3 Microbiological Evaluation of the Nail Bed Infection

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Introduction

Nails, hair, and superficial skin layers are unique structures composed of keratin. The etiology and morphology of nail disorders are different. Infections can range in severity from mild to potentially fatal. Most diseases begin small and are curable. Failure to correctly treat these infections can result in lifelong impairment or even amputation of the finger. A meticulous and evidence-based microbiological evaluation detects the pathogen early and guides the surgeon to treat nail bed infections, thereby avoiding complications. This chapter will discuss the technical nuances of microbiological applications in fingertip diseases.

Fingertip Infections

Fingertip infections have varied presentations ranging from nail bed infections (paronychia) to felon, herpetic whitlow, cutaneous warts, bite wounds, osteomyelitis, bacterial fungal and mycobacterial infections. Of these, nail bed infections are the most common disease seen by medical professionals. The etiology of nail bed infections is multifactorial. A thorough understanding of the involved structures, microscopic examination, and cultures help to treat and prevent complications.

Onychomycosis is a fungal infection of the fingernails caused by different organisms with varying nail involvement. Several classes based on the morphological patterns and nail invasion assist in treating them.[1–3] The most common classes are:

- Distal and lateral subungual onychomycosis.
- White superficial onychomycosis.
- Proximal subungual onychomycosis.
- Candidial onychomycosis.

Microbiology—Diagnosis

Various organisms cause onychomycosis (Chapter 5), of which the most common pathogen is the genus *Trichophyton*. *Candida* is more common in fingernail infections and is often associated with chronic mucocutaneous candidiasis. Although nondermatophyte molds are a less common pathogens, they dominate in causing infections in HIV patients.

Nail Preparation and Specimen Collection

Nail specimens from nail bed infections are collected after cleaning with 70% isopropyl alcohol to prevent contamination (**Fig. 3.1**). About 8 to 10 nail clippings should be collected in a sterile black craft paper for diagnosis (**Table 3.1**).

Diagnostic precision is enhanced if the sample is collected with a nail drill from the proximal nail in distal and lateral onychomycosis.[1–5] The collected debris or specimen is divided into two portions; one goes for direct microscopy and the other for culture. The specimen is collected from patients who have been off both oral and topical

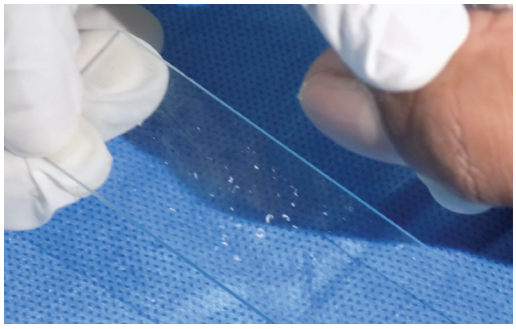

Fig. 3.1 Method of obtaining nail clipping from a suspected case of onychomycosis.

Table 3.1 Specimen collection

Classes	Method of collection
Distal and lateral subungual onychomycosis	Sterile nail clippers or curettes (1–2 mm serrated curette) to collect nail clippings and subungual debris. Nail plate penetration and bleeding must be avoided. Advancing infected edge, close to the cuticle, has the greatest number of viable hyphae
Proximal subungual onychomycosis	A number 15 surgical blade pares the normal surface of the nail plate at the level of the lunula and collects the white debris from the deep part of the nail and proximal nail bed
White superficial onychomycosis (WSO)	The white parts on the nail are scrapped and the white debris underneath is collected
Candida infection	The material close to the proximal and lateral nail edges must be obtained
Total dystrophic onychomycosis (TDO)	The abnormal nail plate and nail bed are used as specimen

antifungal (2–4 weeks) and must not be kept in moist media to prevent bacterial and fungal spore multiplications.

