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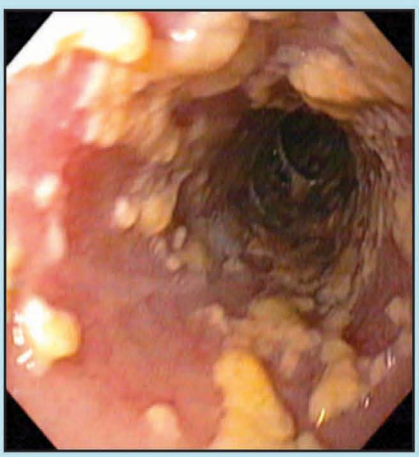
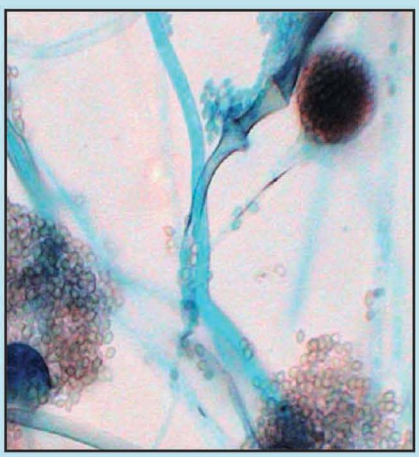
SYSTEMIC FUNGAL INFECTIONS

Principles, Pathogenesis & Practice







Editor
Monica Mahajan

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Contents

Section 1: Introduction

1. Introduction to Mycology 3
Monica Mahajan

• Structure of Fungi *3* • Growth *4* • Reproduction *4* • Nutrition *5*
• Classification of Fungi *6* • Classification of Mycoses on the Basis of Site of Infection *7* • Fungal Dimorphism *9*

Section 2: Diagnosis of Fungal Infections

2. Diagnosis of Fungal Infection 13
Monica Mahajan

• Specimens for Diagnosis *13* • Methods of Fungal Isolation *17*

3. Conventional Methods for Direct Examination of Fungi 19
Monica Mahajan

• Presumptive Identification of Fungi by Direct Microscopic Examination *23*
• Histopathology *23*

4. Fungal Culture 25
Monica Mahajan, Bansidhar Tarai, Pragnya P Jena

• Fungal Growth Requirements *25* • Fungal Culture Media *26* • Characteristics for Classification of Fungi Based on Conventional Methods *27* • Identification of Fungi *27* • Microscopic Examination *29* • Biochemical Studies *29* • Special Tests *30* • MALDI-TOF *30* • Automated Fungal Cultures *30* • Interpretation of Positive Fungal Cultures *31*

5. Serodiagnosis in Mycology 32
Monica Mahajan, Bansidhar Tarai, Pragnya P Jena

• Antibody Testing for Fungal Infections *34* • Antigen Testing for Fungal Infections *35* • Pan-Fungal Detection of β-D-Glucan *35* • Specific Serodiagnostic Tests *36*

6. Molecular Techniques for Diagnosis of Systemic Fungal Infections 43
Monica Mahajan, Bansidhar Tarai, Pragnya P Jena

• Nucleic Acid Detection *43* • Other Techniques *50* • Practical Utilization of Molecular Techniques *51*

7. Radiodiagnosis of Systemic Fungal Infections: An Organ-based Approach 54
Monica Mahajan, Anandamoyee Dhar
• Central Nervous System Manifestations *55* • Sinus Manifestations *57* • Pulmonary Infections *58* • Bone and Joint Infections *64* • Cardiac and Pericardial Infection *66* • Gastrointestinal Infections *67* • Genitourinary Infections *68* • Clinical Pearls *69*

Section 3: Antifungal Drugs

8. Antifungal Agents: Classification 73
Monica Mahajan
• Milestones in the Development of Antifungal Therapy *73* • Classification of Antifungal Agents *73*

9. Azoles.....76
Monica Mahajan
• Mechanism of Action *76* • Microbiological Activity *77* • Pharmacokinetics *78* • Adverse Effects of Azoles *81* • Dosing *83* • Serum Drug Concentration Monitoring *86* • Drug Interactions *86* • Clinical Pearls *87*

