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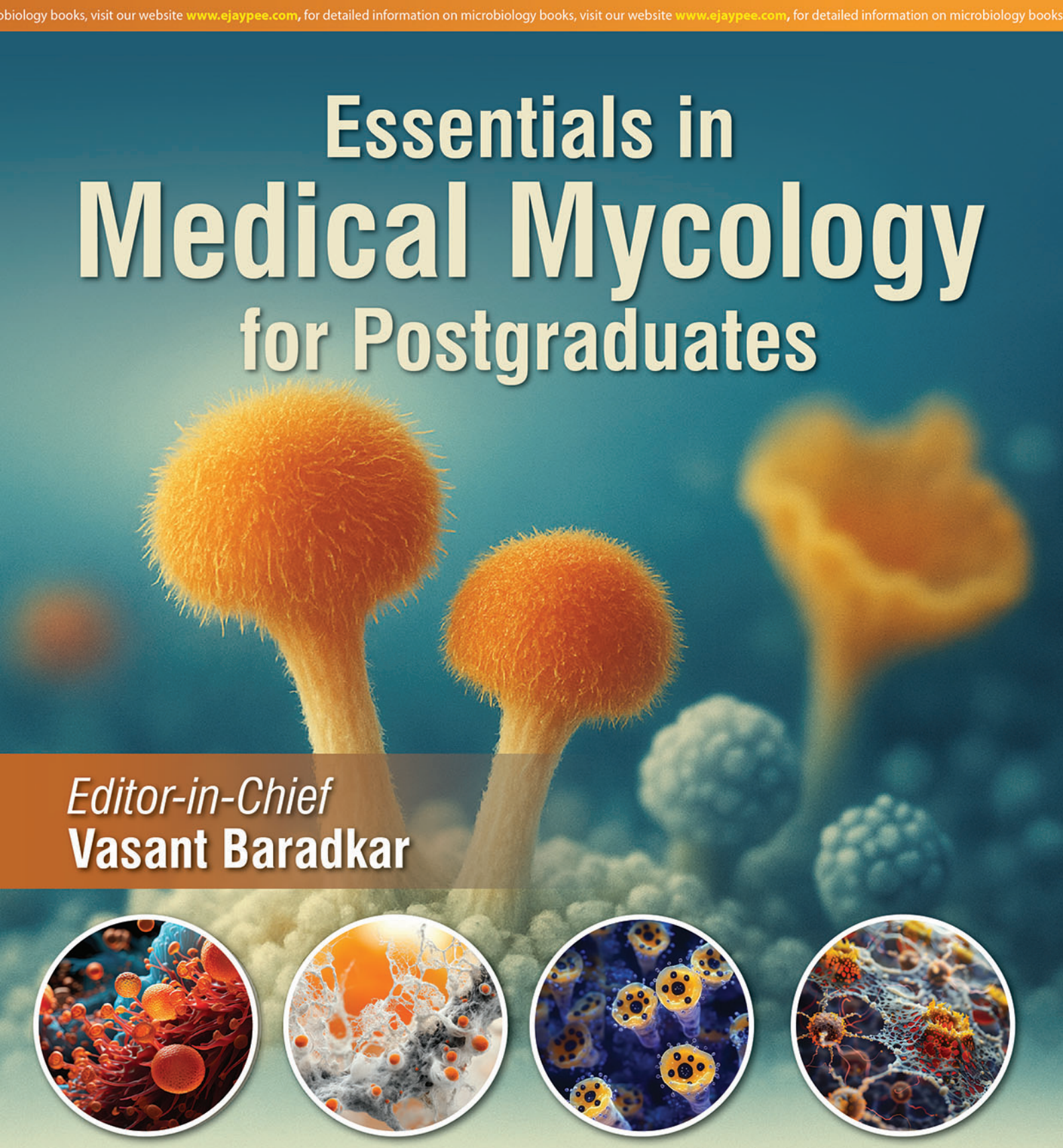

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Opportunistic Mycoses

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CHAPTER

20 Candidiasis

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INTRODUCTION

Candidiasis is caused by Candida species. *Candida* is a yeast-like fungus that occurs as commensal in the human body in some persons. The common sites of its location are mouth, vagina, and intestinal tract. Even at these sites, it can produce symptoms when the numbers increase to a great extent. It is therefore challenging to know whether the agent is occurring as a commensal or it is responsible for infection. When the immunity is compromised it can produce invasive disease. Chronic illnesses, malignancy, anti-cancer drugs, immunosuppressants, acquired immunodeficiency disease (AIDS) are some predisposing causes of invasive candidiasis. *Candida* is probably one of the commonest opportunistic fungi causing human infections. Though *Candida albicans* is the commonest species seen in humans, there are many other species that cause illness in humans. These are referred to as non-albicans *Candida*. They are reported in increasing numbers in recent years. Their speciation is essential as many are showing drug resistance. Some species recognized in last few years like *C. auris* are even showing multi-drug resistance. Newer technologies are available for rapid and accurate identification of *Candida* species. These aspects are covered in this chapter.

HISTORY

For ages, the presence of clinical symptom "thrush" or "moniliasis" has been known as a "clinical entity." In the year 1844, Bennett firmly showed fungal etiology of thrush. The developing yeast-like fungus was termed *Odium albicans* by Robin in 1853. Earlier, Hill (1751) misclassified *Candida* as *Monilia*, a genus that contains fungi that grow on plants. Berkhout (1923) established the genus *Candida* for asporogenous yeasts with few hyphae that lie flat and crumble apart into longer or shorter pieces.

COMMON *CANDIDA* SPECIES

There are 163 species of *Candida*. The common species which produce human infections include:

- *Candida albicans*
- *Candida tropicalis*
- *Candida parapsilosis*
- *Candida glabrata*
- *Candida krusei*
- *Candida guilliermondii*
- *Candida kefyr (previously known as pseudotropicalis)*
- *Candida dubliniensis*
- *Candida auris*
- *Candida haemulonii*
- *Candida rugosa*
- *Candida famata*
- *Candida lusitaniae*
- *Candida viswanathii*

Candida stellatoidea is now included in *C. albicans.*

TAXONOMY

The genus *Candida* is included in the phylum Fungi Imperfecti, order Moniliales, and family Cryptococcaceae. Serotype A and serotype B are the two varieties of *C. albicans*. The areas in which they are found differ. India is home to both serotypes. When it comes to antigens, *C. tropicalis* and serotype A are very similar.

Candida species are common in human gut, vagina, and skin flora. Even at these locations, they may infect whenever they get an opportunity. More and more cases of infection have been documented in the recent years due to *Candida* species other than *albicans*.

EPIDEMIOLOGY

In humans, *Candida* species occur as commensals. Common sites of their presence include the skin, the

digestive system, and the female reproductive system. The majority of cases of candidiasis is endogenous, meaning they originate within or on human tissues.

It is known that strains can be transmitted in hospitals and communities through direct contact between people or through fomites.

As the third most common cause of bloodstream infections (BSIs) contracted while receiving medical treatment, *Candida* species are serious disease-causing microorganisms. In intensive care units, it has the potential to spread like wildfire. Nonalbicans *Candida* species have recently become more common than *C. albicans*. Species such as *C. auris* and the *C. haemulonii* complex show remarkable resistance to therapy.

IMMUNITY

The immune system's ability to fight candidiasis depends on both innate and adaptive responses. *Candida* infections affect a large number of people when the immune system of the host weakens. Infection with *C. tropicalis* can occur in neutropenic individuals. *Candida* infections have been on the rise since the acquired immunodeficiency syndrome (AIDS) pandemic began. People who have primary immunodeficiencies, which impact the immune system's cell-mediated responses, are also more likely to get *Candida* infections.

Candida species antigens are of two main categories:

1. Cell wall antigens
2. Cytoplasmic antigens

The main antigenic component of the cell wall is mannan. The cell wall contains glucan polymers, which have a lower immunological activity. Antigenic characteristics are exhibited by a number of cytoplasmic products.

PATHOGENESIS

Candida albicans undergoes polymorphism when it switches from a commensal to a pathogenic form in response to environmental changes. During this stage, blastopores undergo a morphological change into hyphae, with pseudohyphae acting as a transitional stage.

Candidalysin, an essential component in the progression of candidiasis, is a toxin that is unique to hyphae. The hyphae of *C. albicans* produce it, and it damages the cells of the host. Candidalysin can weaken the cytoplasmic contents by intercalating with, permeabilizing through, and forming holes in the epithelial membrane. It is the first peptide toxin produced by any human pathogenic fungus to be identified.

Proteins, fibrinogens, and fibronectins are used as adhesions by *Candida*. *C. albicans* attaches to host cells by the action of adhesins (Als3 and Hwp1), which are mostly produced during hyphae development.

An important mechanism in the promotion of pathogenicity in *Candida* species is the thigmotropism of *C. albicans*. Thigmotropism is the ability of an organism to respond to touch or other physical stimuli. It is also the ability to sense changes in the surface contours and respond to them. Probably, *C. albicans* utilizes this property for tissue invasion. This is also the process that helps it to form biofilms on inanimate surfaces. Thigmotropism is a process that helps bacteria grow biofilms on inanimate surfaces and then spread those biofilms throughout host tissues.