Microscopy

Potassium hydroxide (KOH) stain is a commonly used office microscopy method. It is economical and easy to perform. The KOH will digest the keratin and leave the fungal cell intact. Nail clippings or scrapings are placed on a microscope slide with a drop of 40% potassium hydroxide solution. This slide is allowed to set for at least 5 to 30 minutes before viewing under a microscope (**Fig. 3.2**). Then heating the slide or adding a dimethyl sulfoxide (DMSO) 40% solution will enhance keratin dissolution. Now view the slide using the optical microscopy at 10× and 40× magnifications (DME—direct microscopic examination). Calcofluor white stain is used with KOH to enhance the fungal components in fluorescent microscopy. The negative samples are stored overnight in a humid chamber and examined again after 24 hours to confirm the presence of fungal elements (hyphae, pseudohyphae, or spores). Although identifying fungal elements confirms the infection it does not identify the organism (**Table 3.2**).

The diagnosis is straightforward from the direct microscopy examination (DME) in certain conditions. Yeast forms are present in candida infections, and conidia may be formed in situ in nondermatophyte infections. To precisely determine the organism, we need to perform the culture in an Emmons' modified Sabouraud's dextrose agar (SDA) medium (**Fig. 3.3**). The culture results are available in 3 to 4 weeks.

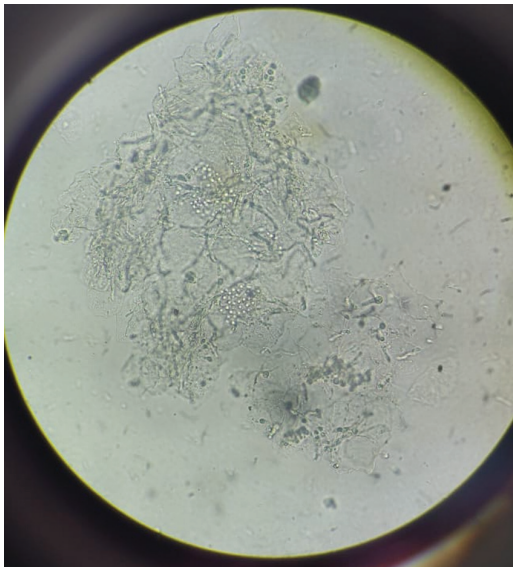

Fig. 3.2 Potassium hydroxide (KOH) mount showing fungal hyphae.

Stains

Periodic acid-Schiff (PAS) stain and methenamine silver stain are the most used methods in fungal infections (**Fig. 3.4**). Histological analysis of nail clippings will determine whether the pathogen

Table 3.2 Direct microscopy and culture

Direct microscopy	Fungal culture
Screening test	Confirmation test
Shows presence or absence of fungal elements	Macroscopic and microscopic appearance of fungi; it also differentiates the pathogen and identifies etiological agents
Easy and economical	Time-consuming (2–30 days) Expensive
False negativity (5–15%) (because of thick nail debris and sparse hyphae in the coarse nails)	False-negative results (30–50%) (because of sampling and collection errors)
Sensitivity: 48%	Sensitivity: 53%

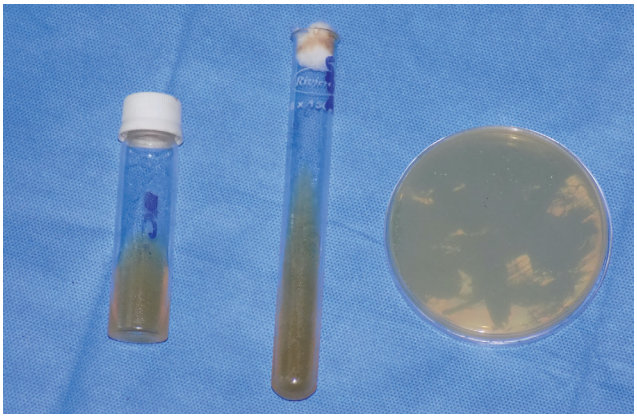

Fig. 3.3 Various types (slant and petri-plate) of Sabouraud's dextrose agar (SDA) medium for fungal culture.