10. Amphotericin B 89
Monica Mahajan
• Mechanism of Action *89* • Spectrum of Activity *90* • Amphotericin B Resistance *90* • Formulations of Amphotericin B *90* • Amphotericin B Deoxycholate *90* • Lipid-associated Formulations of Amphotericin B *93* • Liposomal Amphotericin B *93* • Amphotericin B Lipid Complex *95* • Amphotericin B Colloidal Dispersion *95* • Comparative Efficacy *95* • Clinical Pearls *96*

11. Echinocandins.....98
Monica Mahajan
• Mechanism of Action *98* • Spectrum of Activity *99* • Echinocandin Resistance *100* • Pharmacokinetics *101* • Dosage *102* • Adverse Effects *103* • Drug Interactions *103* • Formulations *104*

12. Flucytosine 105
Monica Mahajan
• Mechanism of Action *105* • Spectrum of Activity *106* • Flucytosine Resistance *106* • Pharmacokinetics *106* • Dosage *107* • Adverse Effects *107* • Drug Interactions *108* • Formulations *108*

13. Antifungal Susceptibility Testing 110
Monica Mahajan

• Definitions *110* • Indications for Susceptibility Testing of Antifungals *111* • Intrinsic Resistance *111* • Methods of Susceptibility Testing *112* • Standardized Methods *112* • Commercial Minimum Inhibitory Concentration Techniques *114* • Interpretation of Minimum Inhibitory Concentration *116*

14. Antifungal Resistance 119
Monica Mahajan

• Assessment of Antifungal Resistance *120* • Mechanisms for Drug Resistance *120* • Azole Resistance *121* • Mechanisms of Echinocandin Resistance *125* • Polyene Resistance *126* • Flucytosine Resistance *127* • Antifungal Stewardship *127*

Section 4: Specific Fungal Infections

15. Candidiasis 133
Monica Mahajan, Rajeev Upreti

• Morphology *133* • Transmission *133* • Virulence Factor for *Candida* *134* • Predisposing Factors for Candidal Infections in Critical Care Patients *136* • Clinical Manifestations of Candidiasis *137* • Chronic Disseminated Candidiasis/Hepatosplenic Candidiasis *145* • Diagnoses of Candidiasis *153* • Treatment and Management *157* • Mortality *168*

16. Cryptococcosis 170
Monica Mahajan

• Taxonomy *170* • Morphology *171* • Life Cycle *171* • Epidemiology *171* • Predisposing Factors *172* • Cryptococcal Virulence *172* • Pathogenesis *174* • Clinical Features *174* • Diagnosis *175* • Differential Diagnoses *178* • Management *180* • Clinical Pearls *187*

17. Aspergillosis 189
Monica Mahajan

• Taxonomy *189* • Pathogenesis *190* • Risk Factors for Invasive Aspergillosis *190* • Clinical Manifestations *191* • Differential Diagnoses *199* • Diagnosis *200* • Prophylaxis *206* • Treatment *207* • Clinical Pearls *213*

18. Mucormycosis 215
Monica Mahajan

• Taxonomy *215* • Epidemiology *215* • Risk Factors *216* • Pathophysiology *217* • Clinical Features *217* • Differential Diagnoses *220* • Diagnostic Workup *220* • Treatment of Mucormycosis *223* • Prevention *226* • Prophylaxis *227* • Prognosis *227*

19. *Pneumocystis Jirovecii* Pneumonia.....229
Monica Mahajan
• Taxonomy *229* • Life Cycle *229* • Transmission *230* • Pathophysiology *230* • Susceptible Patient Population *230* • Epidemiology *230* • Clinical Features *231* • Differential Diagnoses *231* • Investigations *232* • Treatment *235* • Prognosis *241*

20. Histoplasmosis 243
Monica Mahajan
• Morphology *243* • Epidemiology *243* • Predisposing Factors *244* • Pathophysiology *244* • Clinical Manifestations *244* • Differential Diagnoses *246* • Management of Histoplasmosis *246*

21. Blastomycosis.....254
Monica Mahajan
• Etiology *254* • Epidemiology *255* • Pathophysiology *255* • Clinical Features *255* • Differential Diagnoses *256* • Diagnosis *257* • Treatment *258* • Treatment of Central Nervous System Blastomycosis *259* • Prognosis *259*

22. Coccidioidomycosis 261
Monica Mahajan
• Etiology *261* • Life Cycle *261* • Epidemiology *262* • Pathophysiology *262* • Predisposing Factors *262* • Clinical Features *262* • Differential Diagnoses *264* • Diagnosis *264* • Treatment *266* • Complications *268*

