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Isolation and Identification of Dermatophyte Fungi in Darussalam District, Aceh Besar

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Abstract

Dermatophytosis has been recognized as one of the most common superficial fungal infections, often found in tropical areas with high humidity and high population density. This observational descriptive study aims to isolate and identify dermatophyte species in dermatophytosis cases in Darussalam District, Aceh Besar. A total of 50 clinical samples were examined using the 20% KOH method as well as inoculation for culture in both Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM). The results showed 35 positive samples on the KOH examination. A positive culture in SDA identified Trichophyton tonsurans in 35 samples (70%) and *Trichophyton violaceum* in 3 samples (6%). Similar to SDA, DTM identified *Trichophyton tonsurans* as the most common isolate, which was found in 36 samples (72%), followed by *Trichophyton violaceum* in 3 samples (6%), and *Epidermophyton floccosum* in 1 sample (2%). These findings demonstrate the predominance of *T. tonsurans* as the primary cause of dermatophytosis, consistent with its anthropophilic properties that facilitate human-to-human transmission. The identification of dermatophyte species through culture plays a crucial role in the diagnosis and control of diseases.

Keywords: Dermatophytosis, Fungi Isolates, Sabouraud's Dextrose Agar, Dermatophyte Test Medium

Introduction

Dermatophytosis refers to a superficial fungal infection of the skin, hair, and nails caused by dermatophyte fungi, which are a group of keratinophilic fungi that utilize keratin as a nutrient source.[1] More than 40 species, belonging to three significant genera (*Microsporum*, *Trichophyton*, and *Epidermophyton*), are known to cause dermatophytosis in humans. The disease has been acknowledged as one of the most common mycotic infections worldwide and may predispose to clinical symptoms that impact a patient's quality of life.[2] The incidence of dermatophytosis accounts for 20-25% of fungal infections in humans globally. Dermatophytes are expected to infect 10-15% of individuals at some stage, making these infections a considerable concern for the population.[3,4]

Dermatophytes thrive ideally in hot and humid environments, so many tropical and developing countries are predisposed to an increase in dermatophyte infections. Tropical countries, such as Indonesia, have faced significant challenges due to the alarming increase in the number of chronic and recurrent dermatophyte infections. The tropical and subtropical climate of the country is very favorable for dermatophytes. Additionally, density, shared living spaces, and urbanization are factors that contribute to the increasing prevalence of dermatophytosis.[5,6]

The diagnostic confirmation of dermatophytosis is generally carried out with a thorough anamnesis and adequate physical examination; however, diagnostic tests may be performed to confirm the specific underlying etiology. Fungal cultures are essential for the definitive diagnosis and effective treatment of the causative fungal infection. Fungal culture is considered the gold standard in laboratory diagnosis of dermatophytosis. Sabouraud dextrose agar (SDA) is a frequently utilized and effective medium that supports the growth of pathogenic fungi, including dermatophytes.[2,7,8] Cultures using dermatophyte test medium (DTM) contain chlorotetracycline and gentamycin to prevent bacterial contamination, cyclohexamide to inhibit the growth of contaminating fungi, and red phenol, a pH indicator.[7,9,10]

Over the past 70 years, *Trichophyton rubrum* has been the most common species that causes dermatophyte infections. Other common isolates include *T. tonsurans*, *T. mentagrophytes,* and *Microsporum audouinii*.[3] Direct skin contact with soil, animals, or other humans is usually what causes the infection. Several previous studies in the Sumatra region have shown the dominance of *Trichophyton rubrum* as the primary cause of dermatophytosis. Identifying the precise type of fungal cause is crucial for determining effective therapy, given the differences in antifungal sensitivity among each species.[10] This study aims to isolate and identify dermatophyte fungi from clinical cases in Darussalam District using culture methods in SDA and DTM, employing macroscopic and microscopic morphological identification.

Methodology

Research time and place

This research was conducted from January to April 2025. Samples were taken from subjects with clinical manifestations of dermatophytosis at the Darul Ihsan Islamic Boarding School in Darussalam District, Aceh Besar, and then examined at the Microbiology Laboratory of Universitas Syiah Kuala.

Identification Procedure

The examination was conducted, which included potassium hydroxide (KOH) testing, fungal culture in Sabouraud Dextrose Agar (SDA), and Dermatophyte Test Medium (DTM).

KOH Test Procedures

Tools and materials

The tools and materials used in this examination include a 10-20% KOH solution, a light microscope, an object glass, and a glass cover.

Sample collection

The sample was collected by scraping the skin at the edge of the lesion, which is where the most active fungal growth occurs. Sample collection in onychomycosis was carried out by extracting small pieces of infected nails. In the case of tinea capitis, an abnormal hair presentation was also extracted as a sample.