- *Enzymes*: There are three main enzymes produced by *C. albicans*: (1) Hemolysin, (2) phospholipase, and (3) secreted aspartyl protease (SAP). The attachment to host tissue and the rupture of the host cell membrane are both facilitated by hydrolytic enzymes. These enzymes help the pathogen invade blood vessel and mucous membrane surfaces while evading the host immune system.
- *Phenotypic switching*: Morphological changes, such as blastopores becoming pseudohyphae and finally real hyphae, aid in adaptation to different host conditions. Such change allows it to evade immune mechanisms and invade the host tissues.
- *Biofilm formation*: Majority of *C. albicans* infections cause significant morbidity and mortality due to the biofilms similar to that the bacteria form on host surfaces or implants.

CLINICAL FEATURES

Candidiasis or Candidosis

Association of *Candida* with humans could be varied. It could be commensalism or acting as an opportunistic infectious agent or as an allergen. The infection caused by *Candida* species is called candidiasis or candidosis. The clinical manifestations of *Candida* species are given in **Box 20.1**.

Among the fungal infections in the mouth, the most prevalent is candidiasis. Oral *Candida* carriers were originally estimated to be between 35% and 80% of the population. It is believed that >80% of oral fungal isolates contain *C. albicans*, making it the most frequent species in both infected and healthy mouths. *C. glabrata*, *C. dubliniensis*, *C. parapsilosis*, *C. krusei*, and *C. tropicalis* are some of the other *Candida* species found in the mouth.

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BOX 20.1 Clinical classification of candidiasis.

- *Infectious diseases*:
  - *Mucocutaneous manifestations*:
    - Oral: Thrush, stomatitis, glossitis, and cheilitis
    - Alimentary: Esophagitis and gastritis
    - Vulvovaginitis, balanitis, and balanoposthitis
    - Chronic mucocutaneous candidiasis
    - Ocular candidiasis
  - *Cutaneous manifestations*:
    - Intertriginous and generalized
    - Paronychia and onychomycosis
    - Diaper dermatitis
    - Candidal granuloma
  - *Systemic manifestations*:
    - Urinary tract
    - Endocarditis
    - Pulmonary candidiasis
    - Meningitis
    - Candidemia
    - Dissemination
    - Arthritis
    - Osteomyelitis
    - Endophthalmitis
- *Allergic diseases*:
  - Candidids
  - Eczema
  - Asthma
  - Gastritis

The most common predisposing factors for candidal infections are the use of prosthetic replacements, orthodontic appliances, dry mouth (xerostomia), poor oral hygiene, and AIDS. Nutritional deficiencies (iron, folic acid, and vitamin deficiency), endocrinopathy (diabetes, hypothyroidism, hypoparathyroidism) and autoimmune diseases (Sjögren's syndrome, etc.), patients on chemotherapy, and steroids therapy are other factors for the promotion of candidal infections.

Infectious Diseases

Mucocutaneous Candidiasis

Oral Candidiasis

The oral cavity and perioral region are the exclusive domains of primary candidiasis, although systemic conditions might give rise to secondary candidiasis.

When an infection impacts just the mouth and the surrounding areas, it is called primary candidiasis. When the infection is a component of a systemic condition, it is called secondary candidiasis. In its most basic form, there are four candidiasis types: (1) Pseudomembranous, (2) acute erythematous, (3) chronic erythematous, and (4) chronic hyperplastic/nodular.

Pseudomembranous candidiasis: It represents the majority. White plaques or patches appear on the oral mucosa and can be gently scraped off. Typical sites for lesions include the tongue, buccal mucosa, and both the hard and soft palates. Sometimes, it is accompanied with an unpleasant aftertaste.

Acute erythematous or atrophic candidiasis: Painful red lesion on the back of the tongue is a common symptom. Denture stomatitis, prosthetic palatitis, and chronic erythematous or atrophic candidiasis are some of the names for the same condition. The mucosal lesions are red and localized to the locations where the prosthesis is placed.

Chronic hyperplastic candidiasis (Candida leukoplakia): The deep insertion of these white deposits into the oral cavity tissue makes mild scraping ineffective in removing them. Its most prevalent locations are the buccal mucosa and the sides of the tongue.

Angular cheilitis: Redness, erosions, and crusts—sometimes coated with white plaque—are clinical manifestations of an inflammatory illness that affects one or both corners of the lips.

Median rhomboid glossitis: It has no known cause and is frequently linked to secondary *Candida* infection. A nodular or smooth lesion is the clinical manifestation of the alteration that is situated in the center of the tongue.

Gastrointestinal Tract Infections

Esophageal candidiasis: Inflammatory plaques form in the esophagus. They may cause dysphagia, odynophagia, retrosternal burning sensation (acid reflux), epigastric pain, and back discomfort. In human immunodeficiency virus (HIV)-positive persons, esophageal candidiasis is an AIDS-defining condition. Lesions in immunocompromised individuals may progress to stomach producing gastritis. *Small intestine* is sometimes affected. Intestinal infections can be superficial, affecting only the mucosa and submucosa, or deep, affecting the entire intestinal wall and causing tissue damage, perforation, or even distant dissemination. In stool of normal persons, *Candida* may be present in small numbers as commensals. In stool of patients with irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), *Candida* is found in higher numbers. *Candida* is implicated as one of the probable causative agents of antibiotic-associated diarrhea.

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Vulvovaginitis, Balanitis, and Balanoposthitis

- Vulvovaginitis is one of the most common infections caused by *Candida* species. There is itching, burning sensation, and dyspareunia along with white curdy vaginal discharge. As varying percentages of females carry *Candida* in their vagina, interpretation becomes difficult. Local examination shows vulvar erythema and edema. Vulvovaginitis is common in young sexually active females.
- In balanitis and balanoposthitis, there is pruritus, edema, and whitish lesions over glans penis. In some patients, erosions and edema of prepuce may occur. It may lead to phimosis later. As this infection may be transmitted by sexual contact, both the partners should be treated.

Chronic Mucocutaneous Candidiasis (CMC)

CMC is a hereditary immunodeficiency disorder, which is a persistent or recurring infection with *Candida* due to malfunction of T cells. CMC is a collection of illnesses that usually manifest themselves in infancy. It is characterized by recurring and chronic *Candida* infections that can affect the skin, nails, and mucosal surfaces. The chances of systemic spread are minimal. The first symptom is usually oral candidiasis or diaper dermatitis. There are hyperplastic, nodular lesions with white patches and deep crevices. Common symptoms can include nails becoming hyperkeratotic and fragmented with paronychia. Cutaneous lesions are hyperkeratotic, erythematous, and serpiginous plaques. Lips get cracks, and angular cheilitis is seen. Many viral, autoimmune, and endocrine diseases are linked to CMC. Some instances of endocrinopathies include hypoparathyroidism, thyroid abnormalities, and Addison's disease. *Candida* granuloma in CMC is characterized by hyperkeratotic lesions that are disfiguring and well-defined; these lesions can grow to be several centimeters in size and manifest on the scalp, lips, eyelids, or face. Mucosal manifestations of CMC include vulvovaginitis and infections of the oropharynx, larynx, and esophagus.

Infection of Eye

Candida is one of the organisms which may cause keratitis. In a patient with fungemia, it can lead to endogenous endophthalmitis.

Cutaneous Candidiasis

Intertrigo

A typical symptom is inflammation of the skin folds. The axilla, submammary, umbilical, perineum, and under the rings on the hands are typical places that are impacted. People who are overweight or elderly, especially those who have diabetes mellitus, are also more likely to experience intertrigo. Folds between fingers and toes are also the likely sites of affection.

Paronychia and Onychomycosis

Paronychia is inflammation of nail folds of fingers and toes. In onychomycosis, the distal part of nail is affected.

Diaper Dermatitis

Diaper rash is seen in infants after use of diapers which leads to maceration and diaper rash. There are maculopapular lesions with vesicles and satellite pustules. Intertriginous areas of gluteal crease, perineum, and inguinal folds show erythematous rash. Causes are mechanical friction, diaper being soiled with urine and feces, variation in skin pH, and secondary infection with bacteria and *Candida*.

Systemic Candidiasis

Candidemia and Septicemia

It occurs in neonates and adults with immunodeficiencies. Many species may cause this condition, the common ones being *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*. *C. parapsilosis* is associated with total parenteral nutrition (TPN), while *C. tropicalis* is seen in neutropenic patients. Newly emerging yeasts are *C. auris*, *C. haemulonii*, *C. glabrata*, and *Pichia anomala*.

Disseminated Candidiasis

There may be serious infections of oropharynx and upper gastrointestinal tract. Various predisposing factors are:

- Patients with catheterization
- Broad-spectrum antibiotics for a long duration
- Hematological malignancies
- Transplant recipients
- Severe burns
- Postsurgery
- Hyperalimentation

Urinary Tract Infection

Predisposing factors are:

- Disseminated candidiasis
- Diabetes mellitus
- Pregnancy
- Prolonged use of antibiotics
- Catheterization

Candiduria: The infection or colonization of the kidneys or lower urinary tract might cause this. Indicators of a urinary tract infection include pus cells in urine. The majority of infections is transmitted through bloodborne

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pathogens. While indwelling drainage devices are prevalent in hospitalized patients, candiduria is rare in otherwise healthy individuals. *Candida* can enter the urine stream through the catheters. The vaginal cavity is a breeding ground for *Candida* species, making women more susceptible to infection.

Endocarditis and Pericarditis

Infection of previously abnormal native valves or prosthetic valves takes place. Pericarditis may be associated with thoracic surgery.

Meningitis

Infection is common in low-birth-weight babies and elderly patients. Meningitis may also follow complicated neurosurgical procedures or ventriculoperitoneal shunts. Chronic meningitis and shunt obstruction may lead to formation of hydrocephalus.

Arthritis

Infection spreads through the hematogenous route or direct inoculation during surgery.

Osteomyelitis

It develops by the hematogenous route. Osteolytic lesions may develop.