23. Paracoccidioidomycosis.....270
Monica Mahajan
• Etiology *270* • Epidemiology *270* • Pathogenesis *271* • Clinical Features *271* • Differential Diagnoses *272* • Diagnostic Approach *273* • Treatment *274*

24. Mycetoma 276
Monica Mahajan
• Historical Background *276* • Etiology *276* • Epidemiology *276* • Pathogenesis *277* • Clinical Features *277* • Complications *278* • Differential Diagnosis *278* • Diagnosis *279* • Treatment *282* • Clinical Pearls *285*

25. Penicilliosis/Talaromycosis 286
Monica Mahajan
• Etiology *286* • Epidemiology *286* • Pathophysiology *287* • Predisposing Factors *287* • Clinical Manifestations *287* • Differential Diagnoses *287* • Diagnosis *288* • Treatment *289*

26. Chromoblastomycosis 291
Monica Mahajan
• Epidemiology *291* • Etiology *291* • Pathophysiology *291* • Clinical Manifestations *292* • Complications *293* • Differential Diagnoses *293* • Diagnosis *293* • Treatment *295*

27. Sporotrichosis 297
Monica Mahajan
• Etiology *297* • Routes of Transmission *297* • Clinical Manifestations *298*
• Diagnostic Workup *298* • Differential Diagnoses *299* • Treatment *299*
• Clinical Pearls *302*

28. Lobomycosis 303
Monica Mahajan
• Etiology *303* • Pathogenesis *303* • Epidemiology *304* • Clinical Features *304* • Differential Diagnoses *304* • Diagnostic Workup *305*
• Treatment *305*

29. Phaeohyphomycosis 306
Monica Mahajan
• Etiology *306* • Epidemiology *306* • Pathogenesis *306* • Predisposing Factors *308* • Clinical Features *308* • Differential Diagnoses *309*
• Diagnosis *310* • Treatment *311* • Prophylaxis *312*

30. Hyalohyphomycosis 313
Monica Mahajan
• Etiology *313* • Pathogenesis *313* • Fusariosis *313* • Scedosporiosis *321*
• *Paecilomyces* Species *322* • *Acremonium* Species/*Cephalosporium* Species *324*
• *Scopulariopsis 325* • *Trichoderma 326* • *Blastoschizomyces Capitatus 327*

Section 5: Invasive Fungal Infections

31. Invasive Fungal Infections: Definitions 331
(Based on EORTC/MSG Consensus Group Recommendations)
Monica Mahajan
• Invasive Fungal Disease *331* • Proven Invasive Fungal Disease *333*
• Proven IFD: Endemic Dimorphic Fungi *334* • Histopatholgy *334* • Probable Invasive Fungal Disease *335* • Possible Invasive Fungal Disease *335*
• Limitations of Revised Invasive Fungal Disease Definitions *335*

32. Invasive Cutaneous Fungal Infections 337
Sunil Choudhary, Soumya Khanna, Raghav Mantri, Prateek Arora, Rohit Jain, Shaunak Dutta
• Epidemiology *337* • Pathogenesis *338* • Clinical Presentation *339*
• Differential Diagnoses *340* • Laboratory Diagnosis *342* • Management *344*

33. Invasive Fungal Infections in Neutropenic Cancer Patients 350
Pramod Kumar Julka, Shaik Maheboob Hussain, Tarun Mohindra
• Epidemiology *350* • Pathogenesis *350* • Risk Factors and Frequency of Infections *351* • Options for Preventing Invasive Fungal Infection in Cancer Patients *352* • Treatment of Fungal Infections in Neutropenic Cancer Patients *352* • Reconstituting Immune Defense Mechanisms *365*

34. Postrenal Transplant Fungal Infections 367
Dinesh Khullar, Sahil Bagai
• Presentation *368* • Risk Factors *368* • Timing of Infections: Post-transplant *369* • Incidence of Fungal Infection *369* • Invasive Mycoses *369*
• Clinical Pearls *375*

35. Fungal Diseases of the Lungs.....378
Ashish Jain
• Clinical Presentation *378* • *Candida* *379* • *Aspergillus* *379*
• *Pneumocystis Jirovecii* Pneumonia *387* • Diagnosis of Fungal Infections *388*
• Treatment *393*

