CURRENT PERSPECTIVES IN THE MICROBIOLOGICAL DIAGNOSIS OF DERMATOPHYTOSES

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Abstract

Globally, dermatophytosis affects approximately 25% of the population, being a category of cutaneous mycoses that affect the skin and nails with a varied epidemiology, influenced both by geographical location and by some populations themselves. The main etiological agent in the case of dermatophytosis is the species Trichophyton rubrum. Although in general the lesions in dermatophytosis are superficial, limited to the keratinized layer, cases of mycoses in which the etiological agent is a dermatophyte fungus are increasingly being reported in which layers such as the dermis or hypodermis are invaded, especially in people with a immunosuppressed immune system.

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1. INTRODUCTION

Dermatophytosis (dermatophyte infection, tinea, ringworm) is a superficial cutaneous mycosis affecting the skin, nails and hair, caused by dermatophyte fungi [2,4,5,8]. Following recent multilocus phylogenetic studies, the taxonomy of dermatophytes has been revised such that species that were previously classified in the three known genera (*Epidermophyton*, *Microsporum* and *Trichophyton*) are now distributed in several genera, respectively: *Arthroderma, Epidermophyton, Lophophyton*, *Microsporum*, *Nannizzia, Paraphyton* and *Trichophyton* [7].

Depending on the location on the body surface, dermatophytosis can be found under different names, namely: tinea pedis (on the foot), tinea cruris (inguinal intertrigo), tinea corporis (affects glabrous skin), tinea barbae (on the chin and mustache), tinea faciei or sycosis (on the face), tinea capitis (can affect the scalp, eyebrows, eyelashes), tinea manuum (on the hand), tinea unghium (on the nails), tinea favosa (favus) and tinea imbricata or tokelau [5,12,13,17].

For a quality laboratory diagnosis, the way in which the sampling is carried out and the amount of material taken are very important. The material taken should be sufficient for both direct microscopy (important in the case of immunosuppressed patients for a rapid diagnosis) and for performing culture in the laboratory. Knowledge of additional data about the patient such as the appearance and location of lesions on the body surface, whether he has come into contact with an animal or whether he has traveled to certain areas, are useful in establishing the diagnosis of dermatophytosis [18].

In order to ensure proper sample collection, it is recommended that samples be collected before antifungal treatment begins, as well as removing any creams or lotions from the sample area using alcohol. Clean and sterile instruments, such as curettes, blunt scalpels, scissors, and forceps, should be used. A sterile swab can also be used to scrape the area from which the specimen was previously collected to ensure that all scales have been collected [18].

From the skin, in patients suspected of tinea corporis, the specimen is collected by scraping the lesion from the center to the edge.

From the foot, in the case of tinea pedis with blisters, the upper part of the blister is removed and collected; in the case of onychomycosis or tinea unguium, the subungual material or nail fragments are collected. From the scalp, in the case of tinea capitis or tinea favosa, the hairs are plucked or epilated if plucking them is not possible [18].

2. MATERIALS AND METHODS

In order to highlight the importance of the laboratory diagnostic in dermatophytosis, a meta-analysis of the specialized literature was conducted, mainly addressing the epidemiology, clinical manifestations and diagnosis of dermatophytosis, in the context in which the clinical manifestations may be similar to those of other dermatoses such as psoriasis or candidiasis, and laboratory examination is essential for identifying the etiological agent and choosing the appropriate treatment.

Laboratory diagnosis of dermatophytosis includes the following stages:

2.1.1. Direct microscopic mycological examination.
2.1.2. Inoculation of the collected materials on culture media (culture).

2.1. **Direct microscopic mycological examination**.
Direct microscopic mycological examination is performed using preparations mounted in a drop of KOH (potassium hydroxide) with a concentration of 10-20%, depending on the type of sample. To prevent crystallization and drying of the preparation, it is recommended to add glycerol.
For this examination, the sampled material (scales, subungual deposit, nail fragments, hairs) is placed on a glass slide, over which a drop of KOH is added and covered with a coverslip, the coverslip is gently pressed, avoiding the formation of air bubbles. The obtained preparation can be slightly heated and examined under a microscope after a contact time of 10-15 minutes, necessary for the degradation of keratin and clarification of the preparation (the time may vary depending on the reagent used, the amount of material taken and the degree of contamination) [10].

Interpretation of results:
In the case of a positive result, when scales or nail fragments are examined under a microscope, the following can be observed: hyaline, septate, branched hyphae and arthroconidia . Arthroconidia result from the fragmentation of hyphae, and their presence is definitive for making the diagnosis of dermatophytosis.

2.2. Inoculation of the collected materials on culture media (culture).
Regardless of the result of the direct microscopic mycological diagnosis, it is recommended to perform a culture for diagnosis and the susceptibility testing. To perform the culture, the samples (scales, subungual deposit, nail fragments, hairs) are inoculated on usual isolation media or on special culture media for dermatophytes, incubated at temperatures of 25-30ºC and observed for 4 weeks.
The identification of isolated strains is carried out by analyzing macroscopic and microscopic characters. When these are not sufficient for species identification, a series of tests can be used such as: the in vitro hair perforation test, the urea hydrolysis test, the rice growth test, tests for determining nutritional requirements and the thermotolerance test.

