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Research Article

Epidemiology of Superficial Mycoses and the Role of Multiplex Real-Time PCR in Diagnosing Dermatophytosis in Our Setting

Romano Mattei*, Gabriele Marchi, Nicola Simoncini, Letizia Paganucci, Giulia Cappellini, Giulia Benelli, Elena Rossi, Jessica Bacigalupi and Moreno Ferroni

Chemical-Clinical and Microbiological Analysis, Laboratory Eurofins Centro Medico LAMM, Italy

***Corresponding Author:** Romano Mattei, Chemical-Clinical and Microbiological Analysis, Laboratory Eurofins Centro Medico LAMM, Italy.

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Abstract

Superficial mycoses affecting the skin, nails, and hair are among the most common fungal infections. This study aimed to investigate the etiological agents responsible for superficial mycoses in select provinces of the Tuscany region, Italy, over a four-year period, and to evaluate the effectiveness of molecular diagnostic methods for dermatophytosis. The study included 1,250 outpatients with clinically suspected superficial mycoses who visited the Eurofins Medical Center in Lucca, Italy, between 2020 and 2024. The results showed that 851 samples tested negative, while 399 were positive. Among the positive cases, 234 were caused by dermatophytes, 123 by yeast-like fungi, and 37 by Non-Dermatophyte Moulds (NDM). The most frequently isolated dermatophyte was *Trichophyton rubrum* (65.8%), while Candida albicans was the predominant yeast (82%), and Fusarium species were the most common NDM (37%). In 2021, we introduced the DERMADYN IVD KIT, a real-time multiplex PCR assay designed to detect seven dermatophyte species: *Trichophyton rubrum, Trichophyton tonsurans, Trichophyton violaceum*, the *Trichophyton mentagrophytes* complex, *Microsporum canis, Microsporum (Nannizzia) gypseum,* and *Epidermophyton floccosum*. The DERMADYN multiplex assay demonstrated a sensitivity of 90.7%, specificity of 99.2%, positive predictive value of 97.5%, negative predictive value of 96.8%, and overall accuracy of 97.0%.

Aim: This study aimed to investigate the etiological agents responsible for superficial mycoses in select provinces of the Tuscany region, Italy, over a four-year period, and to evaluate the effectiveness of molecular diagnostic methods for dermatophytosis.

Keywords: Dermatophytes; Yeasts; Non-Dermatophyte Moulds; Culture; Real-Time Multiplex PCR

Introduction

Superficial mycosis is a common fungal infection worldwide, considered a major public health problem, whose distribution depends on geographic, demographic and environmental factors and is often associated with underlying comorbidities. According to investigations conducted by the World Health Organization, approximately 25% of the world's population has been found to be affected by dermatophytes [1-3]. Onychomycosis represents more than 50% of the nail pathologies [4-6]. Epidemiology of dermatophyte infections in Italy and in Europe has changed rapidly due to the increase in mass tourism, social and economic improvements and immigration., in particular the appearance of rare agents, like *Trichophyton violaceum* and *Trichophyton soudanense* [7,8]. Although dermatophyte do not cause mortality, their clinical significance lies in their morbidity, recurrence and cosmetic disfigurement. In addition to their high distribution in various parts of the world and the associated clinical manifestations, dermatophytes have recently started to show antifungal resistance [9-11], thus making

it essential to understand virulence factors and pathogen-host interactions in order to introduce new therapeutic approaches [12]. The etiological agents of dermatophytosis, also known as tinea, are dermatophytes fungi classified into three distinct genera, namely *Trichophyton*, *Microsporum*, and *Epidermophyton*. Dermatophytes require keratin for nutrition and must live on stratum corneum, hair, or nails to survive and transmission occurs from person to person, from animal to person, or from soil to person. After inoculation into the host's skin, favourable conditions promote the progression of infection through the phases of adherence and penetration. The host's immune response primarily develops through a delayed-type hypersensitivity reaction mediated by T-cells. However, antibody production does not seem to offer protective effects. Natural defence mechanisms against dermatophytes rely on a combination of both immunological and non-immunological processes [13]. Recent studies highlight a shifting trend in fungal infections, with a growing prevalence of yeast and non-dermatophyte moulds as causative agents (e). While dermatophytes remain the primary cause of onychomycosis, *Candida* species are increasingly recognized as emerging pathogens responsible for skin and nail mycoses [14-16]. Infections caused by Non-Dermatophyte Moulds, once considered mainly contaminants, are on the rise [17,18]. Although their definitive role in skin infections is not yet fully established, their involvement in nail infections is well documented [19-21]. Given the importance of a sensitive and specific diagnosis of fungal infections, our aim in this study is to investigate the epidemiology in some provinces of Tuscany and at the same time describe our diagnostic approach.

