the ribosomal DNA, provides quick and precise methods for diagnostics or, identification of candida species and candidiasis and hence leading to proper treatment. This review deals with the taxonomy, morphology, identification, genome and life cycle of *C. albicans* for the proper treatment of candidiasis in humans [29].

Systematic Position: *C. albicans* is anamorphic yeast classified in the order Saccharomycetales along with the Saccharomyces cerevisiae because of its molecular closeness. In the classification system, it has been classified in the class Blastomycetes **Table 1**.

Morphological forms of *C. albicans*: Morphologically *C. albicans* is a dimorphic fungus mainly present in three forms- yeast cells, pseudohyphae and true hyphal cell **Fig. 1**. Yeast cells are round to ovoid in shape and separated readily from each other. Pseudohyphae resemble elongated, ellipsoid yeast cells that remain attached to one another at the constricted septation site and usually grow in a branching pattern. True hyphal cells are long and highly polarized, with parallel sides and no constriction between cells. Presence of some environmental factors cylindrical outgrowth is initiated on the surface of a blastospore forming a germ tube.

TABLE 1: CLASSIFICATION OF *C. ALBICANS*

Domain	Eukarya	Eukarya
Kingdom	Fungi	Fungi
Phylum	Ascomycota	Deuteromycota
Class	Saccharomycetes	Blastomycetes
Subclass	Saccharomycetidae	-
Order	Saccharomycetales	Cryptococcales
Genus	*Candida*	*Candida*
Species	*albicans*	*albicans*

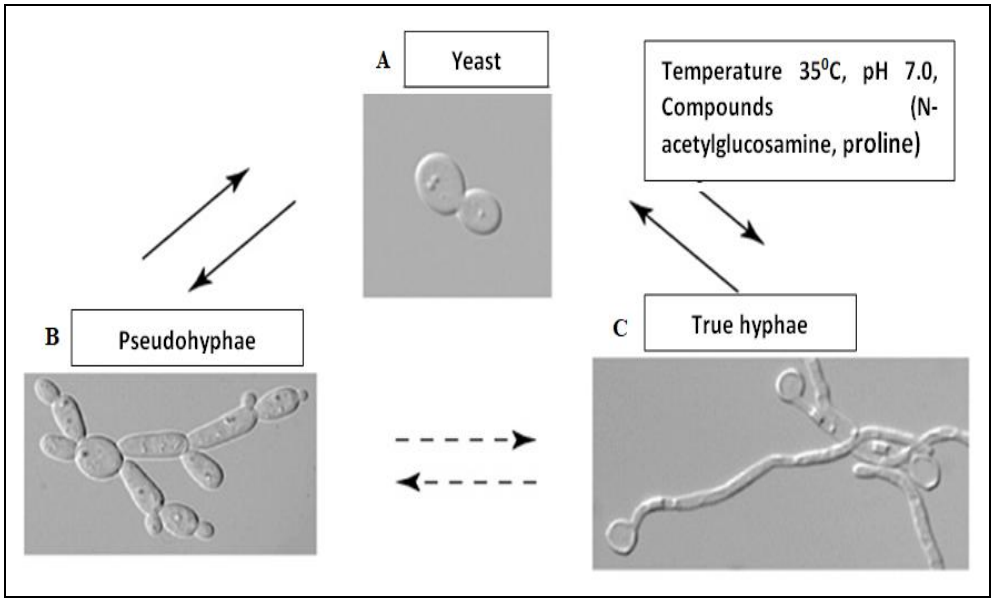

FIG. 1: DIMORPHISM IN *C. ALBICANS* AND THE MAIN FACTORS GOVERNING THE DIMORPHISM

Germ tubes grow and septa are laid down behind the extending apical tip to form a hypha. Hyphal branches and/or secondary branches are produced just behind newly laid-down septa, constituting a mycelium. Secondary blastospores become separated from the filament. An interesting feature of *C. albicans* is its ability to show the phenomenon of dimorphism *i.e.* to grow in two different ways; growth by budding and forming an ellipsoid bud **Fig. 1a** and in hyphal form, which can periodically fragment and give rise to new mycelia, or yeast-like forms **Fig. 1b, c**. Transitions between the two phenotypes can be induced *in-vitro* in response to several environmental factors such as pH, temperature, or different compounds such as N-acetylglucosamine or proline. However, perhaps the most critical criterion for pathogenicity is the induction of the mycelial form by serum or macrophages. In addition to the intrinsic biological interest of this dimorphism, its ability to switch between the yeast and the hyphal mode of growth has been implicated in its pathogenicity.

Identification of *C. albicans*: *Candida albicans* is the most often isolated yeast species from clinical specimens and is of clinical importance, so its rapid and true identification is the most important point for clinical laboratories and mycologists. Rapid presumptive identification of *C. albicans* is required for early diagnosis and therapy.

Preliminarily identification of *C. albicans* in a clinical specimen begins with direct microscopic examination of stained and unstained samples of the specimen, which also includes their macroscopic features (morphology, colour, size, and texture) and other conventional methods such as germ tube formation test, growth on cornmeal agar, culture media containing fluorogenic or chromogenic substrates specific for *C. albicans*, sugar fermentation and assimilation tests. Emphasis is being laid on the molecular techniques for its rapid identification [40].

I. Direct Microscopy: Direct microscopy provides a tentative diagnosis prior to growth in culture and it may give enough information for the clinician to begin correct immediate patient management. Microscopic examination of Gram-stained cells and KOH preparations are mainly used for the identification of yeasts [30].

(A) Gram Stain: *C. albicans* cells stain purple and appear as round or oval, often with buds. Under oil, immersion yeasts appear as giant Gram-positive cocci.

(B) Potassium Hydroxide (KOH) - A Wet Mount: A 10% or 15% solution of KOH is used, which acts as a clearing agent of the tissues and cellular debris but does not damage the fungal cells. Specifically, the KOH digests proteinous debris, bleaches pigment, and dissolves the "cement" that holds keratinized cells together and makes pseudohyphae, hyphae, and spores more apparent.

(C) Calcofluor White + KOH Wet Mount: Calcofluor white is a fluorescent dye that binds to the cellulose and chitin in the cell walls of yeasts (and molds). When viewed under a fluorescent microscope, it is easy to see the intense yellow-green fluorescent yeast and mold elements. This is still a wet-mount preparation, and the KOH is necessary for its clearing ability. The calcofluor white greatly enhances the ability to detect fungi.

(D) Potassium Hydroxide + Lactophenol Cotton Blue (LPCB) Wet Mount: This is used for the same purpose as the other KOH preparations. The LPCB stains the fungi, the lactic acid is a clearing agent and phenol kills the fungi.

II. Colonial Characteristics on Different Media: Five media commonly used for culturing and identification of *C. albicans* are Sheep blood agar, Sabouraud's dextrose agar (SDA), CHROM agar, methyl blue-sabouraud agar and candi Select 4. Of these, Sabouraud's dextrose agar is the most frequently used medium for primary isolation of candida spp. On CHROM agar, *C. albicans* strains produce β-N-acetylgalactosaminidase, which interacts with the chromophore (chromogenic hexosaminidase substrate) incorporated into the agar, and with incubation produces green colonies. This culture medium is also used to differentiate different species of candida. In methyl blue-Sabouraud agar, the exact reaction between the methyl blue-Sabouraud dye and *C. albicans* is not known, there is a possible reaction with specific cell wall polysaccharides which produces the fluorescent metabolite **Fig 2**. However, in the case of candi Select 4, which contains chromogenic

substrates react with enzymes secreted by *C. albicans* that leads to specific enzymatic activity resulting in pink to purple colored colonies. On Sheep blood agar many isolates of *C. albicans* produce colonies with feet or extensions [35].

III. Production of Chlamydospores on Corn Meal Tween 80 Agar: Cornmeal agar with Tween 80 is used for demonstration of chlamydospore production as it stimulates sporulation of *C. albicans*. The suspected Candida cultures are inoculated on cornmeal Tween 80 agar medium (pH, 7) containing 4% cornmeal powder, 1% Tween 80 and 1.5 % agar in double-distilled water. The plates are incubated at 37 °C for 48 - 72 h and observed for the production of chlamydospores, blastospores, branched pseudohyphae and true hyphae.

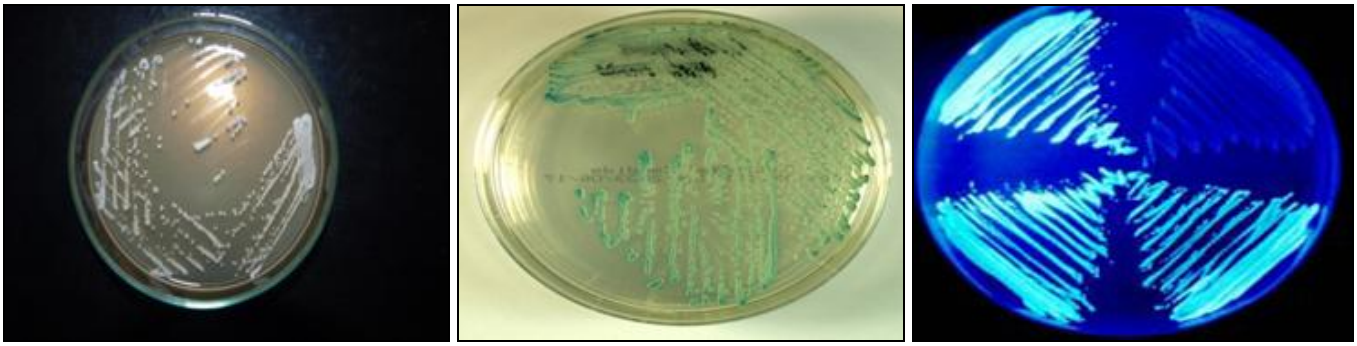

FIG. 2: COLONIAL CHARACTERISTICS OF *CANDIDA ALBICANS* ON THREE AGAR MEDIA: (A) GROWTH ON SDA; (B)APPLE GREEN COLOUR COLONIES ON CHROMAGAR; (C) APPEARANCE OF THREE *C. ALBICANS* STRAINS ON METHYL BLUE AGAR

IV. Germ Tube Test: Germ tube test is a rapid screening procedure for differentiating *C. albicans* from other species **Fig. 3**. It provides a simple, reliable and economical procedure for the presumptive identification of *C. albicans*. A germ tube represents the initiation of a hypha directly from the yeast cell. It is a filamentous, cylindrical outgrowth from the yeast cell with no constriction present at the base. The suspected Candida cultures are grown on sabouraud dextrose agar and inoculated into 0.5 ml of human serum in a small tube and incubated at 37 °C for 2 h. After a desired period of incubation, a loop-full of culture is placed on a glass slide and overlaid with a cover-slip. The preparation is examined for the production of germ tubes (one/yeast cell) from the germinating blastospores.

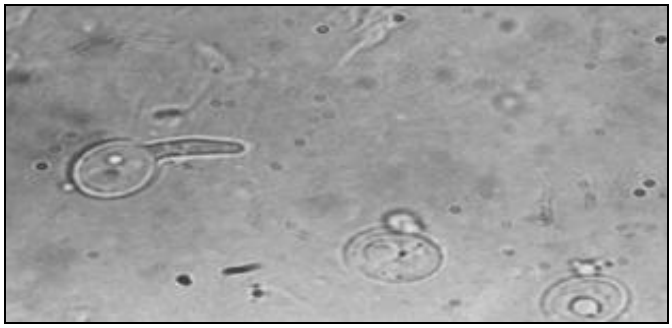

FIG. 3: GERM TUBE PRODUCTION FROM A YEAST CELL OF *C. ALBICANS* IN HUMAN SERUM

V. Physiological Tests:

(A) Carbohydrate Assimilation Tests: The carbohydrate assimilation test measures the ability of yeast to utilize a specific carbohydrate as the sole source of carbon in the presence of oxygen. Carbohydrate assimilation profiles for candida species can be obtained by examining zones of candida growth around discs or wells impregnated with various sugars on basal agars. Sugars used for assimilation tests include dextrose, maltose, sucrose, lactose, galactose, melibiose, cellobiose, inositol (a form of sugar, carbocyclic polyol, and cyclohexanehexol), xylose, raffinose, trehalose and dulcitol (or galactitol, sugar alcohol, the reduction product of galactose). In this test, the candida cultures are suspended in saline (NaCl) to which 1.5 ml of basal medium containing 67.8% yeast nitrogen base is added. This suspension is then transferred to 13.5 ml of molten, cooled agar containing 2% agar powder, mixed well, poured into a petri dish and allowed to solidify. After the solidification of the medium, 20 % sugar-soaked filter paper discs are placed on the medium. The plates are incubated at 25 °C for 10 to 24 h and observed for the zone of growth of Candida around the wells. Growth of the yeast indicates the assimilation of sugar. Sugars utilized by *C. albicans* are given in **Table 2**.