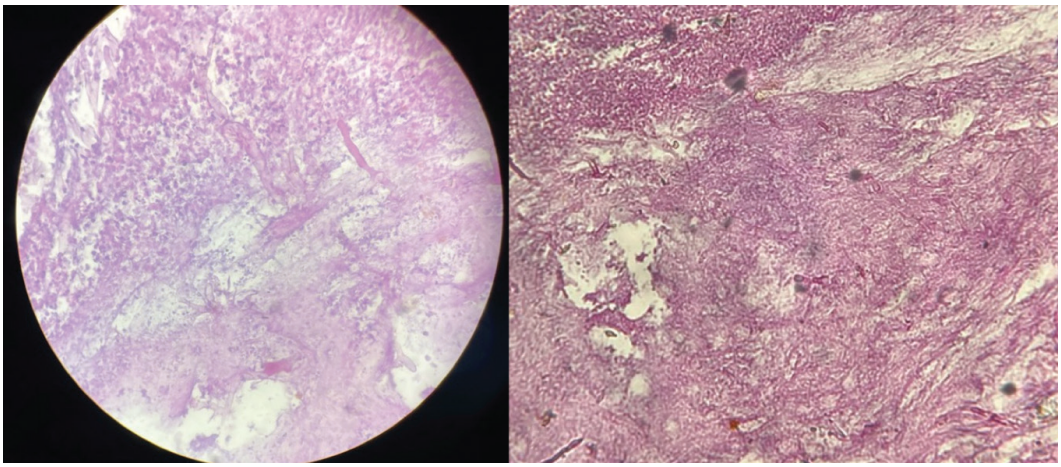

Fig. 3.4 Periodic acid-Schiff (PAS) stain image showing aseptate hyphae.

is a fungus or not. These stains are helpful when the microscopy or culture results are inconclusive. PAS stains glycogen and mucoprotein in the fungal cell wall and requires a short time (<24 hours) to render a diagnosis. Additionally, the PAS stains demonstrate whether a fungus is an invasive or merely normal commensal/colonizing debris. The methenamine silver and calcofluor white stain are also more selective in determining the fungus.

Sensitivity of Tests

- KOH light microscopy: 48%.
- Culture: 53%.
- PAS staining: 82%.
- PAS and culture: 96%.[4]

Polymerase chain reaction (PCR) is more accurate than cultures and PAS stains. The results are available in 3 days.

Bait Thread Test

Microbiological examination of a swab from the nail fold remains the basis for treatment. Bait thread test uses a sterile cotton thread soaked in Sabouraud's broth underneath the nail fold for 24 hours. Alkiewicz introduced this bait thread test for detecting nail fold fungal infections. The sterile cotton thread taken from the nail fold is placed in the broth medium for initial proliferation and incubated for 24 hours.[5] The broth is cultured on MacConkey agar, Sabouraud's agar with gentamicin and chloramphenicol, and blood agar. This culture is incubated at 37°C for 24-48 hours.

Bacteria

The fingernail swab is cultured in mannitol salt agar (MSA) for isolating *S. aureus* and coagulase-negative *Staphylococci*. For gram-negative bacteria, the swab is cultured in MacConkey agar, and blood agar, incubating for 24 hours at 37°C. The bacterial colonies are identified by the morphology, gram staining, and a panel of biochemical tests. The biochemical tests include reaction on oxidase, catalase, Simmons' citrate, indole production, Christensen's urease medium, motility, and TSI (triple sugar iron agar medium for gas and hydrogen sulfide [H_2S] production).[6–9]

Antimicrobial Susceptibility Testing

Mueller-Hinton agar is used for antimicrobial susceptibility tests using the Kirby-Bauer disk diffusion method.[7] The antimicrobial agents used for gram-positive isolates (**Fig. 3.5**) are:

- Cefoxitin (30 µg).
- Penicillin (10 µg).
- Erythromycin (15 µg).
- Ampicillin (30 µg).
- Ciprofloxacin (10 µg).
- Tetracycline (30 µg).
- Cotrimoxazole (25 µg).
- Vancomycin (30 µg).

The antimicrobial agents used for gram-negative isolates are:

- Ampicillin (10 µg).
- Tetracycline (30 µg).
- Chloramphenicol (30 µg).
- Gentamicin (10 µg).
- Norfloxacin (10 µg).
- Cotrimoxazole (25 µg).
- Ciprofloxacin (10 µg).

