Diagnostic Microbiology and Infectious Disease 83 (2015) 170–174

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Diagnosis of dermatophytosis: an evaluation of direct examination using MycetColor® and MycetFluo®

Marc Pihet [a,b,*], Nathalie Clément [b,c], Catherine Kauffmann-Lacroix [d], Sandrine Nail-Billaud [b,c], Agnès Marot [b,c], Françoise Pilon [e], Raymond Robert [b,c]

[a] Laboratoire de Parasitologie-Mycologie, Institut de Biologie en Santé, CHU d'Angers, 4 rue Larrey, 49933 Angers Cedex 9, France
[b] Groupe d'Etude des Interactions Hôte-Pathogène, UPRES-EA 3142, l'UNAM Université, Université d'Angers, France
[c] Laboratoire de Parasitologie-Mycologie, Faculté de Pharmacie, 16 boulevard Daviers, 49100 Angers, France
[d] Laboratoire de Parasitologie-Mycologie, CHU de Poitiers, 2 rue de la Milétrie, 86021 Poitiers Cedex, France
[e] Laboratoire Tharreau, 4 rue Jules Ferry, 49500 Segré, France

ARTICLE INFO

Article history:
Received 31 March 2015
Received in revised form 22 June 2015
Accepted 29 June 2015
Available online 3 July 2015

Keywords:
Dermatophytes
Dermatophytosis
Mycological diagnosis
Direct examination
Stain
Fluorochrome

ABSTRACT

Dermatophytes are an important cause of superficial fungal infection. Direct examination of skin, nail, or hair samples remains essential in diagnosis, as it provides a quick response to the clinician. However, mycological analysis, including direct examination and culture, often lacks sensitivity. The use of stains or fluorochromes may enhance the performance of direct examination. We analyzed 102 samples from patients with suspected dermatophytosis in 4 different diagnostic mycology laboratories. Two reagents, MycetColor® and MycetFluo®, which use Congo red and calcofluor dye, respectively, were evaluated for the direct microscopic examination of skin, hair, and nail specimens. The results were compared to those of culture and conventional direct examination. Both reagents were able to clarify the specimens and also to specifically stain fungal elements. Microscopic examination of the specimens was greatly facilitated with MycetFluo®, which allowed a higher number of positive cases to be detected compared to the other methods.

1. Introduction

Dermatophytoses are superficial mycoses affecting human skin (tinea faciei, tinea corporis, tinea cruris, and tinea pedis), hair (tinea capitis), beard (tinea barbae), or nails (onychomycosis or tinea unguium). Onychomycoses are particularly common, as they represent about 30% of superficial mycoses in dermatology consultations and 50% of etiologies of nail disease (Moreno and Arenas, 2010). According to the recent study of Farhi et al. (2011), the prevalence of onychomycosis among patients consulting their general practitioner is 16.8%. Although rare in children, the frequency of onychomycoses increases with age, with a prevalence of about 48% in patients over 70 years of age (Levy, 1997).

Nail disease is not synonymous with onychomycosis, and examination of biological samples is essential to make a diagnosis and establish a specific treatment (Hainer, 2003). The diagnostic approach, which includes examination of the patient, must be completed by mycological analysis and/or histology of nail samples (Welsh et al., 2010). Histological analysis of nail biopsies, which is considered the "gold standard" for the diagnosis of onychomycoses, is seldom performed, and routine diagnosis of dermatophytosis involves direct microscopic examination of samples, followed by culture. Due to the low sensitivity of direct microscopic examination and the slow growth of dermatophytes in vitro, molecular methods such as polymerase chain reaction (PCR) have been developed to enhance and speed up the diagnosis of these superficial mycoses (Verrier et al., 2013). However, microscopic identification of fungal elements directly in clinical specimens is still the first approach in most laboratories because of its simplicity and rapidity (Feuilhade de Chauvin, 2005).

In this study, we evaluated 2 reagents, MycetColor® and MycetFluo® (SR2B, Avrillé, France), for their ability to clarify preparations of skin, nail, and hair samples obtained from patients with clinically suspected dermatophytosis and to stain fungal elements in the samples.