(B) Carbohydrate Fermentation Tests: Fermentation tests are generally performed in broth and to detect whether or not the yeast can produce acid or gas from the carbohydrate under anaerobic conditions **Table 2**. Fermentative yeasts recovered from clinical specimens produce carbon dioxide and alcohol. Production of gas rather than a pH shift is indicative of fermentation. Dextrose, maltose, sucrose, lactose, galactose and trehalose are used in the test. The 5 ml of carbohydrate (pH, 7.4) containing 1% peptone, 1% sugar, 0.3% beef extract and 0.5% NaCl, 0.2% Bromothymol blue in distilled water is dispensed in sterilized Durham tube and 0.2 ml of saline suspension of the test organism is added and incubated at 37 °C for 10 days and observed for the production of gas. Fermentation of various sugars brought about by *C. albicans* is given in **Table 2**.

TABLE 2: CARBOHYDRATE ASSIMILATION AND FERMENTATION TEST FOR *C. ALBICANS* IN DIFFERENT TYPES OF SUGARS

Sugars	Assimilation of	Fermentation of
Glucose	+	+
Maltose	+	+
Sucrose	+	-
Lactose	-	-
Galactose	+	+
Melibiose	-	Nt
Cellobiose	-	-
Inositol	-	Nt
Xylose	+	Nt
Raffinose	-	Nt
Trehalose	+	+
Dulcitol	-	Nt

+ Positive; - Negative; Nt- Not tested (Bhavan *et al*., 2010; Lennette *et al*., 1985)

VI. Molecular Methods: Molecular techniques are considered to be more stable approaches as of identification. It includes restriction fragment length polymorphisms (RFLPs) using gel electrophoresis or DNA-DNA hybridization and polymerase chain reaction. These are mainly used for the identification of candida strains but have been used less frequently for differentiation of species

(A) Restriction Fragment Length Polymorphisms (RFLPs) Using Gel Electrophoresis: Restriction fragment length polymorphism is based on the digestion of DNA. Every organism possesses unique nucleotide sequences that distinguish it from every other organism on the basis of the number and size of the fragments. In this method, DNA is extracted from isolates and cleaved into fragments by restriction endonucleases, the fragments are separated by gel electrophoresis. This method has been successfully applied for the exact identification of Candida species. The differences in the restriction patterns for the rDNA regions of the various Candida species serve as a rapid means of differentiating among these organisms. A HaeIII digest is definitive for distinguishing *C. albicans* species from other non-*C. albicans* species. DdeI digestion also seems to be efficient to identify *C. albicans* species

(B) Polymerase Chain Reaction: It has been used to identify candida based on detection of candidal genes encoding for chitin synthase, actin and cytochrome P450LIAI. Ribosomal DNA (rDNA; genes encoding for ribosomal RNA) is also a frequent target in PCR systems since, rDNA sequences exist in multiple copies within the genome, thereby offering greater sensitivity.

A relatively small divergence of the small subunit rDNA (l6S-like) sequences has occurred during evolution and therefore conserved regions exist between distantly related species. However, spacer regions between rDNA conserved sequences evolve faster and variations in the primary structure of these spacers can exist between genera and species. Primers that target conserved rDNA sequence adjacent to the spacer regions permit the incorporation of the spacers into the PCR product. RFLP studies have shown that rDNA sequences differ between Candida species and PCR amplification of 18S rDNA, enables discrimination of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. dubliniensis*. Despite the advantages of PCR- based identification over phenotypic features that may be subject to variable expression and as such may lead to incorrect identification, the application of such molecular techniques is still relatively limited due to certain limitations such as lesser technical staff training and non availability of the facilities in the laboratories, especially in the developing countries of the world.

Genome in *C. Albicans*: Genome is defined as the total inheritable genetic material of an organism, and a haploid set of chromosomes in eukaryote. *C. albicans* is polymorphic yeast with its genome organized in eight diploid chromosomes.

In this yeast, the genome is 14.3 to 16 Megabases (Mb) coding for 5,733 to 6318 genes **Table 3**. The size of diploid species of *C. albicans* shows difference of nearly 50% with haploid species of Candida (*e.g. C. guilliermondii* and *C. lusitaniae*) having small genomes that range from 10.6 to 12.1 Mb [17].

C. albicans has two mating-type-like (MTL) alleles, MTLa, and MTL α. The MTL locus is on the left arm of chromosome 5 (Chr5), approximately 80 kbp from the centromere. Most *C. albicans* isolates are heterozygous for the MTL locus, but approximately 3 to 10% of clinical isolates are naturally homozygous at MTL. Mating can occur between strains carrying the opposite MTL locus, and most strains that were found to be naturally MTL homozygous are mating competent. MTL-homozygous strains have also been constructed from MTL-heterozygous strains by deletion of either the MTL a or MTL α locus or by selection for Chr5 loss on sorbose. Mating between these diploid strains of opposite mating type can occur both *in-vitro* and *in-vivo*. The products are tetraploid and do not undergo a conventional meiotic reduction in ploidy. Rather, they undergo random loss of multiple chromosomes, a process termed "concerted chromosome loss," until they reach a near-diploid genome content.

TABLE 3: GENOME FEATURES OF IMPORTANT CANDIDA SPECIES (BUTLER *ET AL.*, 2009)

Species	Genome size (Mb)	No. of genes	Ploidy
C. albicans WO-1	14.4	6,159	diploid
C. albicans SC 5314	14.3	6,107	diploid
C. tropicalis	14.5	6,258	diploid
C. parapsilosis	13.1	5,733	diploid
C. guilliermondii	10.6	5,920	haploid
C. lusitaniae	12.1	5,941	haploid

Mating and Meiosis: *C. albicans*, diploid and dimorphic yeast, reproduces asexually by budding Fig. 4 and not known to reproduce sexually. But recent genomic studies have provided convincing evidence for the potential for a sexual cycle. A region of genome has been detected that encoded genes similar to those found at the mating locus of saccharomyces cerevisiae hence classified in the saccharomycetales. In *C. albicans* a well-defined mating system has been identified that allows the conjugation of mating-type locus homozygous diploid cells **Fig. 4**, however, there is currently no evidence for a functional meiotic pathway that allows reductional division and a return to the diploid state from the tetraploid. *C. albicans* has a parasexual cycle (mating of diploid cells followed by mitosis and chromosome loss instead of meiosis). In this yeast, efficient mating occurs between α and β forms, yet the completion of the sexual cycle occurs by a parasexual mechanism of random chromosome lose rather than conventional meiosis. In *C. albicans* mating occurs due to the presence of necessary mating genes where MTL a locus expresses MTLa2, which directs mating function, and the MTL α expresses MTLα1 controlling α mating functions. The resulting tetraploid products of mating breakdown to diploids through spontaneous chromosome loss, not meiosis. Diploids from tetraploids through chromosome loss, indicating that mitotic crossing-over gave rise to progeny thus showing the operation of the parasexual cycle. On the basis of genomic evidence, *C. albicans* either has a crytic meiotic program that has yet to be identified or conserved meiosis-specific genes have been programmed to function in the parasexual cycle.

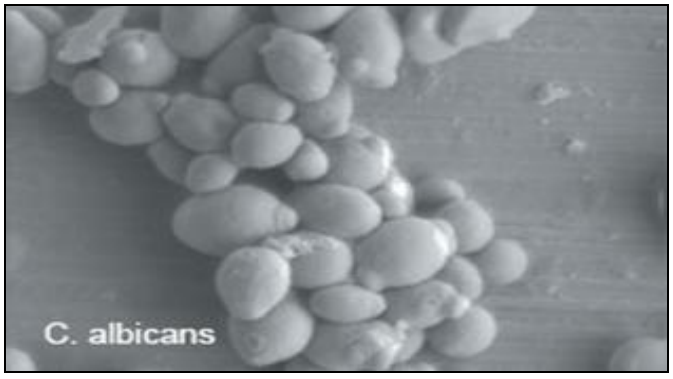

FIG. 4: SCANNING ELECTRON MICROSCOPIC IMAGE OF *C. ALBICANS* SHOWING BUDDING

***Candida Glabrata*:** *Candida glabrata* was first named *Cryptococcus glabrata* by Anderson in 1917. Lodder and deVries in 1938 called it *Torulopsis glabrata*. The original separation of the genera was based on the inability of *Torulopsis sp*. to produce pseudohyphae, like other species of candida. It is small, haploid, monomorphic yeast

with 13 chromosomes and a genome size of 12.3 Mb.

***Candida Tropicalis*:** Aldo Castellani (1877-1971), while he worked in Ceylon (Sri Lanka), differentiated several species of Candida; including Candida tropicalis in 1910. He called it Oidium tropicale. Other names given to this species have been *Monilia tropicalis*, *Candida vulgaris*, Mycotoruladimorpha, Candida paratropicalis. 58 synonyms have been applied to *C. tropicalis*. *C. tropicalis* is diploid with 10 - 12 chromosomes and a haploid genome size of 15 Mb. Yeast and pseudohyphal forms are produced.

Candida Krusei: Castellani described *C. krusei* in 1910 as Sacharomyces krusei, and as Endomyces kruseiin 1912 named it Monilia krusei. 18 other synonyms were proposed before Berkhout renamed it in 1923 as *C. krusei*. Colonies of *C. krusei* appear similar to *C. albicans* and other pathogenic candida species on Sabouraud's agar, but on cornmeal-Tween 80 agar *C. kruseiform* pseudohyphae with elongated blastoconidia, giving the appearance of crossed match sticks or trees. *C. krusei* is inherently resistant to fluconazole. *C. krusei* is diploid yeast with 3 - 5 chromosomes. It is capable of growing both as yeast and a psuedohyphae. On cornmeal agar, it grows as extremely long, rarely branched psuedohyphae.

***Candida Parapsilosis*:** Synonyms include Monilia onychopila (Poll and Nann, 1926), Monilia parapsilosis (Ashford, 1928), Mycocandida parapsilosis. Candida parapsilosis introduced by Langeron and Talice in 1932 is the current name of this diploid yeast. Colony morphology resembles that of *C. albicans*, but *C. parapsilosis* differs microscopically with their crooked or curved short pseudohyphae and occasional large hyphal elements called giant cells. It is diploid or anueploid yeast with 14 chromosomes and a genome size of 16 Mb. Both yeast and pseudohyphae forms are produced. On cornmeal agar, it grows as elongated, curved pseudohyphae with blastoconidia at the septa.

Dimorphism and Pathogenesis: Some fungi, especially human pathogens, can exist in two forms *e.g.* yeast form or in mycelial form and are said to be dimorphic (Gr. dis = twice + morphe = form). This phenomenon is termed as dimorphism. *C. albicans* has the ability to adopt a spectrum of morphologies; hence, *C. albicans* can be considered a "polymorphic" or "pleomorphic" organism. Morphologically, this fungus shows three forms- yeast cells, pseudohyphae and true hyphal cell **Fig. 1**. A number of different factors influence the conversions between morphological forms.