Fungal Culture

The specimen is stab inoculated on the Emmons' modified SDA medium with antibiotics (chloramphenicol, gentamycin, and cycloheximide). These antibiotics suppress the growth of bacteria and saprophytic fungi and identify dermatophytes (**Fig. 3.6**). SDA with chloramphenicol and gentamycin is used to culture nondermatophyte

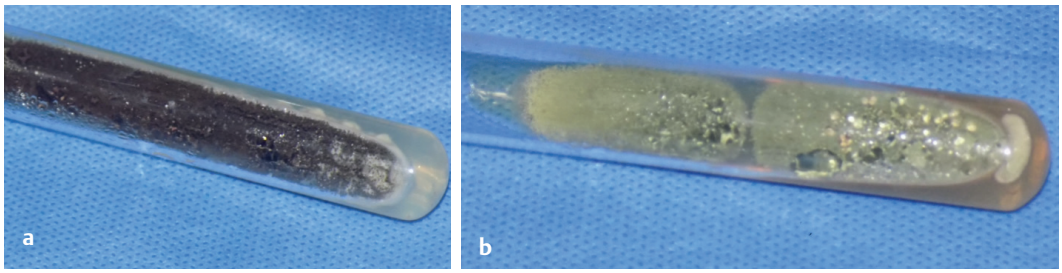

Fig. 3.5 **(a)** Growth on SDA of *Aspergillus niger*. **(b)** Growth on SDA of *Aspergillus flavus*.

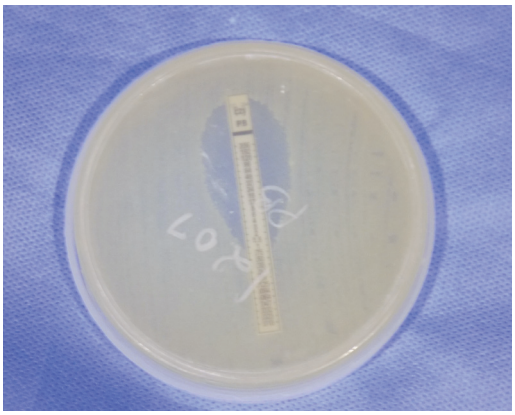

Fig. 3.6 Picture of antibiotic susceptibility testing using E-strip for determination of the minimal inhibitory concentration (MIC).

molds. Dermatophyte test medium (DTM) is a screening medium for dermatophytes (**Fig. 3.7**).

Nail clippings and underlying debris collected are inoculated in two SDA media with chloramphenicol and cycloheximide and incubated at 25°C for 21 to 30 days. A daily fungal growth check is mandatory. The isolated colonies of the fungus are identified by observing the macroscopic appearance and reverse pigment production. Lactophenol cotton blue (LPCB) stain is used to identify microscopic features of the isolates (presence of the hyphae, microconidia, and macroconidia arrangements) (**Fig. 3.8**). If necessary, additional biochemical tests can be performed.[7–11]

The culture results are positive, mixed growth, contamination, or no growth. Positive culture reports are species-specific to the fungi. For example, *Aspergillus, Fusarium*, or *Penicillium* spp. are nondermatophyte fungi; *Microsporum* and *Trichophyton* spp. are dermatophyte fungi; *Candida* or *Tricosporum* spp. are yeasts. False-negative results are seen in 30 to 50% of the samples because of sampling and collection errors and extensive keratinization. Repeat sampling is ideal in cases of suspected onychomycosis.

In certain situations, laboratory contaminants may be present as nondermatophyte fungi. Also, these fungi may cause nail dystrophy and onychomycosis in 2 to 12% of cases. Therefore, KOH testing and cultures must be repeated, and the findings must be clinically correlated.[8–11] Additionally, three consecutive samples may be collected at intervals of 2 to 5 days to increase diagnostic accuracy.[11]

Fig. 3.7 Dermatophyte test medium showing the growth of dermatophyte fungi (*Microsporum gypseum*).