Specimen preparation

The prepared specimens are then left for 15–30 minutes to allow the KOH solution to work, softening or dissolving the keratin. When needed, this process can be accelerated by providing light heating. However, heating must be done carefully so that excessively high temperatures do not damage the fungus's structure.

Microscopic examination and interpretation

After the digesting process is completed, the preparations are observed under a microscope using 40× and 100× magnification. At this stage, the observer looks for a special structure of the fungi. A positive image of dermatophytes under a microscope will show septate, branching hyphae, which are long, narrow filaments that are typically clear but may appear purplish-blue, along with arthroconidia (fungal spores).

Culture Examination Procedure Using SDA

Preparation of Equipment and Materials

The materials and equipment required for this examination include Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol and cycloheximide to inhibit the growth of bacteria and saprophytic fungi. Additional items include sterile Petri dishes, sterile inoculating needle or sterile forceps, an incubator maintained at 25–30 °C, a microscope, and lactophenol cotton blue solution.

Table 1. Interpretation of dermatophyte species based on culture with SDA[10]

Dermatophyte Species	Colony Color	Microscopic Morphology
Trichophyton sp.	Reddish white	Teardrop-shaped microconidia
Microsporum sp.	Yellowish brown	Thick-walled macroconidia
Epidermophyton sp.	Greenish yellow	Club-shaped macroconidia

Sample Collection and Slide Preparation

The examination begins with the collection of skin scrapings from the active margin of the lesion. The specimen is then aseptically inoculated onto the surface of Sabouraud Dextrose Agar (SDA). Following inoculation, the culture plates are incubated at a controlled temperature of 25–30 °C for 2–4 weeks. During the incubation period, fungal growth is monitored every 2–3 days to assess colony development.

Macroscopic Colony Examination

Colony morphology is evaluated by observing the characteristic macroscopic features of dermatophytes. *Trichophyton sp.* typically produces white, cottony colonies on the upper surface with a deep wine-red pigmentation on the reverse. *Microsporum sp.* forms white to yellowish colonies with sharply fringed margins, whereas *Epidermophyton sp.* develops yellow-green, velvety colonies.

Microscopic Examination

For microscopic evaluation, a small portion of the fungal colony is transferred onto a clean glass slide and treated with a drop of lactophenol cotton blue solution. The preparation is then examined under a microscope to identify characteristic fungal structures, including microconidia, macroconidia, and septate hyphae. *Trichophyton sp.* is characterized by microconidia with a teardrop-like shape. *Microsporum sp.* exhibits thick-walled macroconidia, while *Epidermophyton sp.* presents club-shaped macroconidia.

Culture Examination Using DTM

Preparation of Equipment and Materials

The materials required for this procedure include sterile petri dishes containing Dermatophyte Test Medium (DTM). DTM is a selective and differential medium composed of Sabouraud Dextrose Agar supplemented with chloramphenicol, cycloheximide, phenol red, and specialized peptone. Additional equipment includes sterile inoculating loop or forceps, an incubator maintained at 25–30 °C, and a microscope for morphological confirmation of fungal colonies.

Sample Collection and Slide Preparation

The examination using DTM begins with preparing the clinical specimen in a sterile container. The sample is then carefully inoculated onto the surface of the DTM using a sterile, pointed inoculating loop, ensuring that the medium is not punctured or damaged. The inoculated plates are subsequently incubated at 25–30 °C. The incubation period typically ranges from 5 to 14 days, during which daily observations are performed to monitor changes in medium color and fungal colony development.

Macroscopic Colony Examination

Observation focuses on both the color change of the medium and the characteristic growth of dermatophyte

colonies. A shift in medium color from yellow to bright red is a key indicator of dermatophyte growth. This occurs because dermatophytes metabolize the proteins within the Table 2. Interpretation of dermatophyte species based on culture with DTM[10]

Dermatophyte Species	Medium Color	Colony Color	Growth Time
Trichophyton sp.	Bright pink	White-colored with a cottony surface	5-10 days
Microsporum sp.	Bright red	Creamy yellow with radial grooves	7-14 days
Epidermophyton sp.	Yellowish-green red	Creamy-coloured with a cottony surface	6-10 days

medium, resulting in an increase in alkaline pH that produces the distinctive color change.

Results and Discussion

Dermatophytes are among the most common mycotic infections worldwide and can cause clinical manifestations that significantly impair patients' quality of life. These fungi are commonly found in tropical and subtropical regions, where warm and humid conditions favour their growth. In addition, population density, shared living spaces, and urbanization contribute to the increasing prevalence of dermatophytosis. In the present study, clinical samples were collected from students residing in an Islamic boarding school with a high population density and shared dormitory facilities, which increase the risk of infectious skin diseases, such as dermatophytosis. Diagnostic confirmation included direct microscopic examination followed by fungal culture. Although direct microscopy plays a crucial role in diagnosing fungal infections, culture remains essential for the definitive and accurate identification of dermatophyte species.