The yeast to filaments morphogenetic switch is initiated by multiple signals such as serum, temperature, pH, and nutrients. Yeast cells are round to ovoid in shape and separated readily from each other. Pseudohyphae and hyphae form, differ in morphology and several aspects of the cell cycle [12]. Pseudohyphae show constrictions between mother and daughter cells whereas hyphae do not. In pseudohyphae, cell walls are parallel throughout the length of the hyphae **Fig. 1a**.

In another aspect *i.e.* yeast cells from filaments on sub-apical fragments, resulting in lateral yeast growth on filaments. The signals resulting in this transition are mainly due to two genes i.e. PES1 and PDE [2]. The filaments-to-yeast transition might also occur in biofilms, facilitating dispersal **Fig. 1b**.

Phenotypic switching plays a role in virulence or considered essential for pathogenesis in *C. albicans*. It is important to recognize that most dimorphic fungi that are human pathogens exhibit growth by budding in the diseased tissues and exist as filamentous mycelial fungi in the external environment. Therefore, filamentous growth is not obligatorily coupled with tissue invasion, and genetic programs associated with growth *in-vivo* have no universal association with specific growth morphology. Furthermore, the switch to filamentous morphology is co-regulated with the expression of genes encoding virulence-promoting functions and those required for the acquisition of essential nutrients.

C. albicans to achieve its adaptability has evolved sophisticated mechanisms of sensing and responding to environmental cues by activating developmental switches that result in coordinated changes in cell physiology, morphology, and adherence.

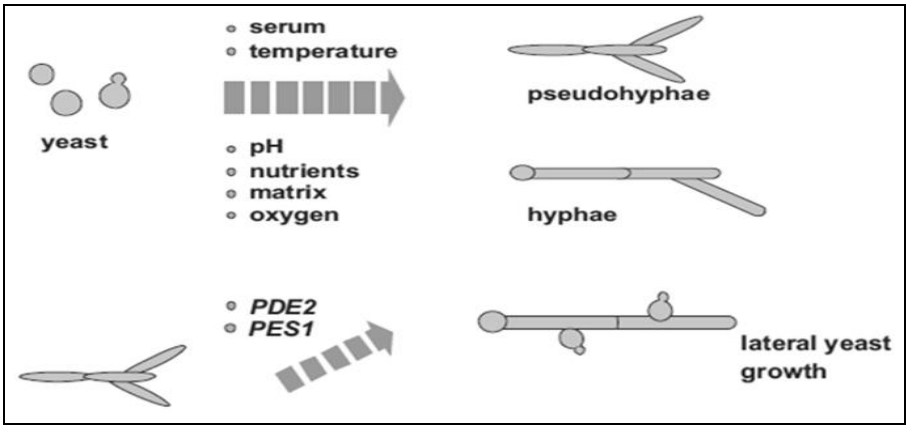

FIG. 1: MORPHOLOGICAL TRANSITIONS IN *CANDIDA ALBICANS*. (A) YEAST TO FILAMENTS; (B) FILAMENTS TO YEAST

Diagnostic Techniques: Species of Candida are present in the normal oral cavity, urogenital areas, upper respiratory passages, intestinal tract, and other deep human infections. Yeast identification today combine phenotypic characters such as colony appearance (*e.g.* filamentation, pigment production) and microscopic characters (*e.g.* cell shape, presence of true hyphae or pseudohyphae, arthroconidia = chlamydospores) or blastoconidia, biochemical (fermentation of selected carbohydrates, assimilation of carbon or nitrogen from selected organic compounds, acid production *etc.*) and molecular testing in a polyphasic approach. In addition to these, rapid and reliable identification of candida species is currently being achieved by culturing on chromogenic media. Although current phenotypic identification methods such as biochemical analysis, germ-tube and chlamydospore examination and evolution of colonial morphology on chromogenic agar represent conventional and reliable approaches, however, are time-consuming and require 48 to 72 h or longer to give definitive identifications. Keeping in view of these limitations, several molecular techniques *e.g.* nested PCR, multiplex PCR, Taq-man PCR, light-Cycler PCR and fluorescent PCR have been developed targeting to detect Candida species in a short period of time, with high sensitivity and specificity.

I. Direct Microscopy: Direct microscopy provides a tentative diagnosis prior to growth in culture, and it may give enough information for the clinician to begin correct immediate patient management. Microscopic examination of Gram-stained cells and KOH preparations are mainly used for the identification of yeasts.

(A) Gram Stain: *C. albicans* cells stain purple and appear as round or oval, often with buds. Under oil immersion yeasts appear as giant gram-positive cocci.

(B) Potassium Hydroxide (KOH) - A Wet Mount: A 10% or 15% solution of KOH is used, which acts as a clearing agent of the tissues and cellular debris but does not damage the fungal cells. Specifically, the KOH digests proteinous debris, bleaches pigment, and dissolves the "cement" that holds keratinized cells together and make pseudohyphae, hyphae and spores more apparent.

(C) Calcofluor White + KOH Wet Mount: Calcofluor white is a fluorescent dye that binds to the cellulose and chitin in the cell walls of yeasts (and molds). When viewed under a fluorescent microscope, it is easy to see the intense yellow-green fluorescent yeast and mold elements. This is still a wet-mount preparation, and the KOH is necessary for its clearing ability. The calcofluor white greatly enhances the ability to detect fungi.

(D) Potassium Hydroxide + Lactophenol Cotton Blue (LPCB) Wet Mount: This is used for the same purpose as the other KOH preparations. The LPCB stains the fungi, the lactic acid is a clearing agent, and phenol kills the fungi.

2. Culturing on Agar Media: Sabouraud, s Dextrose agar (SDA) with chloramphenicol (antibacterial antibiotic) was the medium of choice for the isolation of pathogenic Candida species. The pathological material is spread on agar slants and incubated at 30 °C or at room temperature and later observed for the candida species.

Identification of candida on SDA requires several days, however, a second-generation, chromogenic medium namely, CHROM agar candida (CAC) medium, is a differential ready to use the medium for rapid and reliable identification for Candida species. This medium incorporates a substrate of β-N-acetylhexosaminidase and one of phosphatase directly into the agar medium.

This medium reveals species-specific enzyme activity, which allows easier discrimination of candida species as poly-fungal populations, than does the traditional medium, Sabouraud agar. The colored colonies with different morphology are produced on a medium that is the result of cleavage of chromogenic substrates by species-specific enzymes. CHROM agar candida, Biggy agar and candi select 4 are the three chromogenic media recently being used for identification of candida species.

On Candi Select 4 medium, colonies of *C. albicans* appear pink to purple; *C. glabrata* colonies are pale turquoise in the centre with white periphery; *C. tropicaliscolonies* appear intense turquoise; *C. parapsilosis* colonies are white to slightly bluish and colonies of *C. krusei* are turquoise blue and rough. On biggy agar medium, colonies of *C. albicans* appear brown-black with slight mycelial fringe; *C. glabrata* colonies are pale to light brown; *C. tropicalis* produce discrete dark brown colonies with black centers and sheen; *C. parapsilosis* colonies appear white to slightly bluish and *C. krusei* colonies are large, flat, wrinkled colonies with silver-black top, brown edge, and yellow halo. CHROM agar candida, a differential medium, pathogenic species of candida are identified by colony colour and morphology **Table 1.**

3. Stimulation of Chlamydospores on Corn Meal Tween 80 Agar: In Candida, chlamydospores production is stimulated on cornmeal Tween 80 agar. In this technique, the suspected Candida cultures are inoculated on cornmeal Tween 80 agar medium (pH 7) containing 4% cornmeal powder, 1% Tween 80 and 1.5% agar in double-distilled water.

The plates are incubated at 37 °C for 48 - 72 h and observed for the production of chlamydospores, blastospores, branched pseudo-yphae and true hyphae **Table 2**.

TABLE 1: THE COLONIAL CHARACTERISTICS OF CANDIDA SPECIES GROWTH ON CHROM AGAR CANDIDA (AT 30 – 37 °C FOR 48 H)

Species	Colony characteristics	
	Colour	Morphology
C. albicans	Green	-
C. dubliniensis	Green	-
C. famata	Pink to lavender	-
C .firmetaria	Pink to lavender, pale border	Some flat, rough, other waxy
C. glabrata	Dark to violet	Small to medium, smooth, convex, creamy
C. guilliermondii	Pink to lavender	-
C. inconspicua	Pink to lavender, pale borders	Flat, rough
C. kefyr	Pink to lavender, often with darkened centers	Large, rough
C. krusei	Pink, pale borders	Medium to large, flat, rough
C. lipolytica	Ivory to pink	Large, flat, rough, wrinkled
C. lusitaniae	Pink to lavender	Some waxy
C. norvegensis	Ivory to pink	Large, rough

- Not observed

TABLE 2: SPORULATING CHARACTERISTICS OF FIVE PATHOGENIC CANDIDA SPP. GROWN ON CORN MEAL AGAR AT 37 °C FOR 48-72 H

Species	Morphological features
C. albicans	Chlamydospores; abundant pseudohyphae and true hyphae and clusters of blastospores present
C. glabrata	Psuedohyphae absent
C. tropicalis	Abundant pseudohyphae with blastoconidia present
C. parapsilosis	Clusters of blastospores seen occasionally as giant cells
C. krusei	Branched psuedomycelium with clusters and chains of blastospores present

4. Germ Tube Test: Germ tube test, first reported by Reynold and Braude in 1956, is a rapid screening procedure for differentiating *C. albicans* from non-albicans candida. A variety of media

have been used for germ tube test as pooled human serum, sterile horse serum, BHI broth, Trypticase soya broth, raw egg white, tissue culture media, peptone media, sheep serum, N-acetyl D-glucosamine, proline mixture of amino acids such as lee's medium and modified Sabourad's dextrose agar [2].

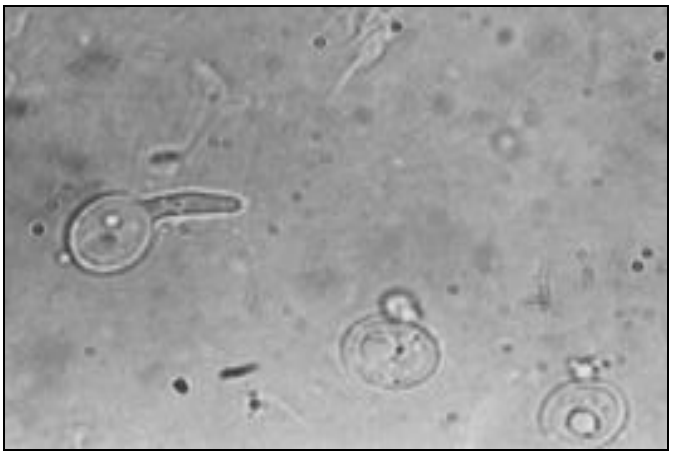

FIG. 2: GERM TUBE PRODUCTION FROM A YEAST CELL OF *C. ALBICANS* IN HUMAN SERUM

In this test, 0.2 ml of sterile human serum is transferred into a test tube followed by inoculation with the test organism and incubating at 37 ℃ for 2 h. After incubation, a drop of the sample is placed on a clean slide and viewed under a microscope for short hyphal strands which were not constricted at the junction of the blastoconidia and the germ tube. *C. albicans* produces short, slender, tube-like structures (germ tubes) as shown in **Fig. 2**.

5. Biochemical Tests: Carbohydrate assimilation and carbohydrate fermentation tests are also used for differentiation of candida species which are described below

I. Carbohydrate Assimilation Test: This test measures the ability of yeast to utilize a specific carbohydrate as the sole source of carbon in the presence of oxygen. Carbohydrate assimilation profiles for candida species can be obtained by examining zones of Candida growth around discs or wells impregnated with various sugars on basal agars.

Sugars used for assimilation tests include dextrose, maltose, sucrose, lactose, galactose, melibiose, cellobiose, inositol (a form of sugar, carbocyclic polyol, and cyclohexanehexol), xylose, raffinose, trehalose and dulcitol (or galactitol, sugar alcohol, the reduction product of galactose). Growth of the yeast in the presence of specific sugar indicates the assimilation of a sugar [9]. Various sugars utilized by species of Candida can be used for its identification as given in **Table 3**.