Endophthalmitis

It is either manifestation of disseminated *Candida* infection or a rare complication of ocular surgery.

Pancreatitis

Fungal infection in a patient with pancreatitis may turn out to be a severe outcome.

Pulmonary Candidiasis

Pulmonary involvement occurs through systemic infection. Presence of *Candida* in sputum is considered nonpathogenic unless we isolate *Candida* from a lung biopsy sample.

Allergic Diseases

Allergic reaction similar to "id" reaction to dermatophytes may occur in some individuals due to metabolic products. Cutaneous urticaria and eczema are reported.

Candida in AIDS

Patients with AIDS may suffer from a variety of infections due to *Candida*. There may be increased oral carriage, oral candidiasis, esophageal candidiasis, infections of urogenital system vulvovaginitis, balanitis, or hematogenous dissemination. Various oral presentations include thrush, erythema, angular cheilitis, or leucoplakia.

LABORATORY DIAGNOSIS

Specimens will depend on the sites affected. These include swabs collected by rubbing lesions on oral or vaginal mucosa. Sputum, cerebrospinal fluid (CSF), urine, skin and nail scrapings, and tissue biopsies are collected for direct demonstration and culture. Blood for culture can also be submitted. Molecular techniques are available for diagnosis of *Candida*. Serum is submitted for serological tests in some situations.

Microscopy

- Potassium hydroxide (KOH) (10%) slide mount is performed on the specimens collected which depend on the sites of involvement. Specimens including skin or nail scraping and whitish patches from oral mucosa and vagina are collected with sterile swab.
- *Tube KOH*: Biopsy is kept in a tube of KOH overnight and after that wet mount is prepared to see budding yeast cells with or without pseudohyphae.
- Gram staining is performed on suitable specimens for detection of gram-positive yeast cells (4–8 µm), pseudohyphae, and pus cells. Initially, it was thought that presence of pseudohyphae signifies the colonization and tissue invasion. But it should be kept in mind that some *Candida* species do not produce pseudohyphae and may still produce invasive infections.
- Other staining methods used are PAP smear and calcofluor staining method.
- Grocott–Gömöri's methenamine silver (GMS) staining is used to demonstrate fungal element in tissue (black against green background).

Fungal Culture

Sabouraud Dextrose Agar

Specimens are inoculated on two sets of Sabouraud dextrose agar (SDA) slants with antibiotics; one slant is incubated at 37°C while the second slant is incubated at 25°C. Smooth, creamy, and pasty colonies **(Fig. 20.1)** appear in 2–3 days. *C. tropicalis* shows hyphal fringe at the edge of colony. Lactophenol cotton blue (LPCB) mount or Gram staining is performed from the colonies. As the colonies are smooth and pasty, Gram staining is preferred.

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FIG. 20.1: SDA slant showing pasty colonies of *Candida* species. (SDA: Sabouraud dextrose agar)

Candida species grow on blood agar at 37°C after just overnight incubation. Hence, in an unsuspected case, *Candida* may be observed accidentally on blood agar inoculated for bacterial culture.

Brain Heart Infusion Broth for Blood Culture

For diagnosis of candidemia, blood is collected in brain heart infusion broth which is incubated and periodically subcultured on SDA slants. After 48 hours of incubation, before the first subculture, Gram staining is performed from smear prepared from blood culture bottle so that a provisional report for the presence or absence of growth can be given. *Lysis centrifugation test* increases the sensitivity of blood culture. It is a laboratory technique used to detect BSIs caused by fungi, bacteria, or mycobacteria, especially in cases where other blood culture methods have limitations.

Automated Blood Culture Systems

Blood is collected in a blood culture bottle and incubated till a signal is "flagged" or till 5 days. Subculture and smears are prepared from the flagged bottles.

Growth Pattern on Cornmeal Agar

Part of colony is taken from growth on SDA slant and inoculated on cornmeal agar (CMA) by a method known as Dalmau method.

Conventional Diagnosis

Further identification of *Candida* species is done by germ tube test, growth pattern (with special reference to the formation of chlamydoconidia) on CMA, growth on CHROMagar, sugar fermentation tests, and sugar assimilation test. Other biochemical tests which are added are rapid trehalose test, urease test, and formation of surface pellicle in liquid medium.

Germ Tube Test (Reynaud's Braude Phenomena)

- It is used for presumptive identification of *C. albicans* and *C. dubliniensis*. Very small inoculum (to provide light suspension containing approximately 10^5–10^6 cell/mL) is picked up from SDA and emulsified in freshly procured pooled serum in a test tube.
- The suspension is incubated at 37°C. After 2 hours of incubation, a drop of suspension is taken on a grease-free slide and cover slip is placed over the suspension to be observed under low and high power of a microscope.
- If a test is negative after 2 hours, make another preparation after 3 hours from the same suspension which is incubated further. Germ tube formation should not be looked for after 4 hours (hyphae formation may start).
- Along with the test strain, the standard strain of *C. albicans* is tested simultaneously with the same serum as positive control and *C. parapsilosis* as negative control.
- *Germ tube test is a tube-like outgrowth from a conidium or spore; it is the beginning of the hyphae and appears as filaments that are not constricted at their points of beginning* **(Figs. 20.2A to C)**. If filaments with constriction are present, they are pseudohyphae.
- Gram staining may be useful from the suspension which has two advantages. One is that under oil immersion, constriction is easily made out in pseudohyphae and the second is for recordkeeping. The other method to preserve the findings of a germ tube test is addition of formalin. Each batch of serum should be tested with known positive strain as serum may contain inhibitory substances such as ferritin.

Germ tube test has low sensitivity and reproducibility. Some variants of *C. albicans* give this test negative. The test can also be performed on Mueller–Hinton agar. **Table 20.1** shows differences between germ tube and pseudohyphae.

Cornmeal Agar Culture (Dalmau Method)

- The suspected strain is streaked on CMA with Tween 80, and cover slip is placed over the streaking.
- Along with the test strain, the control strain of *C. albicans* is also inoculated.
- The plate is incubated at 25°C for 2–3 days.
- The growth pattern is observed under an inverted microscope. Also, production of chlamydoconidia is observed. If an inverted microscope is not available,

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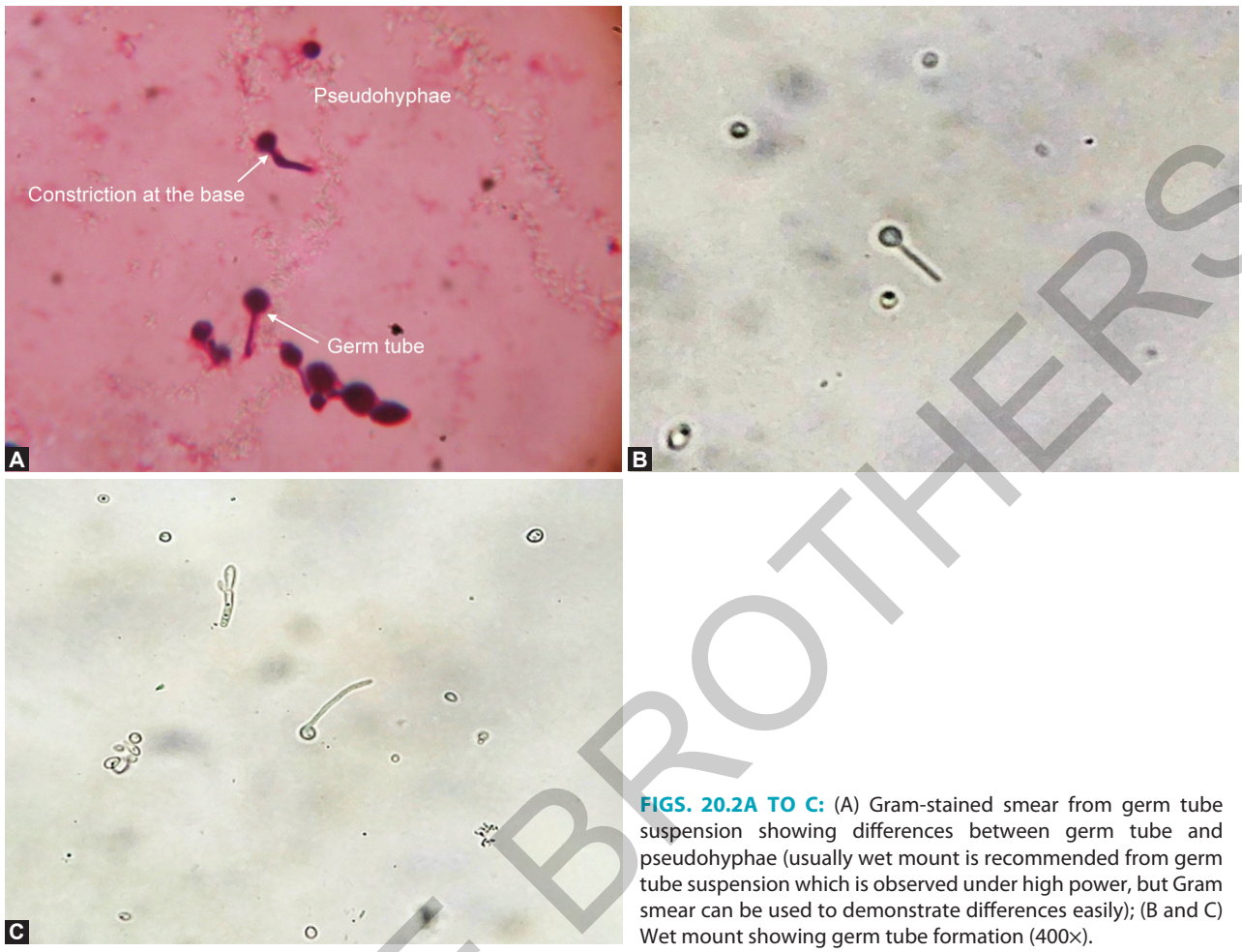

FIGS. 20.2A TO C: (A) Gram-stained smear from germ tube suspension showing differences between germ tube and pseudohyphae (usually wet mount is recommended from germ tube suspension which is observed under high power, but Gram smear can be used to demonstrate differences easily); (B and C) Wet mount showing germ tube formation (400×).