In this study, dermatophytosis detection was performed on 50 clinical specimens using potassium hydroxide (KOH) examination and fungal culture on SDA and DTM. KOH examination revealed that 35 samples demonstrated clear, septate, branching hyphae and were therefore interpreted as positive. Potassium hydroxide acts as a keratin-digesting reagent, dissolving proteins and lipids while lysing epithelial cells. Fungal elements resist the KOH solution (10%–30%) because their cell walls contain chitin and glycoproteins. KOH rapidly and irreversibly clears keratinized tissue without staining the specimen, thereby enhancing the visibility of fungal structures against the background. This contrast improves the quality of microscopic visualization and facilitates the detection of fungal elements.[11] A meta-analysis by Velasquez et al.[12] reported that the sensitivity of KOH examination for diagnosing dermatophytosis is approximately 61%, while its specificity reaches 95%. Other studies have shown that the sensitivity of KOH examination ranges from 44% to 100%.[13] The relatively low sensitivity and specificity of KOH testing are influenced by sample quality, examiner experience, and the presence of microscopic artifacts such as fibers or debris that can mimic fungal hyphae. Moreover, KOH only dissolves keratin to highlight fungal elements, but it cannot identify fungal species, which may lead to false-positive or false-negative results. Although rapid, simple, and cost-effective, KOH examination cannot replace fungal culture, which remains the gold standard due to its higher accuracy and ability to accurately identify species.[14,15]

Microscopic examination of 50 dermatophyte specimens cultured on SDA revealed colonies with distinct morphological characteristics. Thirty-eight isolates exhibited white colonies with a reddish reverse, consistent with the morphology of *Trichophyton sp*. Identification was confirmed by microscopic evaluation with lactophenol cotton blue staining, which demonstrated septate hyphae and species-specific conidial structures. Microscopically, the isolates were successfully identified as *Trichophyton tonsurans* in thirty-five samples and *Trichophyton violaceum in 3 samples*. Twelve samples showed no fungal growth.

Culture on DTM demonstrated similarly distinct morphological variations. Thirty-nine isolates produced white, fluffy colonies typical of *Trichophyton sp.*, and one

isolate developed cream-colored, downy colonies consistent with *Epidermophyton sp*. Thus, DTM culture confirmed the distribution of dermatophyte species through microscopic identification, as follows: *T. tonsurans* (36 isolates), *T. violaceum* (3 isolates), and *E. floccosum* (1 isolate) from a total of 50 samples, while 10 samples showed no fungal growth.

Table 3. Detection rate proportions of each examination

Examination Method	Sample	Positive Result	Negative Result	Details
KOH 20%	50	35 (70%)	15 (30%)	Rapid detection (can be underdiagnosed)
DTM	50	40 (80%)	10 (20%)	Media color turns red (positive)
SDA	50	38 (76%)	12 (24%)	Can be contaminated with other fungi (need confirmation)

These findings indicate that *Trichophyton tonsurans* is the most frequently isolated dermatophyte species in Darussalam District, Aceh Besar. The predominance of *T. tonsurans* aligns with numerous previous studies that have reported this species as one of the most common causes of dermatophytosis in humans, particularly in cases of tinea capitis and tinea corporis. Foster et al.[16] reported a prevalence of *T. tonsurans* in 95% of tinea capitis cases in the United States. The incidence showed a seasonal elevation during the summer months and was especially high among residents of rural areas. The anthropophilic nature of *T. tonsurans*, which facilitates human-to-human transmission, and its ability to persist in humid environments likely contribute to the high prevalence observed.[17] A study conducted by Pilz et al.[18] also demonstrated a tenfold increase in T. tonsurans infection over a 2-year period. The isolation of *T. violaceum* as the second most common species suggests the possibility of transmission of these Asian and African endemic, anthropophilic dermatophytes.[19] Although less prevalent, *Epidermophyton floccosum* remains an important etiologic agent of dermatophytosis, despite its lower prevalence.[17]

Conclusion

This study demonstrates that dermatophytosis is prevalent in Darussalam District, Aceh Besar, particularly in densely populated settings such as Islamic boarding schools. Of the 50 samples examined, 35 were positive on KOH preparation, and culture identified *Trichophyton tonsurans* as the most frequently isolated species, followed by *Trichophyton violaceum* and *Epidermophyton floccosum*. The dominance of *T. tonsurans* is likely attributable to its anthropophilic nature.

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