36. Fungal Infections of Central Nervous System 396
Tanmay Trivedi, Puneet Agarwal
• Epidemiology and Classification *396* • Pathogenesis *397*
• *Candida* Species *397* • *Cryptococcus* Species *398* • *Aspergillus* Species *401*
• *Mucormycosis* Species *402* • *Exserohilum Rostratum* *403*

Section 6: New Frontiers in Mycology

37. Vaccines, Immunotherapies, Nanobiotechnology and Newer Antifungals 409
Monica Mahajan
• Fungal Vaccines—The Unmet Need *410* • Immunotherapy *412*
• Nanobiotechnology *413* • Newer Antifungals *414*

Index.....*419*

4

Fungal Culture

Monica Mahajan, Bansidhar Tarai, Pragnya P Jena

INTRODUCTION

Fungi take more time than bacteria to grow on culture. Since fungal cultures may require incubation periods ranging from days to weeks, the rapidly growing bacteria in the specimen may interfere with the isolation of fungi. Hence, fungal culture media are designed to favor the growth of fungi over bacteria.

The media are either in petri dishes or tubes. The advantages of tubed media over petri dishes are as follows:

- Easier storage and transport
- Less chances of dehydration
- Less chances of release of spores into the environment.

The disadvantage of screw-top tubes is the difficulty in preparing stained mounts for microscopic examination. However, petri dishes provide a larger surface for growth resulting in better separation of colonies. This makes it easier to subculture and to tease mounts or make transparent tape preparations. Petri dishes are more prone to dehydration.

FUNGAL GROWTH REQUIREMENTS

Temperature

Many fungi have an optimum growth at temperatures below 37°C. Therefore, culture tubes (Fig. 4.1) are incubated at two temperatures:

1. One tube at 25°C (room temperature)
2. One tube at 37°C (incubator)

This is essential to study fungal dimorphism. Any fungus that is capable of growth at 37°C is always considered pathogenic. *Nocardia* spp. and some dimorphic fungi grow best at 37°C.

The ability to withstand freezing and thawing or tolerating refrigeration varies among different fungi. *Histoplasma capsulatum* and *Mucorales* are especially susceptible, and can be killed/inhibited on storage of samples at low temperatures.

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Fig. 4.1: Sabouraud dextrose agar (SDA) media for fungal culture.

Atmosphere

True fungi are aerobes, whereas few bacteria-like fungi are anaerobes.

Time

Fungi may require days to weeks of incubation before the initial growth occurs. Cultures should be examined for fungi for at least 4 weeks. Saprophytes grow fastest, and the growth may be visible over a span of days. *Paracoccidioides* brasiliensis grows over 4–5 weeks, and *Histoplasma capsulatum* may require up to 10 weeks for growth.

FUNGAL CULTURE MEDIA (TABLE 4.1)

Table 4.1: Culture media and their characteristics.

Culture media	*Characteristics*
Sabouraud's dextrose agar	Classic media for all fungi, contains glucose and peptones, pH 5.6, insufficiently rich to recover fastidious pathogenic species/dimorphic fungi
Sabouraud's dextrose agar with chloramphenicol	Inhibits bacterial growth; alternatively, streptomycin or penicillin may be used
Mycosel agar	Commercially prepared agar containing chloramphenicol and cycloheximide that inhibits saprophytic fungi and some yeasts including *C. neoformans*
Brain heart infusion (BHI) slant	More enriched than Sabouraud's dextrose, for recovery of *H. capsulatum*
Potato dextrose agar (PDA), corn meal agar	Used in microcultures to induce sporulation; may be too rich for some fungi and cause excessive mycelial growth rather than sporulation
Caffeic acid agar	Photosensitive, protect from light. *C. neoformans* produces black colonies due to melanin

Contd...

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Culture media	*Characteristics*
Bird seed agar	To isolate *C. neoformans* from contaminated cultures
Kelley agar/KT medium	To convert dimorphic fungus *Blastomyces dermatitidis* from mycelial to yeast form
Levine's modified converse liquid medium	To promote spherule production by *Coccidioides immitis*

Antimicrobials including chloramphenicol, streptomycin, gentamycin, and penicillin inhibit bacterial growth, whereas cycloheximide inhibits saprophytic growth. Culture media may be enriched with 5–10% sheep blood to support the growth of certain fungi.