TABLE 20.1: Differences between germ tube and pseudohyphae.

Features	*Germ tube (later forms hyphae)*	*Pseudohyphae*
Growth process	Apical elongation with continuous growth at the tip	Budding with formation of constricted septa
Terminal cell	Elongated and cylindrical	Shorter and more spherical
Cell wall	Uniform and parallel	Constricted at septa, irregular
Septa	Straight and perpendicular, typically complete	Curved, pinched, and often incomplete
Origin of branches	No constriction at the point of branching	Branches arise from constricted areas

one can seal the plate and observe under 10× objective of a light microscope.

- Other different media used for the demonstration of chlamydoconidium are rice starch agar, Staib agar, and tobacco agar.

Flowchart 20.1 shows an algorithm used for the identification of yeast isolated from clinical specimen. Biochemical tests such as sugar fermentation and sugar assimilation which are used for the identification of common *Candida* species are shown in **Tables 20.2 and 20.3**, respectively.

CHROMagar

Colonies of various colors are produced when enzymes from *Candida* species react with a medium that contains diverse chromogenic substrates. Subculturing an isolate colony on this medium and incubating it for 1–2 days comprise the standard procedure. In contrast to the blue colonies produced by *C. tropicalis*, those of *C. albicans* are green in color. One cannot obtain the desired effects if the medium is not from a reputed manufacturer.

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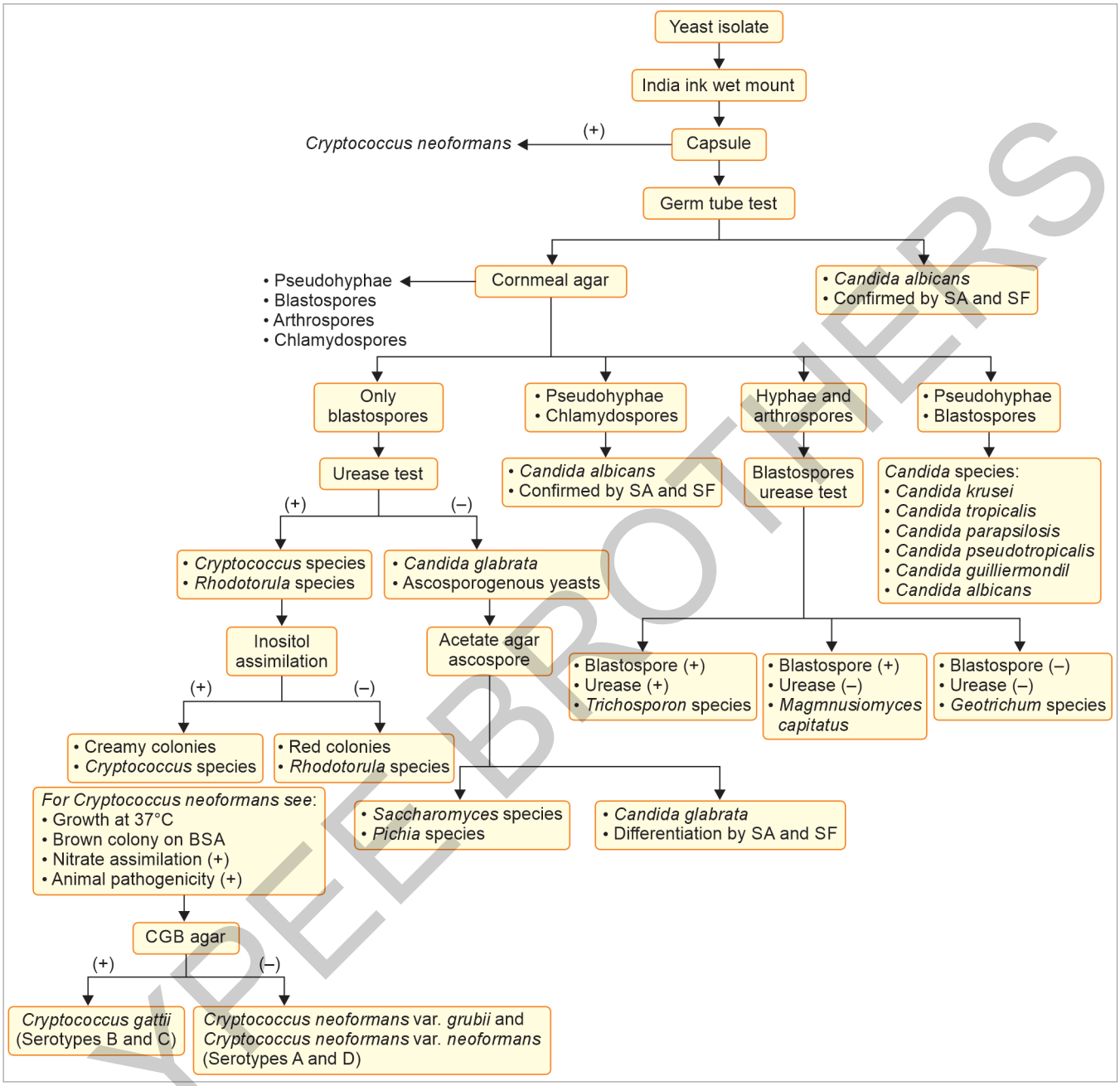

FLOWCHART 20.1: Identification scheme of commonly encountered yeast isolates.
(SA: sugar assimilation; SF: sugar fermentation)

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MYCOLOGICAL CHARACTERISTICS

Candida albicans

- *Colony morphology on Sabouraud Dextrose Agar*: The rate of growth is rapid, the colony matures in 3 days. Cream-colored, pasty, smooth colonies are produced on SDA **(*see* Fig. 20.1)**. Initially after overnight incubation, colonies are small, but after 24–48 hours, the size increases. Sometime, confluent pasty growth may cause confusion as it may closely resemble colonies of *Cryptococcus*; hence, confirmation is done by microscopic examination. Negative stains such as India ink and nigrosine are used to demonstrate capsule. Capsule is absent in *Candida* species. Old colonies may appear wrinkled and show extensions

TABLE 20.2: Fermentation reactions of *Candida* species.

Candida species	*Glu*	*Mal*	*Suc*	*Lac*
Candida albicans	AG	AG	–	–
Candida tropicalis	AG	AG	AG	–
Candida kefyr	AG	AG	AG	–
Candida guilliermondii	AG	–	AG	–
Candida parapsilosis	AG	–	–	–
Candida krusei	AG	–	–	–
Candida glabrata	AG	–	–	–

[A: acid production; G: gas production; Glu: glucose; Lac: lactose; Mal: maltose; Suc: sucrose; –: no reaction)

TABLE 20.3: Assimilation reactions of *Candida* species and growth on TRM.

Candida species	*Glu*	*Mal*	*Suc*	*Lac*	*Cel*	*Gal*	*Tre*	*Raf*	*Mel*	*Xyl*	*Ino*	*Dul*	*TRM*
Candida albicans	+	+	+	+	+	+	+	–	–	+	–	–	PP
Candida tropicalis	+	+	+	–	+	+	+	–	–	+	–	–	M
Candida kefyr	+	–	+	+	+	+	–	+	–	+	–	–	SP
Candida parapsilosis	+	+	+	–	–	+	+	–	–	+	–	–	RP
Candida guilliermondii	+	+	+	–	+	+	+	+	+	+	–	+	PsP
Candida krusei	+	–	–	–	–	–	–	–	–	+	–	–	DP
Candida glabrata	+	–	–	–	–	–	+	–	–	+	–	+	PP

(Cel: cellobiose; Dul: dulcitol; DP: pink and dry; Gal: galactose; Glu: glucose; Ino: inositol; Lac: lactose; M: maroon; Mal: maltose; Mel: melibiose; OP: orange pink; PP: pale pink; PsP: pink and pasty; Raf: raffinose; RP: rose pink; SP: Salmon pink; Suc: sucrose; Tre: trehalose; TRM: tetrazolium reduction medium; Xyl: xylose; +: assimilation positive; - : assimilation negative)

on enriched media as blood agar which are commonly called "feet" which develops at the border of colony.