Methods

Sample collection

Collection [26], transport and storage of samples was carried out according to the UK Standards for microbiological investigations used to isolate dermatophytes, non-dermatophyte moulds and other fungi from skin, nail and hair samples. Skin scraping, nail clipping, and subungual scraping samples were divided into three portions: one for KOH smear, one for culture, and one for DNA extraction and RT-PCR. The samples were obtained from patients at various medical centers and sent to our laboratory for microbiological and molecular analyses.

KOH smear

Clinical specimens were placed on a glass slide with one drop of 30% potassium hydroxide (KOH) and left to stand at room temperature for 20 minutes. The samples were then examined under a microscope for the presence of fungal elements.

Culture

At present, the gold-standard method to identify the specific type of dermatophyte is the culture [27]. Clinical specimens were cultured on two agar plates: Sabouraud Dextrose Agar containing chloramphenicol and gentamicin and on Sabouraud Dextrose Agar with chloramphenicol and cyclohexamide. The cultures were incubated at 28°C for 1 to 4 weeks. Malassezia species were cultured by overlaying Sabouraud Dextrose Agar containing cycloheximide (actidione) with olive oil. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) [28,29] was used in some doubtful cases for identification of dermatophytes from cultures, Vitek 2 was used for identification of yeasts while for non-dermatophytic fungi the diagnosis was based only on the macroscopic and microscopic characteristics of the colonies.

PCR

Primers and probes were custom-designed by DYN R&D Laboratories (DYN R&D Ltd. Beit Jacqueline building, Sagi 2000 industrial park, 2310000 Migdal Haemeq, Israel) to detect seven dermatophyte strains, including *Trichophyton rubrum*, *Trichophyton tonsurans*, and *Trichophyton violaceum*, *Trichophyton mentagrophytes* complex (var. *interdigitale, mentagrophytes, erinacei*), *Microsporum canis, Microsporum gypseum, Epidermophyton floccosum*].

Results

Thirty-six scalp samples were examined, only three patients tested positive for dermatophytes, respectively for *Microsporum canis*, *Tricophyton soudanense* and *Trichophyton violaceum*, no non-dermatophyte yeasts or fungi were detected. The skin samples examined were 545 of which 113 (20.7) were positive for dermatophytes, 45 (8.3%) for yeasts and 7 (1.3%) for non-dermatophyte fungi. The nail samples examined were 670 of which 118 (17.6%) were positive for dermatophytes, 78 (11.6) for yeast and 30 (4.5%) for non-dermatophyte fungi Table 1.

Prior to routinely introducing multiplex real-time polymerase chain reaction (RT-PCR) with the DERMADYN IVD Kit, we validated the test on dermatophyte colonies previously identified by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS). Concordance between the two tests was 100% (Table 2).

Table 1: Frequency and causative agents of superficial mycoses of skin and nails due to dermatophytes, yeasts and non-dermatophyte molds (NDMs).

Fungi groups	Total	Total %	Skin	Skin (%)	Nails	Nails (%)
Dermatophytes	231	72.2	113	48.9	118	51.1
Epidermophyton floccosum	3	1.3	3	2.66	0	0
Microsporum canis	2	0.87	2	1.77	0	0
Microsporon gypseum	7	3.0	4	3.5	3	2.5
Microsporon fulvum	1	0.4	1	0.9	0	0
Tricophyton interdigitale	35	15.2	18	15.9	17	14.4
Tricophyton mentagrophytes	25	10.8	11	9.7	14	11.9
Tricophyton rubrum	155	67.1	72	63.7	83	70.3
Tricophyton soudanense	1	0.4	1	0.9	0	0
Tricophyton tonsurans	1	0.4	1	0.9	0	0
Tricophyton violaceum	1	0.4	0	0	1	0.85
Yeasts	123	38.4	45	36.6	78	63.4
Candida albicans	44	35.8	16	35.6	28	35.9
Candida guilliermondii	12	9.8	5	11.1	7	9.0
Candida glabrata	2	1.6	0	0	2	2.6
Candida lipolytica	9	7.3	2	4.4	7	9.0
Candida parapsilosis	36	29.3	11	24.4	25	32.0
Candida tropicalis	2	1.6	0	0	2	2.6
Cryptococcus terreus	2	1.6	1	2.2	1	1.3
Kloeckera species	1	0.8	0	0	1	1.3
Malassezia species	10	8.1	10	22.2	0	0
Trichosporon asahii	2	1.6	0	0	2	2.6
Tricosporon mucoides	3	2.4	0	0	3	3.8
Non-Dermatophyte Moulds	37	11.6	7	18.9	30	81.1
Acremonium species	1	2.7	0	0	1	3.3
Alternaria	1	2.7	0	0	1	3.3
Aspergillus flavus	3	8.1	0	0	3	10
Aspergillus fumigatus	1	2.7	0	0	1	3.3
Aspergillus niger	4	10.8	0	0	4	13.3
Aspergillus terreus	15	40.5	3	42.9	12	40
Aspergillus versicolor	2	5.4	0	0	2	6.7
Cladosporium	1	2.7	0	0	1	3.3
Fusarium	9	24.3	4	57.1	5	16.7
Total	391	100	165	42.2	1	57.8

Table 2: Reference microbial strains used for analytical kit validation.