CHARACTERISTICS FOR CLASSIFICATION OF FUNGI BASED ON CONVENTIONAL METHODS

- Colony morphology
- Chlamydospore formation
- Germ tube formation
- Adhesive tape preparation
- Microculture
- *Biochemical features*: Carbohydrate fermentation, carbohydrate assimilation, and urease test.

IDENTIFICATION OF FUNGI

The visible colonies are inspected for their morphology.

Yeasts can be identified on the basis of their growth pattern, color, colony morphology on chromogenic media, and biochemistry testing (Figs. 4.2 and 4.3). Filamentous fungi are

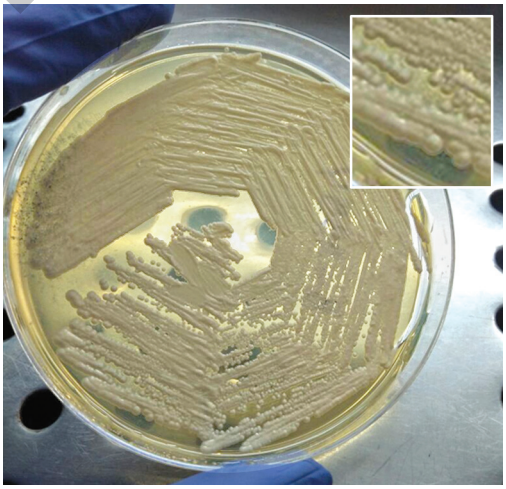

Fig. 4.2: Creamy, smooth, and pasty colony of yeast.

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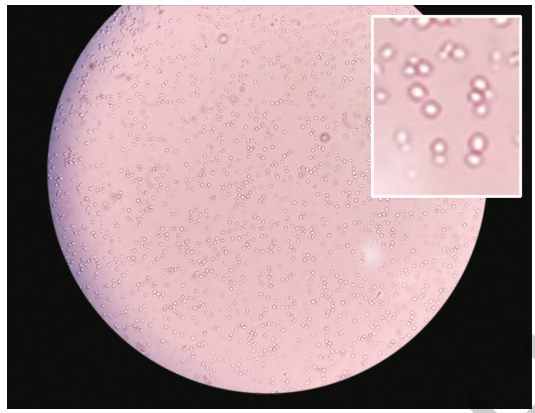

Fig. 4.3: Budding yeast cells on KOH mount.



Fig. 4.4: Cottony growth of mycelium.

more difficult to identify. Without spores, the only real distinction is whether the hyphae are septate or not. Media that assist in sporulation include potato dextrose agar. Once the colony has sporulated, staining with lactophenol cotton blue helps identify the genus.

Colony Morphology

Macroscopic and microscopic feature.

Surface Topography

Free-growing fungi cover the entire surface of agar, whereas other fungi grow in a restricted manner (Fig. 4.4).

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Surface Texture

It may be cottony or woolly (floccose), chalky, granular, powdery, velvety, silky, glabrous, or waxy. Yeast colonies are generally smooth, creamy, viscous, or pasty in appearance. Dimorphic fungi grow in 7–14 days and have a cobweb aerial mycelium.

Pigmentation

Fungi vary from colorless to brightly colored. Color may be visible from the fungus itself, its sporulating apparatus on the agar, or on the bottom of the colony called "reverse pigmentation."

MICROSCOPIC EXAMINATION

Wet Mount-teased Preparation

Adhesive/Scotch Tape Preparation Method

The adhesive side of a small length of transparent tape is touched to the surface of the colony. The tape is adhered to the surface of a microscope slide, stained with lactophenol cotton blue, and observed (Fig. 4.5). The spores remain intact by this technique and their arrangement can be useful in species identification.

BIOCHEMICAL STUDIES

To identify yeast and yeast-like organisms.

Carbohydrate Fermentation

Yeast metabolizes sugars both aerobically and anaerobically. The growth of yeast and utilization of carbohydrate under anaerobic conditions is studied by inoculating the specimen under a broth. The production of acid and gas by the process of fermentation is observed.