- *Microscopic morphology*: Yeast cells are round to oval (3.5 × 4–8 µm); pseudohyphae and true hyphae may be present. The germ tube test is positive.
- *Growth pattern on CMA*: Different growth patterns are shown in **Figures 20.3 and 20.4**. Pseudohyphae and (sometimes) real hyphae, along with clusters of spherical blastoconidia (yeast cells) spaced regularly at septa, provide a "spidery" appearance when cultured at 25°C for 72 hours. At the end of hyphae or short lateral branches, the majority of isolates produce terminal thick chlamydoconidia, which are asexual spores with thick walls. Sometimes, these conidia are produced in clusters similar to *C. dubliniensis*. Hence, one cannot rely on these findings. It is observed that in the case of *C. albicans*, chlamydoconidia are produced at the sites where microaerophilic conditions are available. Because microaerophilic conditions are created around the edge of the coverslip placed over CMA, it increases chlamydoconidium production; CMA plates examined under a low-power microscope are likely to show these features. Chlamydoconidium is produced in different areas below the coverslip by

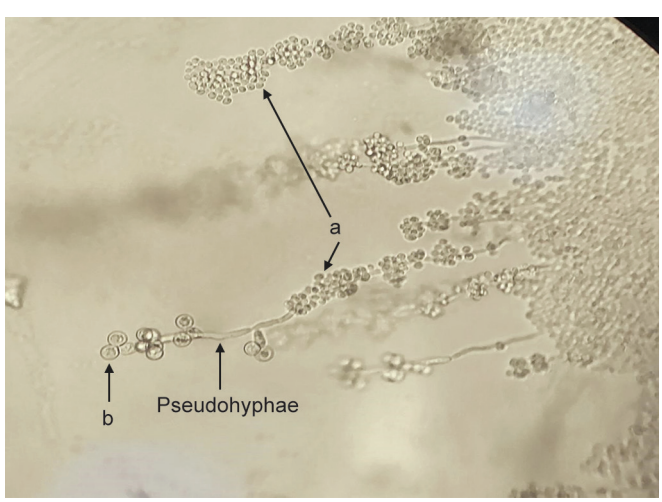

FIG. 20.3: Growth of *Candida albicans* on CMA. (a) Clusters of blastoconidia. (b) Terminal chlamydoconidia (under low power). (CMA: cornmeal agar)

C. dubliniensis. The location of chlamydoconidia may be terminal or intercalary (within hyphae). When Tween 80 is added to corn meal agar it enhances production of chlamydoconidia. **Figures 20.3 and 20.4** show morphological features of *C. albicans* on

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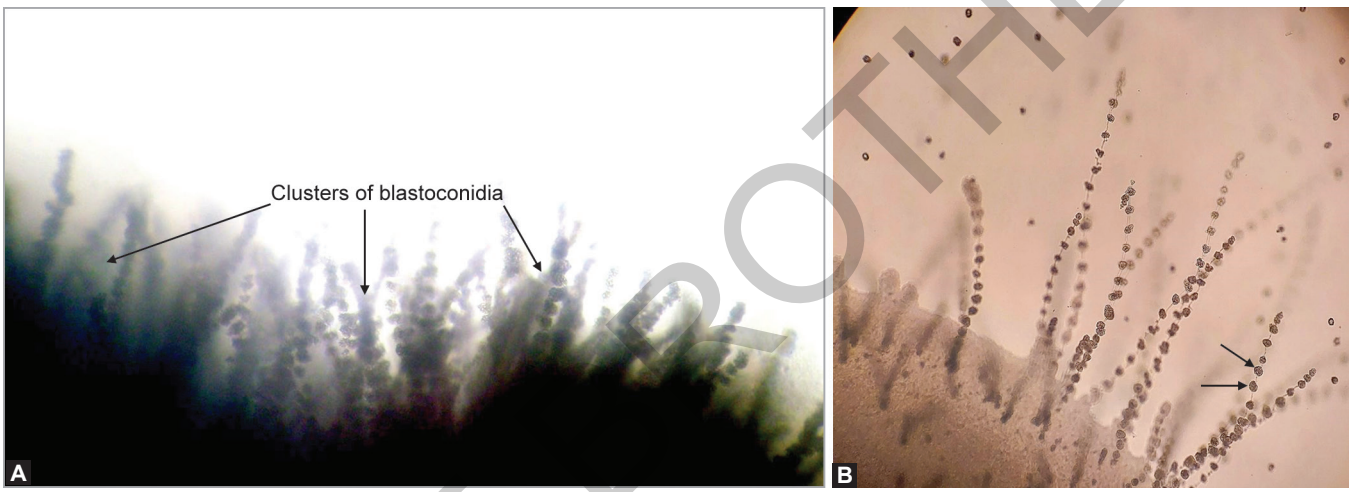
CMA. **Figure 20.5** shows abundant production of chlamydoconidia by *C. dubliniensis* on CMA.

Candida tropicalis

- *Colony morphology*: Rate of growth is rapid, the colony matures in 3 days. Colonies are cream colored, wrinkled near the edge. They may have "mycelial fringe."
- *Microscopic morphology*: Yeast cells are round to oval (3.5–7.0 × 5.5–10.0 µm). The germ tube test is negative.
- CMA (Dalmau plate) at 25°C for 72 hours shows blastoconidia singly or in very small groups all along "long graceful" pseudohyphae **(Fig. 20.6)** (*C. parapsilosis* has short pseudohyphae). A few teardrop-shaped chlamydoconidia may be seen in some isolates only. Their wall is thin as compared to those produced by *C. albicans*.
- *CHROMagar*: Blue-colored colonies are produced.
- *Growth in SABI broth*: *C. tropicalis* produces surface pellicle in SABI broth **(Fig. 20.7)**. This test may be used to differentiate it from *C. parapsilosis*.



FIGS. 20.4A AND B: One more pattern of *Candida albicans* on CMA (low power). Clusters of blastoconidia at regular intervals giving "spidery appearance".
(CMA: cornmeal agar)

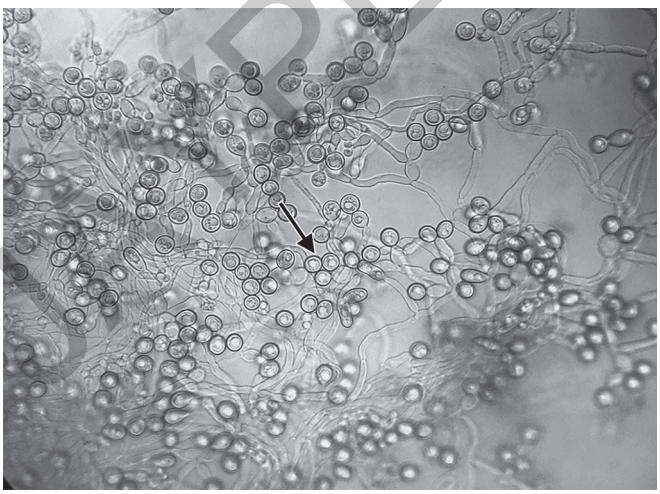


FIG. 20.5: Growth of *Candida dubliniensis* on CMA showing clusters of chlamydoconidia.
(CMA: cornmeal agar)

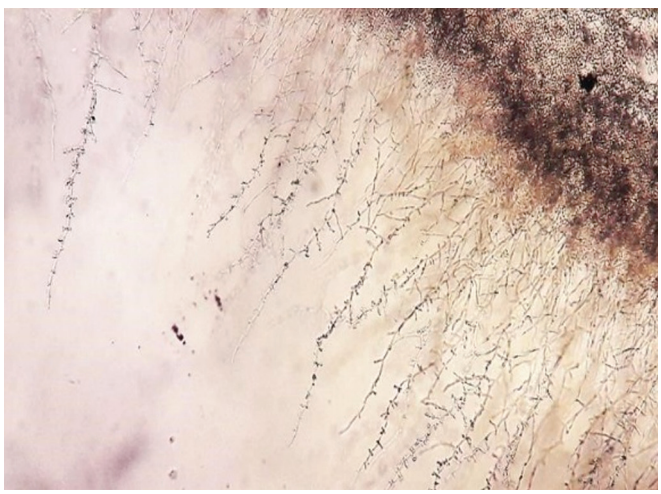


FIG. 20.6: Growth pattern of *Candida tropicalis* on CMA (100×) low power.
(CMA: cornmeal agar)

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Candida parapsilosis

- *Colony morphology on SDA*: Rate of growth is rapid, the colony matures in 3 days.