Microscopic characters

In the case of dermatophyte fungi, microscopic examination can show macroconidia, microconidia, arthroconidia, chlamydospores, "rocket" hyphae, spiral hyphae, "deer antler" hyphae and nodular organs (structures that may be present or absent depending on the genus and species of dermatophyte fungus). For example, the species *Epidermophyton flocossum* does not show microconidia. Very important for identifying the species, microscopic characters can be observed using extemporaneous preparations mounted in lactophenol blue (a) or by performing a culture on a slide (b).

(a). The extemporaneous preparation is the most used and is made by detaching a portion of the dermatophyte colony. The preparation is examined under a microscope with a 10x, 20x, 40x objective.
Another way in which the microscopic examination can be performed is the scotch tape mount technique, usually used for filamentous, sporulating fungi, it prevents the destruction of the spores, which happens to a large extent in the case of the tearing of the mycelial fragments. This technique is performed as follows: cut a small fragment of a transparent adhesive tape (scotch); lightly touch the surface of the colony with the sticky side of the tape, taking care not to touch the colony (for this, you can use tweezers or a wooden stick, which can be moistened with a small amount of alcohol to facilitate detachment from the tape fragment); place the

tape fragment on a slide in a drop of liquid (lactophenol blue is preferred); apply a drop of liquid over the adhesive tape, cover with a coverslip and examine under a microscope [18].
(b). The slide culture is used for the in situ observation of conidia (macroconidia ormicroconidia) and is performed by performing the following steps:
• placing a fragment of sterile glass in the shape of the letter V or U in the wet chamber, over which a sterile microscope slide is placed.
• placing a fragment of a culture medium (preferably a medium that stimulates sporulation) measuring approximately 1cm over the glass slide.
• the medium fragment is inoculated with the strain to be identified and may or may not be covered with a flamed coverslip.
• incubating the Petri dish at a temperature of 30˚C, until mycelial growth and sporulation are observed.
• extemporaneous preparations are made both by applying the slide, removed from the seeded medium fragment, to a drop of lactophenol blue and by directly observing the respective fragment under a microscope.
Conventional diagnostic methods are not always accurate, having some limitations, making it useful to use molecular diagnostic techniques in particular cases [18]. Table 1 present a summary of the advantages and disadvantages of the main diagnostic methods.

Table 1. Diagnostic methods and characteristics
Comparison of diagnostic methods for dermatophytosis

Methods	*Response time*	*Sensitivity*	*Main limitations*	*References*
Microscopy/culture	*Days-weeks*	*Variable*	*Slow, depends on expertise*	*(Aboul-Ella et al., 2020; Begum et al., 2020; Moskaluk & Vandewoude, 2022; Robert & Pihet, 2008; Pihet & Govic, 2017)*
PCR (various types)	*Hours-days*	*High Cost*	*requires equipment*	*(Petrucelli et al., 2020; Aho-Laukkanen et al., 2024; Gnat et al., 2020; Spanamberg et al., 2023)*
MALDI-TOF MS	*Minutes-hours*	*High*	*Limited database*	*(Chen et al., 2021; Machová et al., 2025)*
Rapid immunoassays	*Minutes*	*Good*	*Variable sensitivity*	*(Aboul-Ella et al., 2023)*
VOCs (fingerprint)	*Hours-days*	*Promising*	*Experimental stage*	*(Machová et al., 2025)*

Additional tests for the identification of dermatophyte species

1. In vitro hairperforation test.

The test is useful in the identification of dermatophyte fungal species, especially in differentiating *Tricophyton mentagrophytes* from *Tricophyton rubrum* species. It is performed by placing sterile hairs in a Petri dish, to which sterile distilled water (approximately 10ml), yeast extract (0.1ml) and a fragment of the dermatophyte colony are added. The Petri dish is incubated at 25ºC and examined weekly for approximately four weeks, by making extemporaneous preparations, preferably mounted in a drop of lactophenol blue.

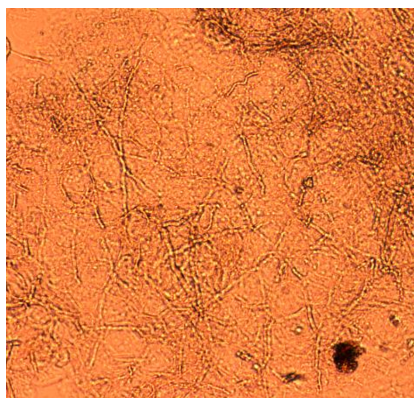
The test can also be performed by applying sterile hairs directly to the surface of the colony, incubating at 25ºC and examining them weekly for approximately four weeks, by making extemporaneous preparations, mounted in a drop of lactophenol blue.
Interpretation of results: in the case of a positive result, conical or wedge-shaped perforations are observed at the level of the hairs [18, 19].