TABLE 3: CARBOHYDRATE ASSIMILATION TEST FOR CANDIDA SPECIES IN DIFFERENT TYPES OF SUGARS GROWN AT 37 °C FOR 24 - 48 H

Species	Assimilation of sugars shown by the growth of the yeast around the discs									
	Glucose	Trehalose	Galactose	Maltose	Lactose	Mellibiose	Xylose	Raffinose	Cellobiose	Inositol
C. albicans	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve
C. glabrata	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
C. tropicalis	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve
C. parapsiolosis	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve
C. krusei	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
C. guilliermondi	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
C. lipolytica	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
C. lusitaniae	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve
C. pseudotropicalis	+ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve
C. rugosa	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve

II. Carbohydrate Fermentation Test: Fermentation tests, which are generally performed in broth, to detect whether or not the yeast can produce acid or gas from a carbohydrate under anaerobic conditions. Production of gas rather than a pH shift is indicative of fermentation. Sugars such as raffinose, glucose, sucrose, trehalose, lactose, maltose and galactose, together with peptone from casein 1% in distilled water, distributed in standard assay tubes containing a Durham tube are employed as substrates in the sugar fermentation test. A 1.5 ml aliquot of each sugar solution containing 6% sugar and sterilized by filtration placed in each tube to produce data set from different carbon sources for each sample. The samples are inoculated using a 200 µl aliquot in each tube, incubated at 37 ºC and readings were taken after 48 h; negative cases were accompanied for up to 15 days 13 Fermentation of various sugars brought about by Candida spp. is given in **Table 4**.

TABLE 4: CARBOHYDRATE FERMENTATION TEST FOR CANDIDA SPECIES IN DIFFERENT TYPES OF SUGARS AT 37 °C FOR 24 - 48 H

Species	Fermentation of sugars shown by the production of gas						
	Glucose	Trehalose	Galactose	Maltose	Lactose	Sucrose	Cellobiose
C. albicans	F	F	F	F	-ve	-ve	-ve
C. glabrata	F	F	-ve	-ve	-ve	-ve	F
C. tropicalis	F	F	F	F	-ve	F	-ve
C. parapsiolosis	F	-ve	-ve	-ve	-ve	-ve	-ve
C. krusei	F	-ve	-ve	-ve	-ve	-ve	-ve
C. guilliermondii	F	F	F	-ve	-ve	F	-ve
C. lipolytica	-ve	-ve	-ve	-ve	-ve	-ve	-ve
C. lusitaniae	F	F	F	-ve	-ve	F	F
C. pseudotropicalis	F	-ve	F	-ve	F	F	-ve
C. rugosa	-ve	-ve	-ve	-ve	-ve	-ve	-ve

F- The sugar is fermented (i.e. gas is produced); -ve negative.

6. Molecular Methods: The advent of molecular biology has provided many new tools for fungal taxonomists. PCR and sequencing of relevant genes provide rapid and accurate identification of a large number of yeast pathogens. New species could not be described without sequence analysis since it provides a more objective separation of genera and species than by phenotypic testing. The most popular targets for PCR-based assays are rDNAs. Interestingly, rDNA subunits contain highly conserved domains separated by more variable domains that display species-specific sequences, making them a target of choice to design universal PCR primers and species or genus-specific primers. The organization of fungal rDNA genes is presented in **Fig. 3**.

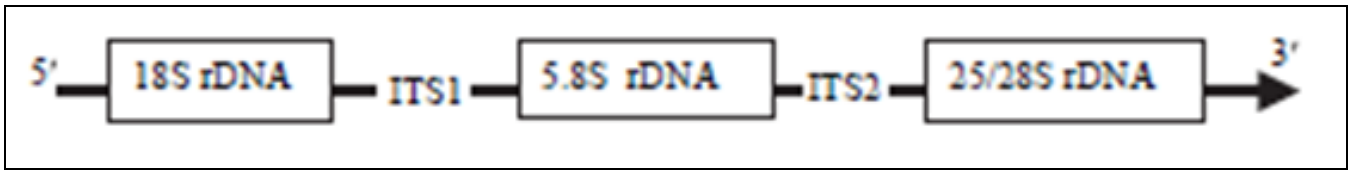

FIG. 3: SCHEMATIC REPRESENTATION OF FUNGAL RDNA REGION

rDNA is present in multiple copies in yeast genomes, with for instance 50 to 100 copies per Candida genome. The ribosomal DNA region consists of genes encoding for small (18S), 5.8S and large (26S) subunits of rDNA separated by the internal transcribed spacers named ITS1 and ITS2. Several universal fungal primers representing highly conserved regions have been designed either in the 18S rDNA subunit, 5.8S rDNA subunit or in the 26S rDNA region. These primers allow the amplification of 18S, 5.8S, 26S rDNA as well as ITS1 and ITS2 regions. Examples of different universal fungal primers described in the literature are presented in **Table 5**.

TABLE 5: EXAMPLES OF UNIVERSAL FUNGAL PRIMERS

Primers	Nucleotide sequence (5' to 3')	rDNA Target
ITS1	TCC GTA GGT GAA CCT GCG G	18S
ITS2	GCT GCG TTC TTC ATC GAT GC	5.8S
ITS3	GCA TCG ATG AAG AAC GCA GC	5.8S
ITS4	TCC TCC GCT TAT TGA TAT GC	26S
ITS5	GGA AGCT AAA AGT CGT AAC AAG G	18S
1	GTC AAA CTT GGT CAT TTA	18S
1a	GTC AAA CCC GGT CAT TTA	18S
2	CCA AGA GAT CCA/GTTGTT	5.8S
3	TTC TTT TCC TCC GCT TAT TGA	26S
Uni-F	GCA TAT CAA TAA GCG GAG GAA AAA G	26S
Uni-R	GGT CCG TGT TTC AAG ACG	26S

Different molecular techniques such as nested PCR, multiplex PCR, Taq-man PCR, light-Cycler PCR, and fluorescent PCR had been developed targeting to detect Candida species. However, in the last decade, several other molecular methods have been developed for the identification of

Candida sp. as nucleic acid sequence-based amplification (NASBA), fluorescence *in-situ* hybridisation (FISH), pulse-field gel electro-phoresis (PFGE), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), DNA microarray, micro-satellite typing and more recently multilocus sequence typing (MLST) and single-nucleotide polymorphism (SNP) analysis. Recent molecular studies have shown that many 'single species' of fungi pathogenic to humans, in fact, contain several phylogenetically distinct entities. Such 'cryptic' species have been described for *Histoplasma capsulatum* and *Coccidioides Candida parapsilosis, C. albicans, Cryptococcus neoformans, Aspergillus flavus, A. fumigatus immitis.* The ability to distinguish these cryptic species, which are extremely difficult or even impossible to identify using conventional phenotypic methods, is of clinical importance as they have been shown to have significantly different antifungal susceptibility profiles or pathogenicity characteristics.

Genomics: Genome is defined as the total inheritable genetic material of an organism, and a haploid set of chromosomes in eukaryote. *C. albicans* is polymorphic yeast with its genome organized in eight diploid chromosomes. In this yeast, the genome is 14.3 to 16 Mega bases (Mb) coding for 5,733 to 6318 genes **Table 3**. The size of diploid species of *C. albicans* shows the difference of nearly 50% with haploid species of Candida (*e.g. C. guilliermondii* and *C. lusitaniae*) having small genomes that range from 10.6 to 12.1 Mb.

TABLE 3: GENOME FEATURES OF IMPORTANT CANDIDA SPECIES

Species	Genome size (Mb)	No. of genes	Ploidy
C. albicans WO-1	14.4	6,159	diploid
C. albicans SC 5314	14.3	6,107	diploid
C. tropicalis	14.5	6,258	diploid
C. parapsilosis	13.1	5,733	diploid
C. guilliermondii	10.6	5,920	haploid
C. lusitaniae	12.1	5,941	haploid

Biofilms: Biofilms are complex microbial communities of single or multiple species of surface-associated microorganisms. Biofilms are characterized by the production of a thick, extra-cellular matrix; they are notoriously difficult to eradicate and are a source of many recalcitrant infections. This sessile mode of life provides biofilm embedded microbes with ample environmental nutrients and protection from host phagocytic clearance, greatly limiting the ability of the host to adequately deal with the infection. A more important consequence of biofilm growth, with profound clinical implications, is the markedly enhanced resistance to antimicrobial agents, where biofilm-associated micro-organisms are estimated to be 50 - 500 times more resistant than their planktonic counterparts.

A variety of microbial infections are caused by biofilms and these can range from the common, such as urinary tract infections, middle-ear infections, and gingivitis, to the more threatening, such as infections of heart valves. Immuno-compromised patients in particular, such as those with cancer or HIV infection, are often the most susceptible. The majority of manifestations of candidiasis are associated with the formation of candida biofilms on surfaces and this phenotype is associated with infection at both the mucosal and systemic sites. Superficial candida infections of prostheses and implanted devices are troublesome and the most frequently encountered. One of the most common is oral denture stomatitis, a Candida infection of the oral mucosa present in 65% of edentulous individuals.

Indwelling intravascular catheters, which provide a route through the body's barrier defenses, present a significant cause of morbidity and mortality among hospitalized patients. Candida species adhere avidly to catheter material and form a biofilm of cells, the detachment of which can result in fungemia. In fact, up to 40% of patients with Candida isolated from their central venous catheter surfaces have underlying fungemia. Electron microscopic evaluation of catheters removed from patients has demonstrated widespread biofilm colonization, confirming the importance of biofilms

in catheter-associated infections see **Fig. 4**. The current recommendations for the eradication of such a potential nidus of bloodstream infections is the removal of catheters infected with Candida. However, catheter removal is not always feasible for all patients and is associated with increased healthcare expenses, as well as complications related to catheter replacement.

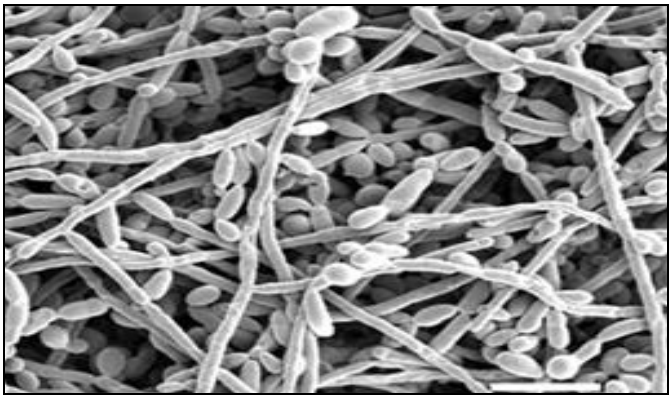

FIG. 4: SCANNING ELECTRON MICROGRAPH SHOWING HYPHAL INVASION OF TISSUE

Major Types of Candidiasis: Candidiasis is an acute or chronic infection produced by candida, generally limited to the skin and mucous membranes, but it could produce serious systemic disease.

(1) Mucosal Candidiasis: Candidal infections are restricted to non-sterile mucosal surface, for example, oropharyngeal and vulvovaginal candidiasis.

(A) Oropharyngeal Candidiasis (OPC): Oral candidiasis is one of the most common, oral mucosal infections seen in persons with HIV. Candida is a commensal organism and part of normal oral flora in about 30-50% of the population. However, the vast majority of infections remain superficial, affecting moist mucosal membranes, particularly of the vagina and oral cavity **Fig. 1**.