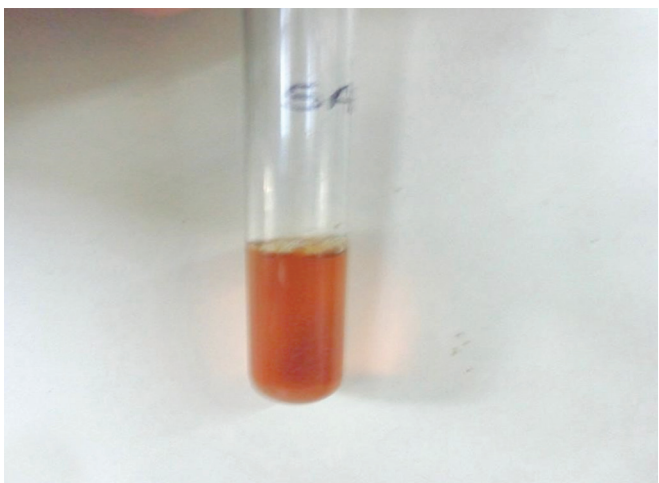
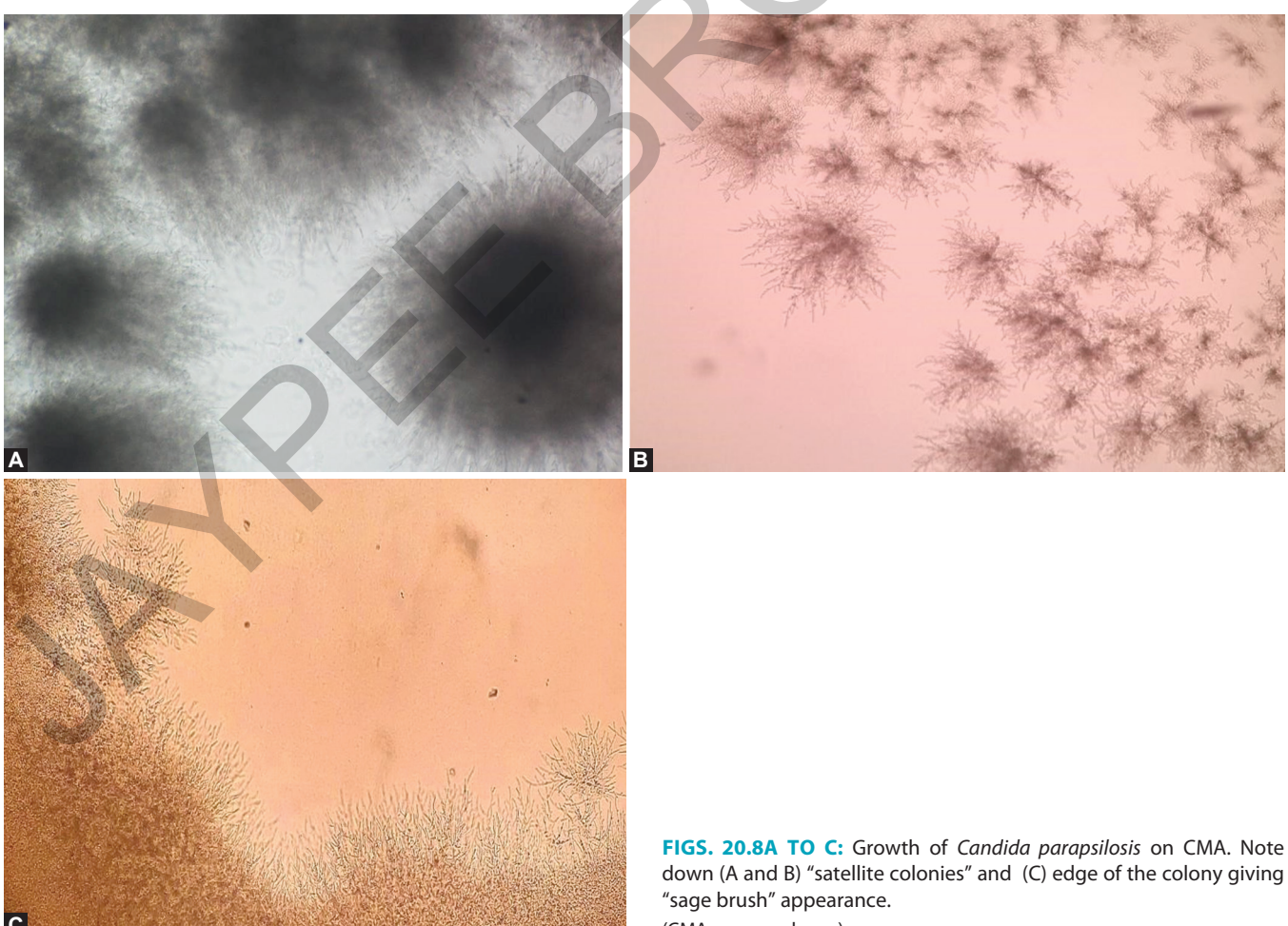


FIG. 20.7: Growth of *Candida tropicalis* in SABI broth.

- Colonies are creamy; sometimes, they show mycelia fringe.
- *Microscopic morphology*: Yeast cells are round to oval, size varying 3–4 × 5–8 µm.
- *CMA*: It shows blastoconidia, single or small clusters along the pseudohyphae. Important characteristics include "sage brush" appearance and some "satellite colonies" away from line of inoculation **(Figs. 20.8A to C)**. Pseudohyphae are relatively short (compared to *C. tropicalis*), crooked or curved, and occasional presence of large hyphal elements called giant cells.

Candida krusei

- *Colony morphology on SDA*: Rate of growth is rapid, and colonies mature in 3 days. Colonies are dry, dull, flat, and creamy to slight tan colored with hyphal fringe.
- *Microscopic morphology*: Yeast cells are oval, size varying 2–6 × 4–10 µm, with single terminal budding.
- *CMA* shows blastoconidia, and pseudohyphae are often present with robust, cells arranged parallel to the main axis. Pseudohyphae with elongated



FIGS. 20.8A TO C: Growth of *Candida parapsilosis* on CMA. Note down (A and B) "satellite colonies" and (C) edge of the colony giving "sage brush" appearance.
(CMA: cornmeal agar)

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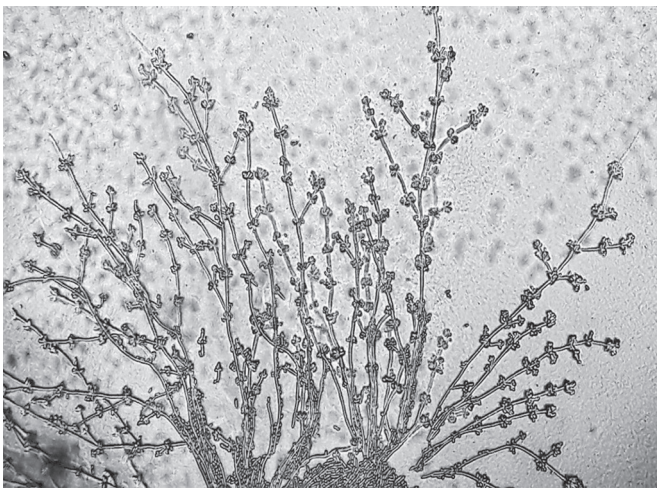

FIG. 20.9: Growth pattern of *Candida krusei* on CMA (low power).
(CMA: cornmeal agar)

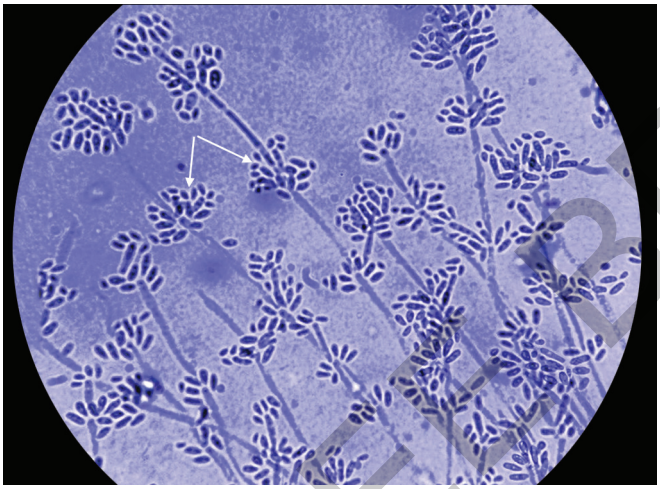

FIG. 20.10: Growth of *Candida krusei* on CMA (high power). Arrangement of pseudohyphae in various directions giving "cross matchsticks" appearance.
(CMA: cornmeal agar)

blastoconidia give "cross matchstick" or (tree)-like appearance **(Figs. 20.9 and 20.10)**. This may be confused with *Blastoschizomyces capitis*. *B. capitis* shows growth in the presence of cycloheximide, assimilates galactose, and shows anneloconidia which are absent in *C. krusei*.

- *Growth in SABI broth*: There is formation of surface pellicle **(Fig. 20.11)** which also extends along the side of tube.

Candida glabrata

- *Colony morphology on SDA*: Rate of growth is rapid; colonies mature in 3–5 days.

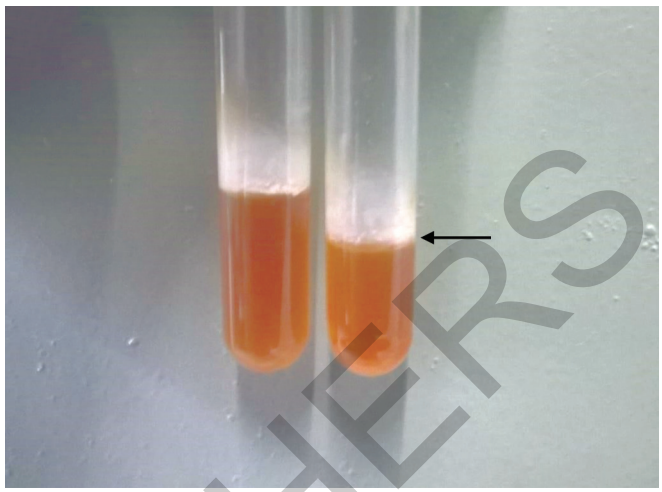

FIG. 20.11: Growth of *Candida krusei* in SABI broth showing surface pellicle.

- Colonies are pastry cream to white smooth.
- *Microscopic morphology*: Yeast cells are small, size varying 2–3 × 3.5–4.5 µm, oval with single terminal budding.
- *CMA*: It shows blastoconidia only, and no pseudohyphae are formed. Short chains of blastoconidia may be formed which may be confused with pseudohyphae. Yeast cells (blastoconidia) on CMA are closely arranged in contrast to that of *Cryptococcus* in which because of capsule produced by each cell, yeast cells show spaces in between. As there are other *Candida* species such as *C. famata* and *C. auris*, one cannot only rely on findings on CMA for identification of *C. glabrata* (*C. glabrata* gives *rapid trehalose* test positive).

Candida guilliermondii

- *Colony morphology on SDA*: Rate of growth is rapid, and colonies mature in 3 days. Colonies are flat, glossy, smooth, and creamy to tan color which may turn slightly pinkish with age.
- *Microscopic morphology*: Yeast cells have size varying 2–5 × 3.3–12 µm, oval or elongate.
- *CMA*: It shows small groups of blastoconidia at septa of pseudohyphae which are few and short.