2. Materials and methods

2.1. Clinical samples

Clinical specimens, including skin and nail scrapings and hair, were collected by physicians (mycologists or dermatologists) and sent to 1 of 3 different mycology laboratories (Parasitology-Mycology laboratory, University Hospital, Angers [Lab-1]; private Tharreau laboratory, Segré [Lab-2]; and Parasitology-Mycology laboratory, University Hospital, Poitiers [Lab-3]). In these laboratories, each sample was inoculated onto Sabouraud's agar for mycological culture and treated with the reagents used routinely for direct examination, namely, chloral-lactophenol (CL), MycetColor®, and potassium hydroxide with chlorazol black (KCB), respectively.

* Corresponding author. Tel.: +33-2-41-35-34-72; fax: +33-2-41-35-36-16.
E-mail address: mapihet@chu-angers.fr (M. Pihet).

http://dx.doi.org/10.1016/j.diagmicrobio.2015.06.022
0732-8893/

The remaining samples were stored in closed glass tubes at room temperature for up to 2 months and then sent to the Parasitology-Mycology Laboratory of the Pharmacy Faculty, Angers (Lab-4), which performed a comparative study between 4 tests for direct examination: CL, KCB, MycetColor®, and MycetFluo®. For this evaluation, only 102 specimens with a sufficient quantity to allow these 4 additional direct microscopic examinations were selected. They included 51 nail specimens, 38 skin scrapings, and 13 epilate hair samples. In this laboratory, each sample was divided into 4 equal parts: 1 part for each test. The observers for these 4 microscopic examinations were unaware of the previous results, direct examination and culture results obtained by Labs 1, 2, and 3.

2.2. Direct microscopy

Small amounts of each specimen were deposited into a drop of CL (chloral hydrate, 20 g; phenol, 10 mL; lactic acid, 10 mL) or KCB (KOH, 20 g; glycerol, 10 mL; chlorazol azole E black [0.1%] 10 mL; sterile distilled water, 80 mL) (both from Polysciences Europe, Eppelheim, Germany), on a glass microscope slide. The preparations were covered with a coverslip and pressed gently to remove air bubbles. Fungal elements were then visualized under a light microscope and by phase-contrast microscopy for CL preparations. Samples were considered positive when fungal hyphae and/or arthroconidia were present.

MycetColor® and MycetFluo® (SR2B, Avrillé, France) contain sodium dodecyl sulfate (SDS) and a dye or fluorochrome (Congo red and calcofluor white, respectively). These reagents are able to digest keratin in skin, nail, and hair samples and also to stain hyphae or spores, which are detected with a conventional microscope for MycetColor® or with a fluorescent microscope for MycetFluo®. Staining was performed according to the manufacturer's instructions. Briefly, the skin, nail, or hair samples were deposited onto a microscope slide placed on a black card. Twenty-five microliters of dissociating solution was added, and the sample was squashed gently with a stick to ensure complete immersion in the dissociating solution. After 15–30 min, 50 μL of dye was added, and the whole preparation was homogenized (sample + dissociating solution + stain) and then covered with a coverslip. After incubation for 15 min at room temperature, the slide was examined under a microscope. For MycetColor®, an Olympus BH2™ microscope or Nikon Fluophot™ microscope with white light (blue filter) was used. With this reagent, samples were considered positive when fungal elements (hyphae or conidia) appeared red on a pink or light orange background. Skin or nail cells usually remain unstained or faintly stained. For MycetFluo®, a Nikon Fluophot™ microscope equipped with an epifluorescence attachment was used. By using a combination of UV-excitation filters (excitation wavelength 365 nm) or a combination of V-excitation filters (excitation wavelengths 410–420 nm), samples were positive when fungal elements (hyphae or conidia) harbored blue-white or green-blue fluorescence, respectively.

All direct microscopic examinations were carried out using 10× or 40× objective lens amplification. Images were acquired using a Nikon M35™ automatic exposure camera with Kodacolor Gold™ ISO 400 film.