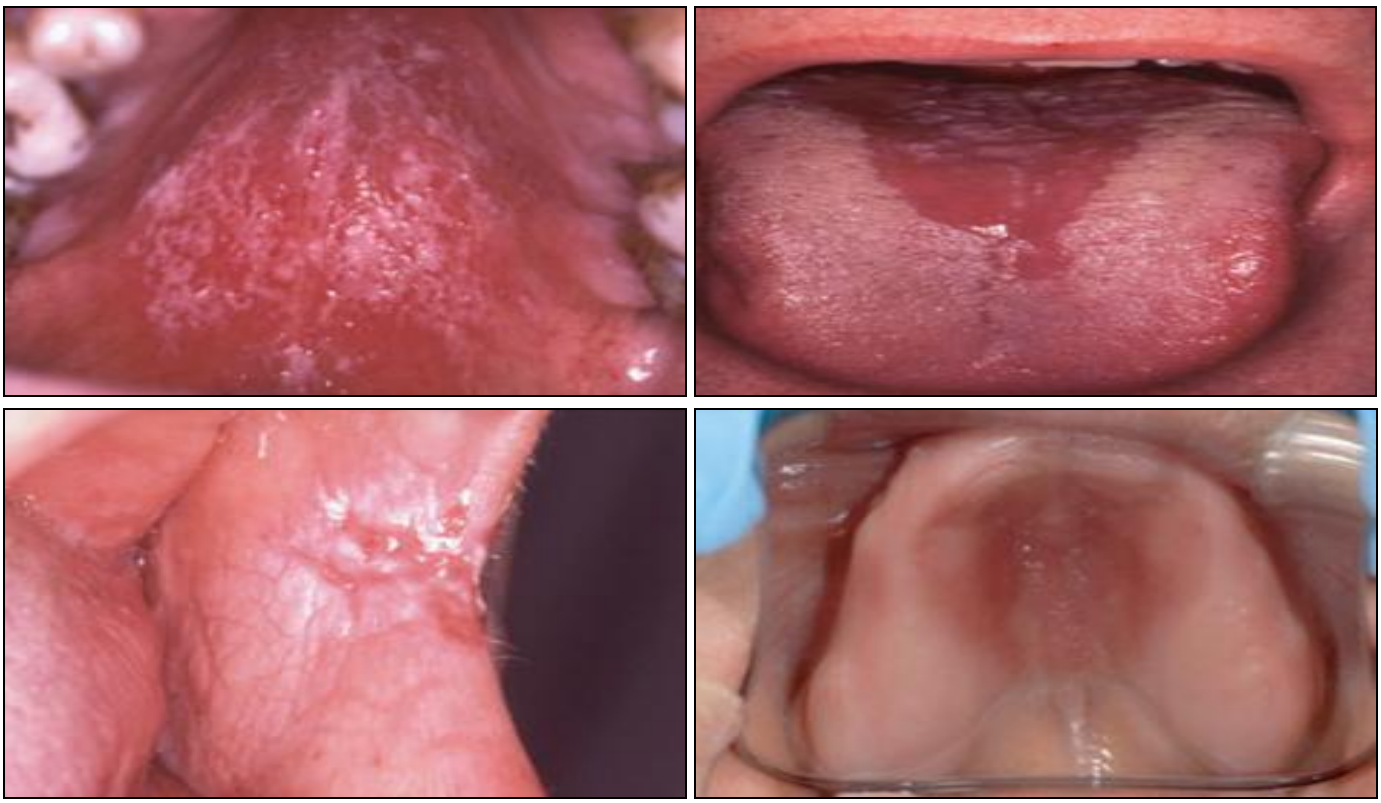

FIG. 5: CLINICAL PRESENTATION OF PRIMARY ORAL CANDIDOSIS: (A) PSEUDOMEMBRANOUS CANDIDOSIS, (B) ACUTE ERYTHEMATOUS CANDIDOSIS, (C) CHRONIC HYPERPLASTIC CANDIDOSIS, AND (D) CHRONIC ERYTHEMATOUS CANDIDOSIS

There are three general factors that may lead to clinically evident oral candidiasis: immune status of the host, oral mucosal environment and a particular strain of *C. albicans* (hyphal form is usually associated with pathogenic infection). The appearance of OPC in HIV-positive patients heralds the onset of AIDS, corresponding to the decrease in the CD4+ T-Lymphocyte count below

200/µl and plasma viral loads of more than 100,000 copies/ml. However, other yeast species have been increasingly identified, such as non - *C. albicans* Candida (NCAC) species (*Candida glabrata*, *C. parapsilosis*, *C. krusei*, *C. tropicalis*, *C. dubliniensis*, and *C. guilliermondii*) [29]. The ability of the yeasts to overcome host clearance mechanisms and to colonize surfaces can be considered as a risk factor for oral infection. The balance between Candida colonization and candidiasis relies on the balance between pathogen characteristics (*e.g.* production of adhesins, secreted aspartyl proteinases) and host factors. Host local predisposing conditions comprise: (i) reduced saliva secretion (ii) epithelial changes and local mucosal diseases, (iii) changes in commensal flora, (iv) high carbohydrate diet (v) denture wearing. There are different types of oropharyngeal candidiasis including acute pseudomembranous, acute atrophic, chronic hyperplastic, chronic atrophic, median rhomboid glossitis, denture stomatitis, and angular cheilitis. The most discrete lesion represents conversion from benign colonization to pathological overgrowth [27].

(B) Vulvovaginal Candidiasis (VVC): Vaginal candidiasis is the most frequent reason for gynecology consultation in primary health care services. The disease is usually associated with considerable morbidity, healthcare cost, discomfort, pain, and sexual functioning; however, it is seldom life-threatening. The symptoms associated with VVC are eczematoid dermatitis lesions that sometimes show vesicular and grey-white pseudo membrane, vulval pruritis, burning, erythema and curd-like discharge. It is a significant problem affecting 75% of women at least once during their lifetime. *C. albicans* is both the most frequent colonizer and responsible for most cases of VVC. Nevertheless, over the last decades, there have been reports demonstrating an increment in the frequency of cases caused by non-albicans species with *C. glabrata* consistently being the leading species while other species are C. glabrata, *C. tropicalis*, *C. krusei* and *C. parapsilosis*. C. dubliniensis is a new species that has been recently reported from the vaginal disease in the world. *C. albicans* possesses the ability to survive and proliferate in physiological extremes of pH, osmolarity, availability of nutrients, and temperature. This versatility may account for the successful behavior of *C. albicans* both as a commensal colonizer of the vagina and as a pathogen.

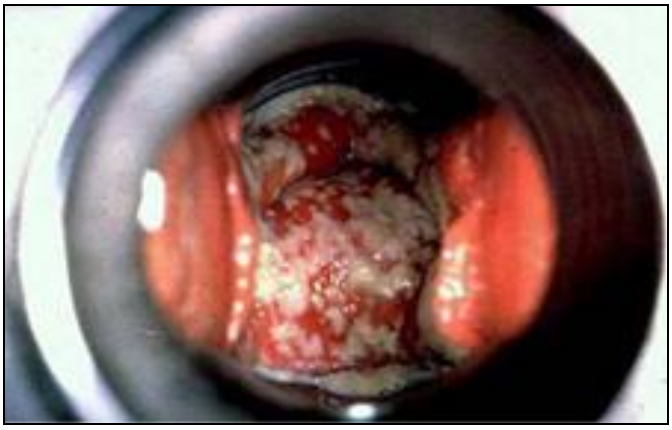

FIG. 6: VULVOVAGINAL CANDIDIASIS SHOWING TYPICAL COTTAGE CHEESE APPEARANCE OF WHITE CLUMPY VAGINAL DISCHARGE

(2) Cutaneous Candidiasis: Cutaneous candidiasis is usually a secondary infection of skin and nail (body folds) in predisposed patients. It occurs as a sub-acute or chronic infection. Disease involvement may be localized or generalized to the skin or nails. The spectrum of cutaneous candidiasis includes; diaper rash, intertrigo candidiasis, candida folliculitis, otomycosis, onychia and paronychia (It usually occurs in the warm, moist and creased area, such as axillary folds, inguinal or intergluteal areas. It is a fairly common opportunistic disease and is usually caused due to maceration and trauma in the skin. It is commonly found in diabetics and obese people. Other predisposing factors are antibiotic and oral contraceptives become macerated.

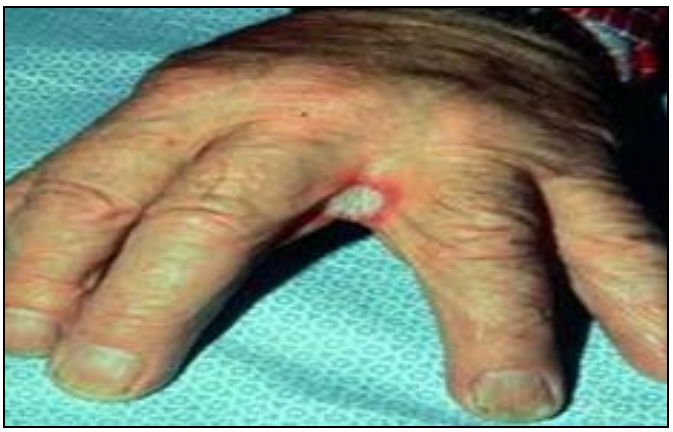

FIG. 7: EROSIO INTERDIGITALIS BLASTOMYCETICA (HTTP://OVERCOMINGCANDIDA.COM/IMAGES)

(3) Invasive Candidiasis: Invasive infections that can involve virtually any organ. *Candida albicans*

continue to account for the majority of invasive fungal infections, there has been a recent increase in disease due to tonon-albicans candida species (and antifungal-resistant candida isolates) [31].

(A) Systemic or Disseminated Candidiasis: Severe organ invasive or systemic hematogenously disseminated candidiasis is characterized by spreading of the candida cells into almost the entire body with a tendency to create abscesses in vitally important organs, inducing their failure which leads to mortality in ≈50 % of all cases, irrespective of administration of intensive antifungal therapy. Clinical signs of ongoing systemic candidiasis are hyper- and/or hypothermia, tachycardia, hypotension, high white blood cell counts, the need for vasopressor, *etc*.

It occurs predominantly as a consequence of some invasive medical procedures, immunosuppressive therapy, and aging. Principal predisposing factors are severe neutropenia and a variety of neutrophil dysfunctions. Besides the innate immunity (neutrophils, phagocytes, complement), the host protection is associated with the patient's ability to produce candida-specific antibodies, as demonstrated by those recovered from systemic candidiasis.

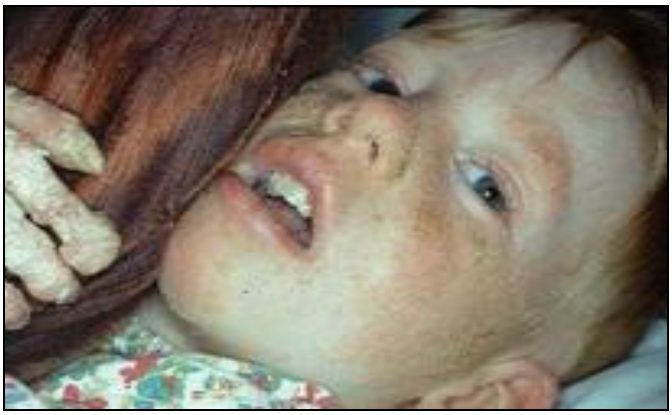

FIG. 8: CANDIDIASIS INFECTIONS OVER THE WHOLE BODY (HTTP://WWW.MEDICALMYCOLOGY.NET.)

(B) Candidemia or BSI (Blood Stream Infections): Candidemia is not only associated with a mortality of about 30% to 40% but also extends the duration of hospital stay and increases the cost for medical care. Candida species are the 4th leading cause of hospital-acquired BSIs reaching to 8 - 10% of all BSIs acquired in hospitals. In recent years, Candida species associated with candidemia have shifted from *C. albicans* to non-albicans Candida species (NAC). Approximately half of the reported cases of candidemia are now caused by NAC. According to Ha *et al.,* candidemia has become an increasingly important infection. The mortality rate of candidemia is higher (range 13-90%). The main entry is the gastro-intestinal tract. The main risk factors for candidemia are serious alteration in cutaneous and mucous barriers (because of surgery wounds, intubation or vascular catheters) and colonization of these barriers due to the use of broad-spectrum antibiotics.