Candida kefyr

- *Colony morphology on SDA*: It has rapid rate of growth; colonies mature in 3 days and round in shape. Colony is cream-colored smooth.
- *Microscopic morphology*: Round-to-oval yeast cells of size 3–8 × 5–12 µm are present.

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- *CMA*: Pseudohyphae with elongate blastoconidia that line up parallel giving "logs in stream appearance."

Candida lypolytica

- *Colony morphology on SDA*: Growth matures in 5 days; the colonies are creamy and smooth.
- *Microscopic morphology*: Round-to-oval yeast cells of size 3–6 × 4–10 µm are produced.
- *CMA*: It shows pseudohyphae and a few true hyphae, bear elongate blastoconidia short-chain forms, and produce a stark, branching appearance. It differs from *C. krusei* by no fermentation of xylose.

Candida dubliniensis

- *Colony morphology on SDA*: It is similar to that of *C. albicans*. Rate of growth is rapid; colonies mature in 3 days.
- *Microscopic morphology*: It is very similar to *C. albicans*.
- *CMA*: It shows pseudohyphae with regular cluster of blastoconidia at regular intervals at septa with large chlamydospores in pairs and clusters, at terminus as well as "intercalary" (within hyphae) **(Fig. 20.5)**. *C. albicans* produces chlamydoconidia on CMA near the edge of the coverslip where microaerophilic conditions are present, while *C. dubliniensis* produces these conidia on all of the area below coverslip as well as edge.
- Differences in colony morphology from *C. albicans* are observed on tobacco agar and Staib agar. *C. dubliniensis* colonies show peripheral fringe and produce large number of *chlamydoconidia.*
- It was first isolated from an HIV patient in Dublin (Ireland), now present worldwide.
- It causes oropharyngeal candidiasis, vaginitis, and BSIs.
- It can rapidly develop resistance to fluconazole.

The differences between *C. albicans* and *C. dubliniensis* are given in **Tables 20.4 and 20.5**.

TABLE 20.4: Differences between *Candida albicans* and *Candida dubliniensis*.

Candida albicans	*Candida dubliniensis*
• Light green or bluish green colonies on CHROMagar • Chlamydospores on appropriate media are arranged singly, occasionally in pairs, and rarely clusters • Colonies on Staib agar are smooth and shiny • Assimilation of xylose • *Growth at 45°C*: Present	• Dark green color on CHROMagar • Chlamydospores usually plenty, intercalary as well as terminal, and present in pairs or clusters • Rough colony on Staib agar with hyphal fringe • No assimilation of xylose • *Growth at 45°C*: Absent

TABLE 20.5: Other differences between Candida albicans and Candida dubliniensis.

Characteristics	*Candida albicans*	*Candida dubliniensis*
Biofilm formation	Strong	Less effective
Virulence factors	Adherence, proteases, and filamentous transition	Similar but less pathogenic
Antifungal susceptibility	Susceptible to azoles, echinocandins, and polyenes	Similar but may vary slightly

Candida lusitaniae

Yeast and pseudohyphae are produced, including distinctly curved pseudohyphae, on CMA. It causes infections in patients with hematological malignancies or in stem cell transplant recipients. *Candida lusitaniae* can develop secondary resistance to amphotericin B (AmB).

Candida pelliculosa

Candida pelliculosa (teleomorph—*P. anomala*) is an uncommon cause of neonatal septicemia. *P. anomala* is a free-living yeast found in plants, fruits, and soil and is associated with food and its products, either as a production organism or as a spoilage yeast. The ability to grow in preserved food and feed environments is due to its capacity to grow under low pH, high osmotic pressure, and low oxygen tension. The sexual stage includes the production of hat-shaped ascospores. Budding cells are spherical or elliptical, and pseudohyphae may be present. This organism has potential to cause outbreaks of fungemia in neonatal intensive care unit (NICU).

The *C. haemulonii* complex includes *C. haemulonii sensu stricto, Candida duobushaemulonii, and C. haemulonii var. vulnera.* There is a concern because of the intrinsic antifungal resistance to AmB, azoles, and echinocandins.

Candida pseudohaemulonii has been isolated from BSIs, and molecular studies showed a close phylogenetic relationship with *C. haemulonii* complex. *C. pseudohaemulonii* can be distinguished from this species by the assimilation pattern of esculin, melibiose, L-rhamnose, trehalose, and glycerol.

The clinical manifestations of infections caused by *C. haemulonii* complex members are similar to those observed in the cases caused by *C. albicans* and other nonalbicans *Candida* species.

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Fungemia, onychomycosis, peritonitis, leukocytosis, and high fever are features of systemic candidiasis caused by *C. haemulonii sensu stricto*.

Candida duobushaemulonii causes onychomycosis and vulvovaginal candidiasis. *C. haemulonii var. vulnera* causes onychomycosis, lower extremity ulcers, and fungemia.

Candida auris

Candida auris has emerged globally as a multidrug-resistant, healthcare-associated fungal pathogen, presenting challenges in organism identification, antifungal resistance, and high patient mortality. Its ability to colonize the skin and persist in the environment promotes transmission within and between healthcare facilities, a feature unique among *Candida* species. *C. auris*, first identified in East Asia in 2009, has since been isolated on five continents and is notable for its multidrug resistance and treatment challenges. Unlike other *Candida* species, *C. auris* spreads within healthcare settings, contributing to BSIs with high mortality rates (30–60%).

Candida auris was first isolated from the ear canal of a patient in Japan in 2009 and soon after reported in South Korea. Retrospective reviews have shown that the earliest isolates date back to 1996. Since its discovery, *C. auris* has been reported in countries, including India, South Africa, the United States, and the United Kingdom. Clinical isolates have been found in various body fluids and tissues, with BSIs being the most common invasive infection. Most cases occur in patients with significant healthcare exposure and underlying comorbidities.

Risk Factors for Candida auris

BSIs include prolonged intensive care unit (ICU) stays, respiratory illnesses, vascular surgery, and prior antifungal use. Clonal transmission within healthcare facilities is common.

Microbiology and Taxonomy

Candida auris has been phylogenetically linked to the *Clavispora/Candida* clade, closely related to *C. haemulonii* and *C. pseudohaemulonii*. Its genetic sequences, particularly from the 26S D1/D2 and 18S ITS regions, demonstrate this relationship. Studies continue to explore its virulence, antifungal resistance profiles, and potential relatedness across strains.

Virulence Factors

Several studies have investigated the virulence factors of *C. auris*, which include germination, adherence, biofilm formation, and the production of enzymes such as phospholipases and proteinases. While *C. auris* does not form germ tubes, pseudohyphae, or chlamydospores in vitro, it may produce phospholipase and proteinase in a strain-dependent manner. *C. auris* can adhere to plastics and form biofilms, but these capabilities are notably weaker compared to *C. albicans*. Interestingly, some strains of *C. auris* form aggregates that resist disruption in vitro, which contrasts with nonaggregating strains.

Identification in the Clinical Laboratory

Identifying *C. auris* in clinical settings is crucial, especially when isolates are from sterile body sites, as antifungal susceptibility can vary significantly by species.

Phenotypic Characteristics

Candida auris appears as nondescript white-to-cream-colored colonies on SDA and pink to beige on CHROMagar. The yeast forms ovoid to elongated budding cells but does not produce pseudohyphae on CMA or rice Tween 80 agar. It grows well at both 37 and 42°C and assimilates sugars such as N-acetylglucosamine, succinate, and gluconate. These traits distinguish *C. auris* from closely related species such as *C. haemulonii*.

Biochemical Methods

Misidentifications of *C. auris* as other *Candida* species, especially *C. haemulonii*, have been common due to limitations in commercial biochemical testing databases.

Possible misidentifications based on various biochemical methods are as follows:

- *API 20C AUX*: *Candida sake* and *Rhodotorula glutinis*
- *BD Phoenix*: *Candida catenulata*
- *Vitek 2*: *C. duobushaemulonii* and *C. famata*

MALDI-TOF MS

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) is an accurate and rapid method for identifying *C. auris* from pure cultures. However, the current Food and Drug Administration (FDA)-cleared libraries for commercial MALDI-TOF systems, such as the Bruker Biotyper and Vitek MS, do not include *C. auris*.

Molecular Methods

Molecular methods, such as rDNA sequencing of the 28S D1/D2 or ITS 1/2 regions, can accurately identify *C. auris*. Polymerase chain reaction (PCR)-based assays have also

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been developed. One real-time PCR assay designed by Kordalewska et al. can differentiate *C. auris* from related species such as *C. haemulonii* and *C. lusitaniae*.

Phenotypic Susceptibility

There are no species-specific antifungal susceptibility breakpoints for *C. auris* as of yet. However, *C. auris* isolates typically show high resistance to fluconazole, with minimum inhibitory concentrations (MICs) of ≥16 µg/mL and variable resistance to other azoles, amphotericin, and echinocandins. Posaconazole and isavuconazole have shown the most potency in vitro. However, studies may be skewed by clonal isolates, and false MIC elevations have been reported when using the Vitek 2 susceptibility testing method.

One more effective way to differentiate *C. auris* isolates from other *Candida* species is by growing them at 40–42°C because only *C. auris* can grow at this temperature.

Another form of differentiation is taking advantage of its ability to tolerate high salt concentrations. A culture in broth containing yeast nitrogen base, 10% sodium chloride (NaCl) supplemented with dextrose, dulcitol, or mannitol, can differentiate *C. auris* from *C. haemulonii sensu stricto*, *C. duobushaemulonii*, *C. albicans*, and *C. parapsilosis*, which cannot grow under these conditions.