2.3. Cultures

For dermatophyte cultures, 3–5 fragments of nail, skin, or hair sample were placed on Sabouraud's dextrose agar plates containing antibiotic (chloramphenicol) and supplemented with cycloheximide (Actidione®; Sigma-Aldrich, St. Louis, MO) to prevent contamination by moulds. Plates were incubated for up to 3 weeks at 30 °C. Isolates were identified to the species level by macroscopic and microscopic examination.

2.4. Performance calculation and statistical analysis

Samples were considered positive when either direct microscopic examination (whatever the technique used) or culture or both were positive. The other samples were considered negative. These positive/negative results were used as the "gold standard" to evaluate the performance of MycetColor® and MycetFluo®.

Statistical analysis was performed using McNemar's test. The χ^2 test was performed to determine whether there was a statistically significant difference between the 4 reagents used for microscopic examination and between microscopic examination and culture. $P < 0.05$ was considered statistically significant.

3. Results

Among the 102 samples tested, cultures were positive for 20 (39.2%), 24 (63.1%), and 13 (100%) nail, skin, and hair samples, respectively. Dermatophytes were identified to the species level using morphological examination (macroscopic and microscopic characteristics).

Sixty-seven of the specimens (65.7%) were positive by at least 1 method of direct examination or by culture. These results of direct examination by Lab-1, Lab-2, and Lab-3 were confirmed by Lab-4 (data not shown). Out of 51 nail specimens examined, 28 were positive by microscopy or culture (54.9%) (Table 1). The results of the comparative study carried out by Lab-4 showed that the CL, KCB, and MycetColor® methods were positive for 14 (27.4%), 13 (25.4%), and 13 (25.4%) of nail samples, respectively (Table 1). The differences were not statistically significant. MycetFluo® was positive in significantly more cases than the other methods: 25 (49%) versus 13 or 14 samples ($P < 0.05$). Twenty-six out of 38 skin samples (68.4%) were positive by direct microscopy or culture (Table 1). A total of 16 (42.1%) skin samples were positive using each of the CL, KCB, or MycetColor® techniques versus 20 (52.6%) positive samples using MycetFluo® ($P = 0.125$). Among the 42 positive cultures from skin and nail scrapings, *Trichophyton rubrum* was the predominant species isolated (52.3%), followed by *Trichophyton interdigitale* (35.7%) and *Trichophyton mentagrophytes* (7.1%). *Epidermophyton floccosum* and *Microsporum canis* were isolated only once each (Table 1). No correlation was observed between the performance of the different methods used for direct examination and the genus or species of dermatophyte isolated. Thirteen hair samples (100%) were positive by each of the 4 methods of direct examination. The species identified in culture were *Trichophyton soudanense*, *Trichophyton tonsurans*, *Microsporum audouinii*, and *Trichophyton mentagrophytes*.

The sensitivity of CL, KCB, MycetColor®, MycetFluo®, and culture was 64.2%, 62.7%, 62.7%, 83.6%, and 85%, respectively (Table 2), and the negative predictive value (NPV) was 59.3%, 58.3%, 58.3%, 76%, and 77.7%, respectively. As samples were considered positive when direct microscopic examination or culture was positive, no false-positive result was, therefore, noted, and consequently, all techniques had a specificity of 100%.

Figs. 1–3 show the images obtained using MycetColor® (Fig. 1) and MycetFluo® (Fig. 2–3) with different samples (skin, nails, and hair). Depending on the specimen, staining of the filaments appeared more or less pronounced with MycetColor®. Observation was greatly facilitated using MycetFluo®, and identification of the fungal elements was easier, as they appeared fluorescent blue or green.

4. Discussion

Treatment of dermatophytosis is frequently prolonged, often weeks or months in the case of tinea capitis or onychomycosis. For this reason, a definitive diagnosis is essential before starting treatment (Hainer, 2003). Despite recent progress in molecular techniques, conventional mycological analysis by direct examination and culture is the standard method used in most laboratories.

For direct examination, which allows a quick response to clinicians, specimens need to be dissociated between a slide and a coverslip in a drop of dissociating agent such as KOH (10–20%) with or without dimethyl sulfoxide or CL. To facilitate observation and increase the