Description of Mucosal Candidiasis:

(1) Vulvovaginal Candidiasis:

Introduction: Vulvovaginal candidiasis, a common pathological condition, afflicting normal women of fertile age, which frequently develops into a chronic, substantially incurable, disease. Candida is isolated from the genital tract of approximately 10-20% of asymptomatic, healthy women of childbearing age. Candida organisms gain access to the vagina from the adjacent perianal area and then adhere to vaginal epithelial cells. More than 40% of affected women will have a history of two or more episodes of VVC, and infection occurs more frequently during pregnancy.

It is believed that higher estrogen levels and higher glycogen content in vaginal secretions during pregnancy increase a woman's risk of developing VVC. Although the pathogenesis of VVC remains controversial, it seems that disruption of the normal vaginal ecosystem may facilitate the overgrowth of Candida species. Antibiotic and steroid therapies, oral contraceptive pills, diabetes mellitus, pregnancy and immune-suppression were reported to increase the risk for the development of VVC. Women with VVC usually complain of thick white caseous (curd-like) vaginal discharge and itching in addition to dyspareunia, vulval redness and edema. Vulvitis and vaginitis in the pediatric population may occur separately or in combination. Symptoms and signs of vulvitis alone include pruritus, tenderness, dysuria, and erythema of the vulva. The presence of discharge is more indicative of vaginitis. The pathologic discharge associated with vaginitis should be distinguished from the physiologic clear, mucoid discharge that occurs during the newborn period and with the onset of pubertal development.

Epidemiology: Vaginal candidiasis is the most frequent reason for gynecology consultation in primary health care services. The disease is usually associated with considerable morbidity, healthcare cost, discomfort and pain, days lost from work and sexual functioning; however, it is seldom life-threatening. The symptoms are eczematoid dermatitis lesions that sometimes show vesicular and grey-white pseudomembrane, vulval pruritis, burning, erythema, and curd-like discharge. It is also known as candidosis or moniliasis and vaginitis. Vaginitis is the most common reason for patient visits to obstetrician-gynecologists and accounts for over 10 million physician office visits annually.

The most frequent cause of vulvovaginal inflammation is infective, being the main organisms: Gardnerella vaginalis (15-50%), candida (20 - 25%) and *Trichomonas vaginalis* (5 - 50%) species, with a frequency distribution that depends on the populations studied. Prevalence of these 3 conditions will vary depending on the clinical setting. National figures show that 40% to 50% of patients with vaginal symptoms have bacterial vaginosis; 20% to 25% have vaginal candidiasis, and 15% to 20% have trichomoniasis.

TABLE 5: EPIDEMIOLOGY AND FACTORS AFFECTING *CANDIDA ALBICANS* INFECTION AT THE VAGINAL MUCOSA

Factors	Rate of Occurrence
Percentage of healthy individuals that are asymptomatically colonized by yeast species in the vagina	5-20%
Lifetime occurrence of at least one episode of vaginal candidiasis in healthy women	50-75%
C. albicans as a causative agent if vaginal candidiasis	85-90%
Predisposing factors for infection with acute vulvovaginal cadidiasis (VVC) or secondary recurrent VVC (RVVC)	
– Antibiotics	+
– High estrogen contraceptive therapy	+
– Steroids	+
– Chronic mucocutaneous candidiasis	+/-
– Chemotherapy	
– Lymphoma/hematologic malignancy	+/-
– Transplantation (allogeneic)	+/-
– AIDS	+
Prevalence of recurrent infection in healthy women (HIV-negative); Primary RVVC	5-10%
Antifungal resistance	Rare

Vulvovaginal candidiasis (VVC) is a significant problem affecting 75% of women at least once during their lifetime.VVC can be recurrent or relapsing. When a woman presents with four or more episodes per year, it is termed recurrent or relapsing VVC. Recurrent VVC is a condition that affects less than 5% of healthy women. In one study [3], almost 30% of the women with symptoms of vulvovaginitis had yeast isolated, confirming the diagnosis of vulvovaginal candidosis. Because of broad range of affected women, VVC now is categorized as an uncomplicated or complicated disease. Approximately 10-20% of women will have complicated VVC. It is estimated that 80 - 90% of women who have symptomatic VVC have an uncomplicated disease. A summary of the epidemiology of VVC and RVVC is shown in **Table 1**.

Microbiology: *Candida albicans* is both the most frequent colonizer and responsible for most cases of VVC. Nevertheless, over the last decades, there have been reports demonstrating an increment in the frequency of cases caused by non-albicans species with *Candida glabrata* consistently being the leading species. The next species are C. glabrata, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*. *C. dubliniensi*s is a new species that has been recently reported from the vaginal disease in the world. The incidence of *C. dubliniensis* varies between different studies from 0.17 to 29.52%. Normal vaginal floras (lactobacilli) colonize the vaginal epithelium and may have a role in defense against infection. They maintain the normal vaginal pH between 3.8 and 4.4. *Candida albicans* possesses the ability to survive and proliferate in physiological extremes of pH, osmolarity, availability of nutrients, and temperature. This versatility may account for the successful behaviour of *C. albicans* both as a commensal colonizer of the vagina and as a pathogen.

Classification: VVC is classified by recurrence and is categorized as either sporadic or recurrent VVC. Each category is classified then into primary and secondary causes. Sporadic VVC, also known as uncomplicated VVC, is defined as women who have two or fewer episodes per year [28]. Primary sporadic is defined as having no known cause, and secondary sporadic is defined as being caused by

pregnancy, antibiotic use or inappropriate lifestyle habits. *Candida albicans* is the primary culprit in 85 percent to 90 percent of sporadic VVC cases. Non-albicans Candida species, such as *C. glabrata* and *C. parapsilosis*, tend to be the cause of recurrent VVC in approximately 33 percent of patients.

According to the CDC, recurrent VVC, also known as complicated VVC, is defined as more than four episodes per year or an infection within two months of a previous treatment regimen. Other sources have to define recurrence when as least four specific episodes occur in one year or more than three episodes unrelated to antibiotic therapy occur in one year. Primary recurrent are frequent cases with unknown etiology and secondary recurrent are frequent episodes caused by immunodeficiency (*i.e.,* HIV/AIDS, chronic steroid therapy), uncontrolled diabetes, oral hormone intake, resistant strain to antifungal therapy or underlying genital condition. Although the estimates vary, approximately [5] percent of women will experience recurrent VVC infection.

The symptoms of sporadic and recurrent VVC do have some differences that can aid the clinician in determining whether self-treatment is appropriate. As previously mentioned, uncomplicated VVC typically presents with a thick, white cottage cheese-like vaginal discharge, discomfort after urination or intercourse, itching and burning. Patients with recurrent VVC may present with more severe symptoms and extreme changes to the vulva upon medical examination, including edema and thickening of the skin. A thorough physical examination is essential when a recurrent infection is suspected.

Recurrent Vulvovaginal Candidiasis: Recurrent vulvovaginal candidiasis (RVVC) is a condition what causes women a great deal of discomfort, inconvenience, and sometimes has psychological sequelae. RVVC is defined as four or more episodes of VVC in the preceding year, with at least one of these episodes well documented with culture, most cultures are positive for *C. albicans*. Some women who have RVVC exhibit an impaired tolerance for glucose despite having a similar incidence of overt or preclinical diabetes than controls.

Seventy percent of women who have RVVC who receive a conventional course of treatment with an antifungal agent can expect to have another episode within 6 months. Treatment options, which have been studied and shown to be effective, include ketoconazole (100 mg orally daily), clotrimazole (500 mg suppositories weekly) and fluconazole (150 mg orally once weekly). Because of liver toxicity associated with the use of ketoconazole, the latter two agents, especially fluconazole, are now preferred as maintenance regimens.

Chronic Vulvovaginal Candidiasis: Chronic VVC is defined as an episode of VVC which does not respond to conventional antifungal therapy after two weeks. With this condition, sometimes symptoms are minimal with flare up at certain periods of time, especially at certain periods of the menstrual cycle.

A history of vulval itching and burning with premenstrual exacerbation has been reported. It may be worsened by courses of systemic antibiotics, and non-specific vulvitis may be present.

Pathogenesis: Candidal vaginitis is a generic term used for vaginal infections caused by Candida species. *Candida albicans* is responsible for 90% of vulvovaginal candidiasis; the remaining 10% corresponds to *C. glabrata* and *C. Tropicalis* [11] Candida albicans colonizes the genital tract of 10 - 55% women in the childbearing age group. Most studies show increased rates of vaginal colonization in women infected with HIV. Symptomatic infections occur in the presence of predisposing factors such as immunodeficiency, pregnancy, uncontrolled diabetes mellitus, use of corticosteroids, antibiotics or high-estrogen oral contraceptive pills. Candida enters the vagina through any of several different sources, including local spread from the perineum and gastrointestinal (GI) tract, digital introduction and sexual transmission. Estrogen is believed crucial in the maintenance of colonization. Estrogen has also been linked to the induction and elongation of hyphae. The concept of pathogenesis may well be the result of these switching phenotypic properties. Little is known regarding the role of candidal proteolytic enzymes, toxins, and phospholipase in determining the virulence of the organisms.

Secreted aspartic proteinases (Saps) are expected to fulfill different tasks during mucosal or disseminated infections. Mycotoxin may act to inhibit the phagocytic activity or suppress the local immune system and has been found in vaginal secretions [16].

Pre-Disposing Factors: Although VC is both treatable and mild when left untreated, is a possible risk for acquisition of HIV/AIDS as well as other complications (UNAIDS, 2003). Other complications include pelvic inflammatory disease, infertility, ectopic pregnancy, pelvic abscess, menstrual disorders, spontaneous abortion, and premature birth. It is now well established that the presence of infective vaginal discharge greatly facilitates transmission and acquisition of HIV between sexual partners. Candida species are part of the lower genital tract flora in 20-50% of healthy asymptomatic women. Carrier rates are higher in women treated with broad-spectrum antibiotics, pregnant women, diabetic women and women with HIV/AIDS. The only well-proven predisposing factors are pregnancy, diabetes mellitus and the use of broad-spectrum antibiotics as well as an oral contraceptive with high estrogen content. Poorly supported risk factors include use of a sponge, intrauterine devices (IUDS), diaphragms, condoms, orogenital sex, douching and intercourse and diet with high glucose content. Unfortunately, in our part of the world, there is a culture of silence, hence in most of the patients, there is a delay in seeking help. A pathological discharge may be ignored by somewhere as normal physiological discharge was considered as abnormal by some fastidious women.

Transformation of Symptomatic Vaginitis: The mechanism by which candida organisms induce vulvovaginal inflammation is still obscure. Psuedohyphae and hyphae appear during a symptomatic episode. Hyphal elements enhance colonization and, although they represent the dominant invasive form that is capable of penetrating intact epithelial cells, only very superficial layers are involved. The clinical spectrum of symptomatic vaginitis varies from an acute florid exudative form with copious white discharge and large numbers of germinated yeast cells to the other extreme of absent or little discharge, fewer organisms, and yet severe pruritus.

Occasionally male partners of asymptomatic female carriers of candida develop transient postcoital penile erythema and pruritus, suggesting that inflammation is due to hypersensitivity mechanisms.

Vaginal Defence Mechanisms: The normal vaginal microflora is best described as a broad spectrum of facultative organisms, dominated by the presence of Lactobacillus. Lactobacilli produce lactic acid from glucose, keeping the vagina at an acidic pH. Glucose is metabolized by vaginal epithelial cells from glycogen, which is deposited under estrogenic control after puberty. In addition to producing acid, some species of Lactobacillus produce hydrogen peroxide (H_2O_2). H_2O_2 of microbial origin interacts with peroxidases produced by the host along with halide ion. The product of this reaction is a potent oxidant that is toxic to many bacteria H_2O_2- positive lactobacilli are capable to kill HIV *in-vitro* as well as Gardnerella vaginalis, anaerobes and *Neisseria gonorrhoeae*. Furthermore, women with H_2O_2 - producing lactobacilli are less likely to have bacterial vaginosis, chlamydia trachomatis, and Trichomonas vaginalis.