New CHROMagar *Candida* Plus medium showed better performance in the *C. auris* identification, although *C. pseudohaemulonii* resembles *C. auris* in mixed culture colonies; it tends to be much smaller and produces a distinctive blue halo after 72 hours. This makes the misidentification of *C. pseudohaemulonii* with *C. auris* less likely when found in clinical specimens.

RAPID IDENTIFICATION SYSTEMS

API 20C Aux System

API 20C Aux system is a process based on the assimilation of carbohydrates. It entails testing the assimilation of 19 carbohydrates in cupules that are cultured at 30°C for 24–72 hours. Turbidity analysis via computer programs is used for identification. This technique reliably distinguishes between various *Candida* species, including *C. dubliniensis* and *C. albicans*.

API Candida System

API *Candida* system, a 10-tube test, was able to identify five carbohydrates using a battery of acidity and enzymatic assays. Sugar acidification and enzyme activity form the basis of the test's principle. Visual observations of changes in color and morphological features allow for interpretation.

Fluorogenic Membrane Filtration Method

This is more accurate than the CHROMagar method. The enzymatic reaction is detected by passing fluorogenic substrates through a nylon membrane filter. It is helpful for identification of *C. albicans*, *C. krusei*, *C. tropicalis*, and *C. glabrata*.

Fungichrom I and Fungi-Fast I Twin Systems

In contrast to the fungi-fast twin system, which has 10 test cupules, the Fungichrom I twin system has 16 test cupules (International Microbio, Parc D'activitésallee D'athenes, France). After being incubated at 30°C for 24–48 hours, the samples are checked for any changes in color.

Vitek

The main concern is that *C. auris* could be incorrectly diagnosed because these tests require distinct fungal colonies and an incubation time of 2–3 days.

MALDI-TOF MS

It is useful for accurate identification of *Candida* species. Even identification of newer species such as *C. auris* is possible.

MOLECULAR METHODS

DNA-based Methods

Various conventional techniques have been utilized for studying the genome of *Candida* species. These include pulsed-field gel electrophoresis (PFGE), restriction enzyme analysis (REA), restriction fragment length polymorphism (RFLP), and amplified fragment length polymorphism (AFLP).

Polymerase chain reaction and its modifications (nested PCR and real-time PCR) that can enhance precise identification and quantification of the *Candida* are available. Microarray is also available now. Nucleic acid sequence-based amplification (NASBA) and peptide nucleic acid-fluorescence in situ hybridization (PNA-FISH) have also been utilized for *Candida*.

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Serological Tests for Invasive Candidiasis

- *Antibody*: There are a number of methods for detecting antibodies, including immunodiffusion, immunoprecipitation, immunofluorescence, and slide agglutination.
- *Antigen*: Cell wall components such as cell wall mannoprotein (CWMP), beta-(1,3)-D- glucan is the target and detected by different tests.
- *Candida* mannan and anti-mannan antibody test. This test detects circulating immune complexes (IC) in patients with invasive candidiasis

Candida Enolase Antigen Testing

The major drawback of this method is that beta-D-glucan can be found in other types of fungi, such as *Aspergillus* species and *Pneumocystis jirovecii*, which makes it questionable to use it for diagnosing *Candida* species infections. Still, this test has high negative predictive value that helps to get rid of invasive candidiasis.

- *The Platelia® Candida antigen sandwich EIA* (Bio-Rad Laboratories, Marnes-la-Coquette, France): It can identify *Candida* species mannan using monoclonal antibodies (mAbs). A positive result from the enzyme immunoassay (EIA) is indicated when two separate blood samples show ≥0.5 ng of mannan per milliliter. There will likely be more testing of the test in future, and the inclusion of more specific mAbs might increase the number of species identified.
- *LightCycler® SeptiFast Test* (Roche Diagnostics): From 3 mL of patient blood collected over the course of 6 hours, this test can identify 25 different pathogens in the bloodstream, including five different species of *Candida* (*C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *and C. krusei*) and *Aspergillus fumigatus*.
- *T2 Candida panel* (T2 Biosystems, Lexington, Massachusetts, United States): It is useful for the diagnosis of candidemia and the detection of *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* in whole blood.

T2 *Candida* is a nonculture-based test. The completely automated T2Dx device is used to collect whole blood samples, which are placed in K2 ethylenediaminetetraacetic acid (EDTA) tubes. It uses a thermostable DNA polymerase and primers for the *Candida* ribosomal DNA operon to amplify the DNA of the lysed *Candida* cells, which were obtained by mechanically beating the cells using beads. The amplicon-induced agglomeration of supermagnetic particles and T2 magnetic resonance (T2MR) measurement are used to detect the amplified *Candida* DNA product.

Each specimen undergoes processing with an internal check to ensure that the results are accurate. The typical turnaround time of the T2 *Candida* panel is <5 hours, and it can detect as few as 1 colony-forming unit (CFU)/mL of whole blood.

Growth in Sabouraud broth: This test is useful for identifying distinct species. After 2 days of incubation at 25°C, *C. tropicalis* in Sabouraud broth generates a thin surface pellicle. On the underside of the tube, *C. krusei* forms broad surface layers. Surface pellicle is not produced by *C. albicans*.

Sugar assimilation pattern: It is a useful and reliable method for the identification of *Candida* species **Figure 20.12** shows the assimilation pattern of tested sugars on yeast nitrogen base agar.

Urease test: *C. krusei* gives this test positive, but it may show strain variation.

Pal's medium (sunflower seed agar) possibly differentiates *C. albicans* from *C. dubliniensis*. Colonies of both species will develop into smooth, creamy-gray colonies after 48–72 hours of development on this medium. On the other hand, a hyphal fringe encircling the colonies is a hallmark of *C. dubliniensis* isolates. Because other *Candida* species (*C. tropicalis*, *C. parapsilosis*, and *C. krusei*) can also produce a fringe on Pal's agar, it may be prudent to restrict its use to germ tube positive and/or chlamydospore positive isolates.

Whenever any yeast is isolated from clinical specimens, **Flowchart 20.1** is followed.

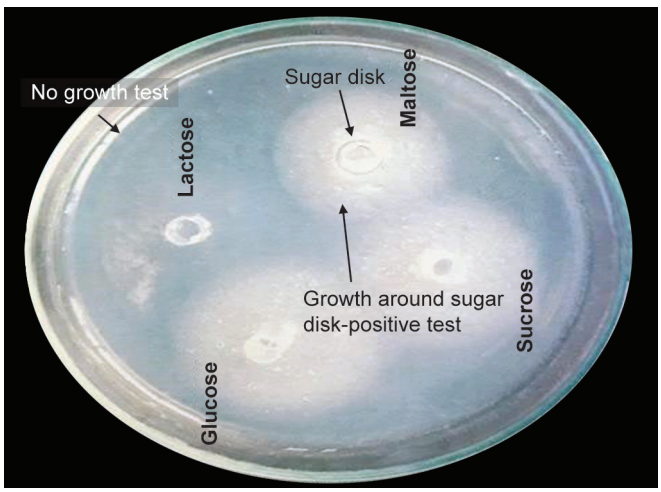

FIG. 20.12: Sugar assimilation test (growth around sugar indicates that sugar is assimilated).

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TREATMENT

Candida infections are treated with antifungal medications such as nystatin, clotrimazole, AmB, and miconazole.

Mild or moderate genital *Candida* infections can be treated with antifungal vaginal cream (1, 3, 7 day treatment) or econazole or fluconazole 150 mg orally one-time dose. For recurrent vaginal infections, fluconazole is given on days 1, 4, and 7 and then weekly for 6 months. This treatment can be used for oral thrush. Even oral lozenges act as a substitute form. For oral and mucocutaneous infections, 1% gentian violet is locally applied. Nystatin can be used for resistant mucosal lesions as suspension containing 200,000 units/mL. Systemic candidiasis requires treatment with oral or intravenous (IV) antifungal medications, including caspofungin, fluconazole, and AmB.

For systemic infections:

- AmB—IV is used for 6–10 weeks.
- 5-fluorocytosine can be added to it for disseminated candidiasis.
- Triazoles such as fluconazole, itraconazole, imidazole, and ketoconazole can also be used.

Fluconazole-resistant Species

Disseminated infections are the primary source of fluconazole-resistant species, including *C. tropicalis*, *C. krusei*, and *C. glabrata*. One potential treatment for fluconazole resistance is voriconazole.

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- Classification and taxonomy of fungi with clinical relevance are given.
- There is detailed discussion of fungal physiology, morphology, genetics, pathogenesis, and virulence mechanisms of fungi.
- Detailed description of fungal infections (superficial, subcutaneous, systemic, opportunistic) is given and diagnostic methods are discussed in details. These include conventional methods as well as recent advances.
- Separate annexures are attached covering topics such as staining methods, culture media, procedures, and antifungal susceptibility testing.
- Diagnostic algorithms and flowcharts are given for quick reference.
- Nosocomial fungal infections and infection-control protocols are given.
- Updates on emerging fungi, climate-linked mycoses, and zoonotic fungal infections are included.
- All the topics of postgraduate MMC curriculum are covered.
- Tables, flowcharts, and high-resolution images have been included, which make morphological diagnosis easy.
- Few fungi although shifted to parasitology are also discussed. New fungi (which were previously considered as parasites) have been added.

Vasant Baradkar is presently working as Additional Professor at Seth GS Medical College and KEM Hospital, Mumbai, Maharashtra, India. He has 26 years of teaching experience. He has 70 publications to his credit. He is the author of Textbook of Microbiology (CBS Publishers, 2017).

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