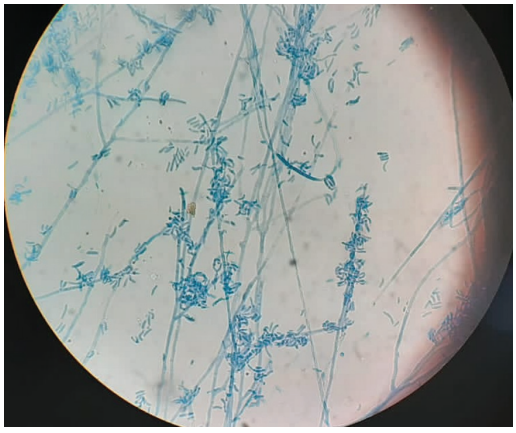

Fig. 3.8 Lactophenol cotton blue (LPCB) stain picture of *Aspergillus flavus*.

Broth Dilution Antifungal Susceptibility

There is a definite need for a standard in vitro antifungal susceptibility assay for optimizing antifungal therapy. Increased/ increasing number of patients with immunosuppression,

development of new antifungal drugs, and emergence of resistance to antifungal drugs are among the most important reasons. There is an interaction between the host, antifungal drugs, and infecting fungi. Clinical and Laboratory Standards Institute (CLSI) 2021 guidelines recommend a standard reference method for testing susceptibility to antifungal agents. The focus is on developing breakpoints and minimal inhibitory concentration (MIC) (by broth dilution method) for both established and newly introduced antifungal agents.[10]

The breakpoint is defined as MIC or zone diameter value needed to categorize fungi or microbes as susceptible, susceptible-dose dependent, intermediate, or resistant. The following antifungals are tested:

- Ciclopirox (32 to 0.0625 µg/mL).
- Terbinafine (0.5 to 0.0001 µg/mL).
- Fluconazole (64 to 0.125 µg/mL).
- Itraconazole (0.5 to 0.0001 µg/mL).

The new triazoles (posaconazole, ravuconazole, and voriconazole) are also included in the updated CLSI documents. The MIC is the lowest antifungal concentration capable of inhibiting 80% of fungal growth (**Fig. 3.9**). This in vitro antifungal susceptibility test is mainly used for epidemiological surveys, surveillance, and further research for the determination of the degree of antifungal activity and prediction of clinical outcome and optimization of antifungal drugs. The standard disk diffusion assay is a reproducible method and correlates with the reference microdilution antifungal susceptibility testing assay. It is also a good model for the investigative purpose of other fungal genera and drugs. CLSI MIC breakpoints and disk diffusion inhibition zone are currently available for certain drugs (fluconazole, itraconazole, voriconazole, flucytosine) against *Candida*. The breakpoints for filamentous fungi and yeast are proposed by CLSI based on the M38-A and M-44 documents, respectively. It will be a standard for predicting clinical outcomes in fungal resistance cases and profound immunosuppressive patients.

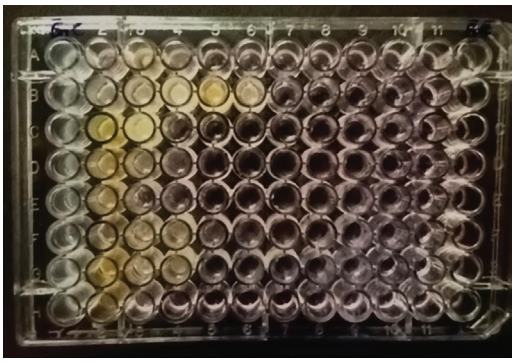

Fig. 3.9 Microbroth dilution method.

Conclusion

Accurate diagnosis of the fingertip infections by clinical examination and culture identification detects the etiological agents and focuses the surgeon on treating them. Histological evaluation using a PAS stain increases the sensitivity of the infection and diagnosis. In addition to the PAS, a fungal culture is a specific method to isolate the microorganism, and it takes several weeks to grow. PCR may speedily indentify the cause of the infection, but it is difficult to differentiate between live and dead microbes. In vitro antimicrobial susceptibility is essential to treat diseases and prevent resistance.

Key Points

- Proper specimen collection is essential for an accurate diagnosis.
- Direct microscopy examination of the nail specimen is a simple method to identify the microorganism.
- PAS stain and fungal culture have a very high sensitivity to detect the microbes.
- A fungal culture accurately confirms and isolates the infective microbes.
- Identifying and assessing the sensitivity to antimicrobials are vital for epidemiological survey, research, surveillance of emerging drug resistance.

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