It has been claimed that some lactobacilli are also protective against candida vaginitis. *In-vitro* studies have demonstrated that *Candida albicans* is inhibited by culture supernatants of lactobacillus acidophilus. Furthermore, H_2O_2 produced by lactobacilli in combination with myeloperoxidase has antifungal effects. Moreover, Candidal vulvovaginitis is a common occurrence after the systemic use of broad-spectrum antibiotics. Antibiotic agents increase vaginal yeast colonization and are thought to act by eliminating lactobacilli, thereby facilitating candida to grow, adhere and germinate. The concept of interaction between lactobacilli and candida includes competition for nutrients and stearic interference of adherence to vaginal epithelial cells.

Other mechanisms comprise the elaboration of bacteriocin by lactobacilli that inhibit yeast proliferation and germination. Nevertheless, clinical studies show that women with yeast vaginitis have the same frequency and concentration of Lactobacillus as women without recurrent infections.

Vulvovaginal candidiasis occurs more frequently in women with a lactobacilli-predominated vaginal flora, as compared with those with a flora change with a mixture of anaerobic and facultative anaerobic bacteria. Moreover, the use of oral or vaginal forms of lactobacilli does not prevent post-antibiotic vulvovaginitis. These findings contradict the hypothesis that the presence of H_2O_2-producing vaginal lactobacilli protects against vaginal candidiasis. The phagocytic system plays an important role in limiting systemic candidal infection and deep tissue invasion, but phagocytic cells are absent from vaginal secretions during vaginal candidiasis.

Since, patients with profound immunoglobulin deficiencies are not predisposed to vaginal yeast infections and patients with recurrent infection do not lack antibodies, it is thought that the humoral system does not protect the vaginal mucosa against Candida colonization either Studies from animal models, women with recurrent VVC (RVVC) and HIV-infected individuals suggest that distinct protective host defence mechanisms may function against vulvovaginal candidiasis. However, while local and systemic cell-mediated immunity appears important for protection against oropharyngeal candidiasis, there is little evidence to indicate that either local or systemic cell-mediated immunity plays a role against VVC.

Risk Factors: Since candida can be either a commensal or a pathogen in the vagina, changes in the host vaginal environment are usually necessary before the organism induces pathologic effects VVC. It is generally thought that high levels of reproductive hormones, by providing higher glycogen content in the vaginal tissue, provide an excellent carbon source for candida organisms. Several studies have shown increased vaginal colonization with species of candida following high-estrogen oral contraceptive use during pregnancy the vagina shows increased susceptibility to infection by species of candida, resulting in both a higher prevalence of vaginal colonization and a higher rate of symptomatic vaginitis. Vaginal colonization is more frequent in diabetic women. As described afore, broad-spectrum antibiotics may also trigger VVC and are thought to act by eliminating the protective vaginal bacterial flora.

Diagnosis: Vulvovaginal examination frequently reveals epithelial erythema, itching and swelling of the labia and vulva, together with adherent whitish discharge. *Candida albicans*, a saprophyte present in the vaginal mucosa, is isolated in 80% of VVC cases. Generally, women are said to be present with clinical symptoms of vulvovaginal candidiasis when the high vaginal yeast count is greater or equal to 105 CFU/ml of vaginal fluid. Factors which predispose to VVC include pregnancy, uncontrolled diabetes mellitus, corticosteroids or systemic/ vaginal antibiotic therapy, HIV infection, and Candida vaginal colonization (CVC). Diagnosis cannot be made solely on the basis of history and physical examination. A medical diagnosis of VVC infection is made by the presence of clinical symptoms, evaluation of vaginal pH, microscopic examination and an amine or whiff test. In VVC infection, vaginal pH typically remains normal at less than 4.5, unlike bacterial vaginosis and trichomoniasis, which causes vaginal pH to rise to greater than 4.5. Other causes of an alkaline vaginal environment include menstruation, ovulation, recent intercourse, douching and infections. Presenting with an odourless discharge is a distinguishing factor of fungal infections. In the amine or whiff test, a drop of 10 percent potassium hydroxide (KOH) is added to the vaginal secretions.

Detecting a fishy odor can differentiate bacterial infection from a fungal infection. Observing vaginal discharge in a saline solution (wet mount test) or a 10 percent potassium hydroxide preparation under a microscope can help confirm the diagnosis of fungal infection. Under the microscope, yeast blastospores and pseudohyphae are characteristic in symptomatic patients. However, due to lack of specificity of these signs all patients with symptomatic vaginitis should be diagnosed on the basis of microscopic examination of vaginal secretions, not only to identify yeast cells but also to exclude the presence of clue cells or trichomonads.

A 10% potassium hydroxide (KOH) preparation can be valuable and may reach a sensitivity of 50 to 60% in identifying germinated yeast. If microscopy of these so-called wet-smears is negative for the presence of Candida but VVC is suspected on the basis of symptoms and signs, a vaginal culture

should be performed. It should be noted however, that vaginal cultures cannot differentiate pathogenic forms of Candida from harmless commensalism. A new diagnostic tool in determining VVC is PCR, which is based on DNA identification of Candida, In addition, noted that methylene blue-stained vaginal smears can also be used to determine the presence of candida. Nevertheless, none of the diagnostic methods described above is superior in detecting VVC and reliance on microscopy, culture or PCR alone in diagnosing (R) VVC can lead to inaccurate results.

Being as many women have yeast as part of normal vaginal flora, microscopic evaluation and cultures may have limited value in those who are asymptomatic. However, any female patient presenting with symptoms of VVC for the first time should always be evaluated by a clinician prior to treatment with a non-prescription antifungal. All clinicians should feel comfortable talking with female patients about their symptoms in order to eliminate the potential of misdiagnosis or treatment of another form of vaginitis or allowing an underlying condition to go untreated. In order to determine appropriate treatment, clinicians should ask patients specific questions about their discharge and associated symptoms.

Questions about discharge should include the presence of odour blood, on-set, duration, amount and previous episodes. Inquiring about associated symptoms should include complaints of itching, burning, fever and pelvic, abdominal or shoulder pain. Asking about age, pregnancy status and medical, sexual and medication history also should be part of the evaluation.

Ideally, pregnant patients should consult with their practitioner, however, non-prescription antifungal in the appropriate dose, formulation, and length of treatment are usually an option for this patient population. Other vaginal conditions, which are non-infectious, could be confused with the symptoms of VVC - specifical complaints of vaginal itching and irritation. These symptoms could be a result of a product hypersensitivity or allergy to latex condoms, spermicides, jellies, use of scented feminine products or frequent douching. These symptoms require a different treatment regimen.

Treatment: VVC should be categorized as complicated or uncomplicated. For patients who have uncomplicated VVC, the broad range of available therapies is summarized in **Table 2**. Current choices are for the most part limited to azole medications, fungistatic drugs that inhibit cell wall metabolism. Azoles are available as topical (creams or suppositories) or oral therapy, vary from 1 to 7 days of treatment, and can be obtained with or without a prescription. In terms of therapeutic efficacy, most commonly defined as clinical cure (resolution of signs and symptoms) and mycologic cure (negative follow up fungal culture), women who have uncomplicated VVC have cure rates of 80% to 90%. Topical therapy causes local burning in 5% to 10% of patients and tends to be messier. Women have complicated VVC for microbial or host factors. Regardless of the reason, women who have complicated VVC exhibit a poorer response to standard courses of antifungal therapy and require more aggressive therapy.

Treatment of acute VVC is available in a variety of highly effective topical azole agents. Overall, cure rates for vaginal azole treatment, defined as eradication of symptoms and Candida-negative cultures, are in the order of 80 - 90%. Oral systemic azole agents achieve comparable or marginally higher therapeutic cure rates and patients enjoy oral administration, which eliminates local side effects and messiness. On the other hand, oral azoles suffer the drawback of potential systemic toxicity, which has limited the use of ketoconazole, although constituting a lesser consideration in prescribing itraconazole and fluconazole. RVVC treatment includes ketoconazole 100 mg daily or either 500 mg General introduction and outline of the thesis. Clotrimazole suppositories or 100 mg fluconazole orally once-weekly.

All three maintenance regimens are effective in preventing a recurrent episode of vaginitis. However, when treatment is stopped, approximately half of the women suffer from symptomatic relapse shortly after cessation of therapy. Sometimes RVVC is due to nonalbicans species such as *Candida glabrata*. In those cases, there is often a reduced susceptibility to all azoles. Boric acid 600 mg administered vaginally once daily in a gelatine capsule has been shown to be highly effective in this clinically resistant infection.

TABLE 6: ANTIFUNGAL DRUGS FOR VAGINAL CANDIDIASIS TREATMENT

Drug	Formulation	Dosage
Butaconazole	2 % cream	5 g/ day for 3 days
	2 % cream (bioadhesive)	Single dose
Clotrimazole	1 % cream	5 g/day for 7-14 days
	10 % cream	5 g single application
	100 mg vaginal tablet	One tablet/day for 7 days
	100 mg vaginal tablet	Two tablets/day for 3 days
	500 mg vaginal tablet	One tablet once
Micoconazole	2 % cream	5 g/day for 7 days
	100 mg vaginal suppository	One suppository/day for 7 days
	200 mg vaginal suppository	One suppository/day for 3 days
	1200 mg vaginal suppository	One suppository once
Ecoconazole	150 mg vaginal tablet	One tablet/day for 3 days
	150 mg vaginal suppository	Single-dose
Fenticonazole	2 % cream	5 g/day for 7 days
Sertaconazole	300 mg suppository	Single dose
Ticonazole (vagistat,Novartis)	2 % cream	5 g/day for 3 days
	6-5 % cream	5 g single application
Terconazole (terazol), Jansen	0.4 % cream	5 g/day for 7 days
Pharmaceutica)	0.8 % cream	5 g/day for 3 days
	80 mg vaginal suppository	One suppository/day for 3 days
Fluconazole	150 mg tablet	Single dose
Ketoconazole	200 mg tablet	Two tablets/day for 5 days
Itraconazole	100 mg tablet	Two tablets/day for 3 days

Non-Albicans Candida Infections: Although clinical and *in-vitro* resistance to *C. albicans, fortunately*, is rare, non-albicans Candida species are less likely to respond to azole antifungal therapy. About half of *C. glabrata* strains isolated from cases of recurrent vulvovaginal candidosis show reduced sensitivity to fluconazole compared with *C. albicans*. Vaginal boric acid (600 mg daily) in a gelatin capsule for 14 days or amphotericin B suppositories are effective (70%) for refractory infection. Vaginal use of flucytosine should be kept to a minimum because of the potential for the acquisition of resistance. The drug can also be combined with amphotericin B. *C. krusei* vaginitis is resistant to fluconazole and flucytosine but usually responds to boric acid or other azoles. *In-vitro* susceptibility testing has not been validated and is not reliable in predicting clinical response in vaginitis. This lack of validation is by sharp contrast with systemic and oral candidosis. These results could be explained in part by the *in-vivo* synergy between fluconazole and organic acids (*e.g.*, acetic acid) normally found in the vagina. The addition of acetic acid to a mixture of yeast and fluconazole *in-vitro* makes fluconazole fungicidal, rather than just fungistatic.

(2) Oral Candidiasis: Oral candidiasis is an opportunistic infection of the oral cavity. It can also be amark of systemic diseases, such as diabetes mellitus and is a common problem among the immunocompromised. Oral candidiasis caused by an overgrowth or infection of the oral cavity by a yeast-like fungus, candida. The important ones are *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. pseudotropicalis*, *C. guillierimondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*and *C. stellatoidea*. *C. albicans*, *C. glabrata*, and *C. tropicalis* represent more than 80% of isolates from clinical infection [1]. Candidiasis is a common oral and perioral opportunistic infection that usually results from an overgrowth of endogenous candida. There are many species of candida but *C. albicans* is the organism most often encountered. It is generally associated with predisposing factors such as the use of immunosuppressive agents, antibiotics, prostheses, and xerostomia.