Urea hydrolysis test
This test is used to differentiate Tricophyton mentagrophytes isolates from *Tricophyton rubrum*. The test can be variable in the case of certain species, Tricophyton mentagrophytes isolates are urease-positive while *Tricophyton rubrum* isolates, depending on the type to which they belong, can be negative or positive.
The fluffy type of *Trichophyton rubrum* is urease-negative while the granular type of *Trichophyton rubrum* isolates are urease-positive [18]. The urea hydrolysis test is also variable for other strains.

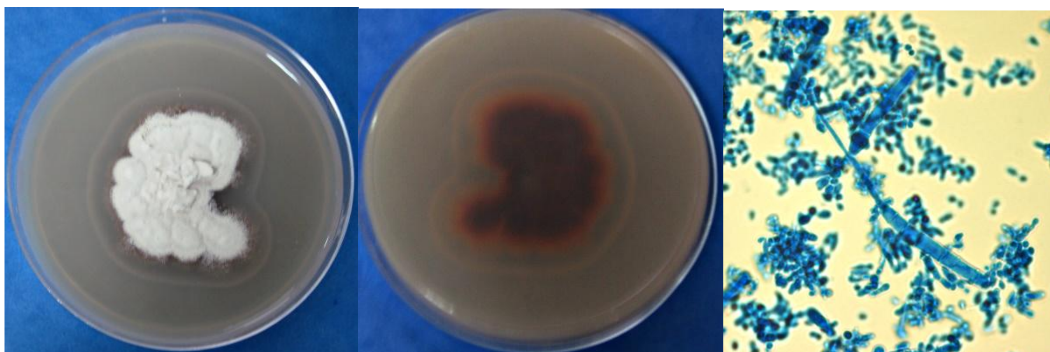
The test is performed by inoculating a colony fragment onto urea medium and incubating at 25º-30ºC for 7-8 days. It is tested in parallel with an uninoculated medium control and examined at 2-3 day intervals to observe whether the color of the culture medium has changed.
Interpretation of results:
In the case of a positive result, the color of the medium changes from light yellow to red-violet [18, 19].

3. RESULTS AND DISCUSSION

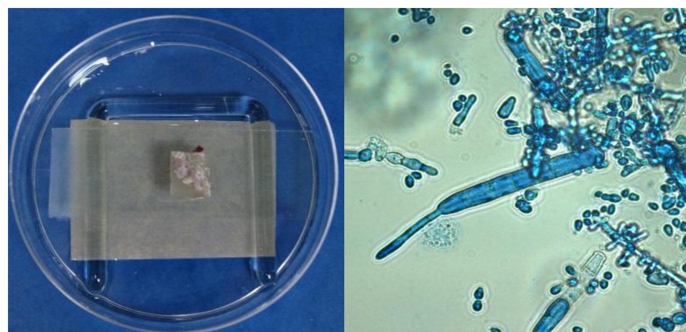
Diagnosis of dermatophytosis has evolved significantly in recent years, moving from laborious and slow methods to rapid and accurate molecular techniques. This change is essential for the effective treatment and prevention of the spread of fungal infections in humans and animals.
In the following images, you can see some of the macroscopic and microscopic characteristics of one of the most commonly isolated species of dermatophyte fungi with clinical importance, namely: *Trichophyton rubrum*.



Direct preparation mounted in 10% KOH, dermatophytosis scales of the trunk, 400X



Trichophyton rubrum, **PDA medium; A-colony avers; B-colony reverse;**
C- macroconidia and microconidia, lactophenol cotton blue, 400x



Trichophyton rubrum**; A- slide culture;**
B-macroconidia and microconidia, lactophenol cotton blue, 400x

5. CONCLUSIONS

For a positive result, the diagnosis-treatment correlation is important, through which the identification of the dermatophyte fungal species agent guides the choice of antifungal used in therapy, knowing that infections caused by *T. rubrum* species respond well to treatment with terbinafine and itraconazole. Also, to monitor the effectiveness of the chosen treatment, it is recommended to repeat the microscopic examination and perform the culture.
Traditional methods include direct microscopic examination and culture on specific media, which remain the gold standard but require up to 4 weeks to identify the etiological agent. These methods are inexpensive and accessible, but have variable sensitivity and specificity, depend on the experience of the personnel and do not always allow identification to the species level [1,4,14].

Molecular techniques, especially PCR (conventional, real-time, multiplex), have revolutionized diagnosis, providing results within hours and a sensitivity far superior to culture. Commercial tests (e.g. DermaGenius) and in-house methods cover most relevant species, reducing turnaround times from weeks to 16 hours [2,9, 17]. Other modern methods include microarrays (e.g. DendrisCHIP®), MALDI-TOF MS (mass spectrometry), and rapid immunochromatographic assays, each with specific advantages and limitations [1,6,15].

Method selection factors and future prospects. The choice of method depends on resources, urgency, the target species spectrum and the experience of the laboratory. Molecular methods are becoming increasingly accessible and can supplement or even replace classical diagnosis, but require extensive databases and standardization [4,14,17,20]. Innovative approaches are also being developed, such as identification based on volatile organic compound (VOC) fingerprinting [15].

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