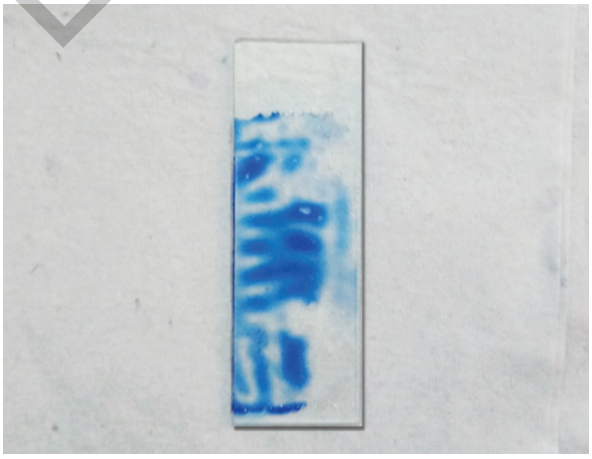

Fig. 4.5: Adhesive tape preparation of mycelium on LPCB (lactophenol cotton blue).

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Carbohydrate Assimilation

It is used for definite species identification of *Candida* and a few other fungi. It determines the ability of a fungus to utilize a carbohydrate (glucose, maltose, lactose, sucrose, etc.) as the only source of carbon. In case of positive test, bromocresol indicator turns from purple to yellow. Tubes unchanged after 10 days are negative.

Nitrogen Assimilation

Bromothymol blue turns yellow on utilization of nitrogen by fungi.

Rapid Urease Test

This test is used to detect the presence of urease enzyme by different *Candida* species. Christensen's urea agar slants are studied for a color change. Urea is hydrolyzed by the yeast to form ammonia, which increases the pH and turns media from yellow to dark pink.

SPECIAL TESTS

- *Germ-tube formation/Reynold–Braude phenomenon*: It is a rapid, efficient, and economic presumptive test for *C. albicans.* True germ tube is defined as hyphal projections from the germinating yeast cell, lacking any constriction at the point of origin from the parent cell. Several colonies of yeast are incubated into a substrate at 37°C. *C. albicans* forms germ tubes within 3–4 hours, while other species do not form germ tubes in that time period.
- *Demonstration of chlamydospores formation for Candida*: Suspected *Candida* species are inoculated on plates or slides with chlamydospore agar (cornmeal with Tween-80), incubated for 3 days, and observed every 24 hours for formation of chlamydospores. *C. albicans* produces abundant chlamydospores.

MALDI-TOF

Many fungi can now be speciated with matrix-assisted laser desorption/ionization time-of-flight (MALD-TOF) mass spectrophotometry. Colonies from a positive culture are mixed with a specialized reagent and directly analyzed in the mass spectrometer. The time to result is approximately 1 minute. MALDI-TOF reduces turn around time and need for subculture (For further details, refer to Chapter 6).

AUTOMATED FUNGAL CULTURES

A number of automated commercial systems are now available. They are continuously monitored systems and have special culture media to enhance fungal growth. They have largely replaced the lysis centrifugation method that was more cumbersome and involved lysis of RBC and centrifugation of blood to isolate *Candida.* The time of positivity of culture will depend on the automatic blood culture system and the causative species. BACTEC 9240 is one of the most validated systems, and the addition of Mycosis–IC/F, a selective medium for fungi, enhances the sensitivity of fungal detection.

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INTERPRETATION OF POSITIVE FUNGAL CULTURES

The growth of fungus on a culture does not necessarily mean it is pathogenic. The results have to be interpreted with caution and compared with the microscopic examination of the specimen. Isolation of an opportunistic fungal pathogen from sterile sites, i.e. blood and CSF is always considered significant. However, the isolation of fungus from an unsterile site such as urine, sputum, or pus must be interpreted in light of the clinical scenario.

Limitations of Fungal Culture

Failure to isolate fungi on an appropriate culture media may be due to the following factors:

- Inadequate specimen
- Inappropriate specimen
- Delayed transport of specimen
- Incorrect isolation procedure
- Inadequate periods of incubation.

The isolation of molds and yeasts can take several weeks, and the results may be available too late. Therefore, there is a need for more automated systems with chromogenic agars for early detection and identification of fungi. Serological tests may be valuable in early diagnosis.

SUGGESTED READING

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- Cites the all relevant updated clinical guidelines
- Includes expert opinions on management of subcutaneous, pulmonary and central nervous system (CNS) fungal infections as well as management in specific clinical scenarios including transplant recipients and neutropenia
- Provides emerging frontiers in the field of mycology
- This book is an educational resource to enhance the knowledge of mycology amongst medical students, physicians, intensivists, respiratory care specialists, surgeons, microbiologists, clinical pharmacologists, and other medical professionals.

"The good physician treats the disease; the great physician treats the patient who has the disease".

—William Osler

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