The development of research in animal models is extremely important for understanding the nature of the fungal pathogenicity, host interactions, and treatment of oral mucosal Candida infections. Many oral candidiasis models in rats and mice have been developed with antibiotic administration, induction of xerostomia, treatment with immunosuppressive agents, or the use of germ-free animals, and all these models have both benefits and limitations.

Over the past decade, invertebrate model hosts, including Galleria mellonella, Caenorhabditis elegans, and Drosophila melanogaster, have been used for the study of candida pathogenesis. These invertebrate systems offer a number of advantages over mammalian vertebrate models, predominantly because they allow the study of strain collections without the ethical considerations associated with studies in mammals. Thus, the invertebrate models may be useful to an understanding of the pathogenicity of candida isolates from the oral cavity, interactions of oral microorganisms, and study of new antifungal compounds for oral candidiasis.

Predisposing Factors: Changes in the oral environment that can predispose or precipitate oral candidiasis include: antibiotics, corticosteroids, dry mouth (xerostomia), diabetes mellitus, nutritional deficiencies, and immunosuppressive diseases and therapy. Saliva contains antifungal proteins including histatins and calprotectin that help protect patients from candida infections. These protective proteins are absent in a patient who has xerostomia. Individuals who use corticosteroid asthma inhalers must rinse their mouths with water after each use to reduce their chances of developing oral candidiasis. Excellent oral hygiene, including brushing and flossing of the teeth twice daily and maintenance of adequate intraoral moisture, is critical in the prevention of candidiasis recurrence in the susceptible patient.

Diagnosis of Oral Candidosis: Diagnosis of oral candidosis can often be made on the nature of the clinical presenting features although micro-biological specimens should be taken if possible in order to both identify and quantify any Candida that may be present and provide isolates for antifungal sensitivity testing.

Epidemiology: Oral candidiasis is the most common human fungal infection especially in early and later life. In the general population, carriage rates have been reported to range from 20% to 75% without any symptoms. The incidence of *C. albicans* isolated from the oral cavity has been reported to be 45% in neonates, 45% - 65% of healthy children, 30% - 45% of healthy adults, 50% - 65% of people who wear removable dentures, 65% - 88% in those residing in acute and long term care facilities, 90% of patients with acute leukaemia undergoing chemotherapy and 95% of patients with HIV. *C. albicans* is a normal commensal of the mouth and generally causes no problems in healthy people. Overgrowth of candida, however, can lead to local discomfort, an altered taste sensation, dysphagia from oesophageal overgrowth resulting in poor nutrition, slow recovery, and prolonged hospital stay. In immune-compromised patients, an infection can spread through the bloodstream or upper gastrointestinal tract leading to severe infection with significant morbidity and mortality. Systemic candidiasis carries a mortality rate of 71% to 79%. It is important for all physicians looking after older patients to be aware of the risk factors, diagnosis, and treatment of oral candidiasis. In a recent study, 30% of doctors said they would prescribe nystatin for oral candidiasis on the request of nursing staff without examination of the oral cavity. This is unfortunate as other pathology may be missed, the diagnosis may be incorrect and failure to address risk factors may lead to the recurrence of the candidiasis [1].

Identification of Candida Species: Identification of yeasts based on primary culture media can be confirmed through a variety of supplemental tests traditionally based on morphological and physiological characteristics of the isolates.

Treatment: Oral candidiasis is one of the most common, treatable oral mucosal infections seen in persons with human immunodeficiency virus (HIV) infection or acquired immune deficiency syndrome (AIDS). Oral candidiasis can be a frequent and significant source of oral discomfort, pain, loss of taste, and aversion to food. *C. albicans* carriage and a history of oral candidiasis are other significant risk factors for oral candidiasis. The infection is caused by *C. albicans*, a dimorphic fungal organism that typically is present in the oral cavity in a non-pathogenic state in about one-half of healthy individuals. Normally present as yeast, the organism, under favorable conditions, has the ability to transform into a pathogenic (disease-causing) hyphal form.

Conditions that favor this transformation include broad-spectrum antibiotic therapy, xerostomia, immune dysfunction (secondary to systemic

diseases such as diabetes or the use of immune suppressant medications), or the presence of removable prostheses. Furthermore, about one in four patients with lichen planus will have superimposed candidiasis. Unless the patient is severely immune-compromised, the infection is generally limited to the superficial mucosa and skin. Invasive candidiasis infection is rare, with the disseminated disease even more so. This superficial nature of the infection makes oral candidiasis so amenable to treatment. Several antifungal agents can be used topically.

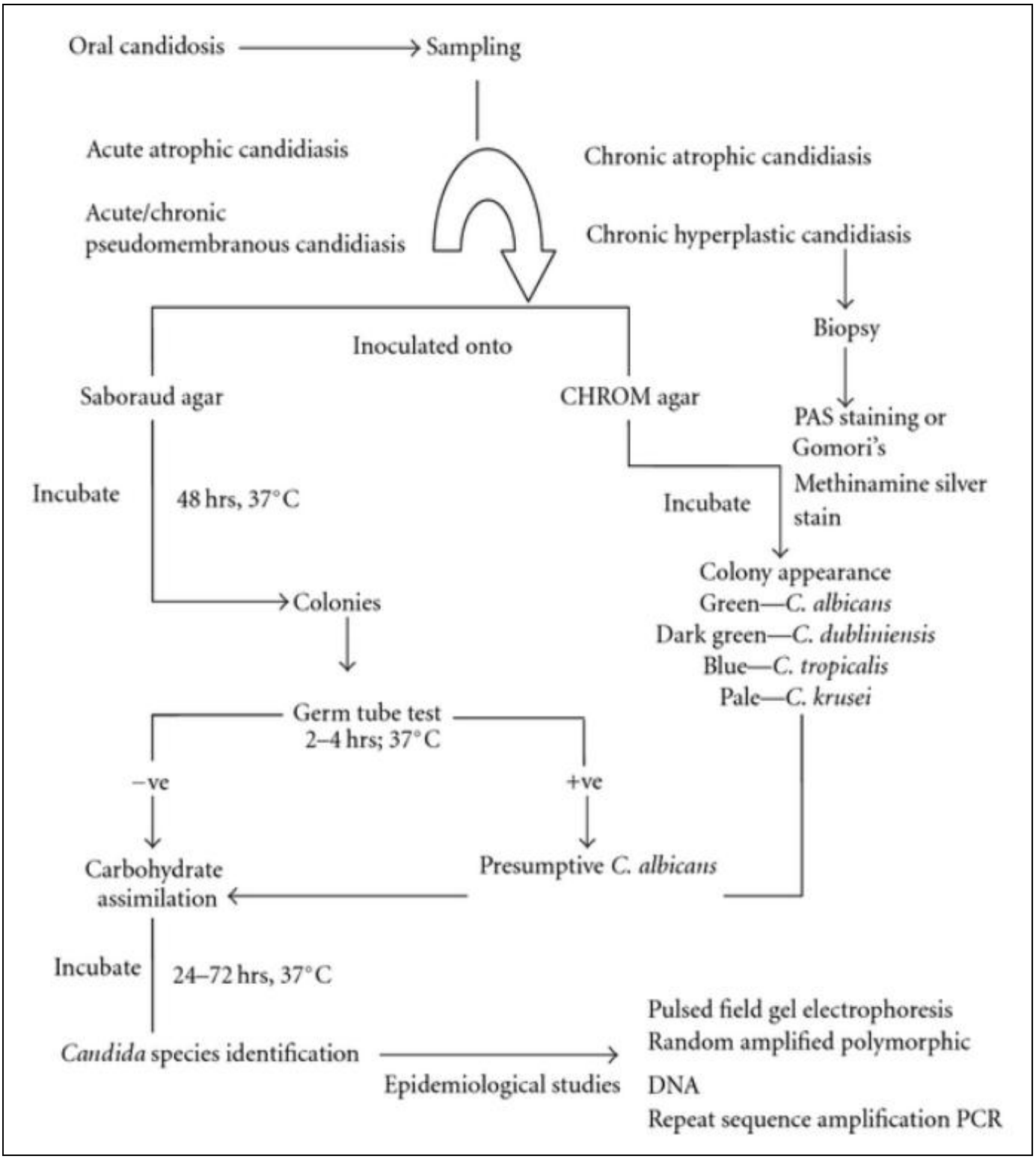

FIG. 9: SCHEMATIC REPRESENTATION OF ISOLATION AND IDENTIFICATION OF CANDIDA SPECIES FROM THE ORAL CAVITY

For topical agents, successful therapy depends on adequate contact time (2 min) between the agent and the oral mucosa. Treatment duration varies from 7 to 14 days, with therapy minimally continued for 2 to 3 days beyond the last clinical signs and symptoms. Topical agents have the benefit of few side effects at normal therapeutic doses because of their lack of gastrointestinal absorption. However, sucrose-containing topical agents can be cariogenic when used over prolonged time periods, such that adjunctive topical fluoride therapy may be needed. Systemic antifungals have the advantage of once-daily dosing and simultaneous treatment of fungal infections at multiple body sites.

However, these antifungals have more side effects, and selection requires consideration of important drug interactions. The present work reviews the common clinical types of oral candidiasis, its diagnosis and current treatment modalities with an emphasis on the role of prevention of recurrence in the susceptible dental patient. The dental hygienist can play an important role in the education of patients to prevent a recurrence.

Fluconazole, a novel bistriazole antifungal agent introduced in 1990, has systemic effects that may be beneficial for other fungal infections. Subjects in the fluconazole prophylactic arm of one antifungal placebo-controlled trial showed improvement of dermatophytosis, such as astinea pedis, onychomycosis, and tinea cruris. In addition, systemic fluconazole prophylaxis may prevent esophageal and vaginal candidiasis, cryptococcosis, histoplasmosis, and other deep fungal infections. Unlike ketoconazole, fluconazole is not altered by changes in gastric acidity and carries less risk of hepatotoxicity; however, many of the same drug interactions are possible. A newly raised concern about the widespread use of fluconazole is the potential for the development of azoleresistant *C. albicans* and selection of non-albicans Candida species, which also increase in prevalence with the immune decline and further complicate the management of some individuals.

TABLE 7: ANTIFUNGAL DRUGS FOR TREATMENT OF OROPHARYNGEAL CANDIDIASIS

Generic name	Proprietary name	Formulation
Amphotericin B	Fungizone	100 mg/ml oral suspension
Clotrimazole	Mycelex	10 mg troche
Fluconazole	Diflucan	100 mg tablet
Itraconazole 100 mg capsule	Sporanox	100 mg capsule
Ketoconazole	Nizoral	200 mg tablet
Nystatin	Mycostatin	100,000 units/ml oral suspension
		200,000 units/ml pastille
		500,000 units/ml tablet
		100,000 units/ml vaginal tablet

CONCLUSION: Candidiasis has emerged as a significant medical problem throughout the globe. The increase of Candida infections might be related to several factors such as the: (1) Human Immunodeficiency Virus; (ii) neutropenic persons due to anticancer treatments; (iii) the abusive use of extended-spectrum antibiotics; (iv) metabolic disorders such as diabetes mellitus. The need of the hour is to understand every aspect of Candidal infections. Early and specific diagnosis is crucial and the decision to treat a patient with these unusual infections is often based on little clinical and microbiological information. Treatment decisions need careful consideration of the epidemiological factors and the immune status of the population at risk. Thus, large prospective epidemiological surveys using a common database and methodologies are needed to monitor the increasing trends in incidence and changes in species distribution, to identify new at-risk patients and to evaluate the impact of the introduction of new antifungal agents into the market.

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CONFLICTS OF INTEREST: With the submission of this manuscript I would like to undertake that all authors of this paper have

directly participated in the planning, execution, or analysis of this study. All authors of this paper have read and approved the final version submitted. The contents of this manuscript are not now under consideration for publication elsewhere. The contents of this manuscript will not be copyrighted, submitted, or published elsewhere, while acceptance by the Journal is under consideration.

There are no directly related manuscripts or abstracts, published or unpublished, by any authors of this paper. My Institute's representative is fully aware of this submission.

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