Sample	Maldi-Tof	Derma -dyn
1	*Epidermophyton floccosum*	*Epidermophyton floccosum*
2	*Microsporum canis*	*Microsporum canis*
3	*Microsporon (Nannizia) gypseum*	*Microsporon (Nannizia) gypseum*
4	*Microsporon fulvum*	*Microsporon fulvum*
5	*Tricophyton interdigitale*	*Tricophyton interdigitale*
6	*Tricophyton mentagrophytes*	*Tricophyton mentagrophytes*
7	*Tricophyton rubrum*	*Tricophyton rubrum*
8	*Tricophyton violaceum*	*Tricophyton violaceum*
9	*Trichophyton soudanense*	Negative
10	*Candida albicans*	Negative
11	*Candida parapsilosis*	Negative
12	*Aspergillus fumigatus*	Negative
13	*Aspergillus terreus*	Negative
14	*Fusarium spp*	Negative
15	*Cladosporium spp*	Negative

Since 2021, we have performed multiplex real-time polymerase chain reaction (RT-PCR) with the DERMADYN IVD kit simultaneously with culture on 157 nail and skin samples. 120 samples were negative in both culture and PCR. In 148 samples (94.3%) there was complete concordance between PCR and culture. RT-PCR identified 10 cases of *Trichophyton rubrum* that did not grow in culture, while culture yielded 4 RT-PCR-negative *Trichophyton rubrum* isolates. One culture positive for *Microsporum gypseum* was misidentified by PCR as *Trichophyton mentagrophytes*.

Distribution of dermatophyte species by cultures and PCR are shown in Table 3, statistic results in table 4.

Discussion

We believe that the pre-analytical phase in particular and the training of operators dedicated to sampling skin and skin appendages is of fundamental importance. Precisely because of the turnover, many healthcare workers have not been sufficiently trained and this has meant that many samples received by our Laboratory were insufficient to be divided into three parts most likely as per operating instructions. Thus, on numerous samples only the cul-

Table 3: Distribution of dermatophyte species by cultures and PCR (N = 157).

Dermatophytes	Culture method	Molecular method
Tricophyton rubrum	4	0
Tricophyton rubrum	0	10
Tricophyton rubrum	18	18
Tricophyton mentagrophytes	4	4
Microsporon gypseum	3	2
Tricophyton iinterdigitale	5	4

Table 4: Statistic results of Dermadyn multiplex NT-RNA.

Statistic	Value	95% CI
Sensitivity	90.70%	77.86% to 97.41%
Specificity	99.17%	95.48% to 99.98%
Positive Likelihood Ratio	109.74	15.55 to 774.63
Negative Likelihood Ratio	0.09	0.04 to 0.24
Disease prevalence	26.22%	19.67% to 33.65%
Positive Predictive Value	97.50%	84.67% to 99.64%
Negative Predictive Value	96.77%	92.18% to 98.71%
Accuracy	96.95%	93.03% to 99.00%

ture test and the PCR were performed and not the microscopic examination. Therefore, we decided not to report the data from direct microscopy both due to the insufficient number of samples tested and the poor specificity of the test.

The gold standard culture test is currently irreplaceable as it is the only test that allows the search for dermatophytes, yeasts and non-dermatophyte fungi and the subsequent identification of species through the macroscopic and microscopic characteristics of the colonies and the possibility of confirming doubtful cases on Maldi-Tof. The disadvantages of the culture test are the long TAT (2-4 weeks), the need to request a new sample in the event of contamination by environmental fungi and the morphological identification which requires significant expertise. The clinical utility of PCR testing for diagnosing fungal infections has been highlighted in numerous studies, particularly for its rapid turnaround time and ability to detect DNA from dermatophytes that are no longer viable due to treatment [22-25]. In our practice, the Dermadin multiplex assay has demonstrated high sensitivity (90.7%) and specificity (99.2%), and high positive and negative predictive value of 97.5%, and 96.8%, respectively. Additionally, the present study shows that PCR method is significantly faster than traditional culture, with an average response time of 1 day compared to 19 days for culture, enabling physicians to initiate timely and appropriate antifungal therapy. A further advantage is that results are not compromised by sample contamination from environmental moulds. However, as with all molecular methods, its main limitations include the inability to detect species not targeted by the test and the requirement for specialized equipment and dedicated laboratory space.

This study confirms the epidemiology observed in several other publications and at the same time the need to combine classical methods, direct microscopy and culture, with PCR-based methods to obtain a better diagnostic sensitivity and a short TAT useful to quickly start antifungal treatment [22-25,30-36].

Conclusion

Many non-infectious conditions affecting the skin and its appendages—such as psoriasis, contact dermatitis, seborrheic dermatitis, and nail trauma—can present with symptoms similar to those of fungal infections. Therefore, accurately confirming a fungal etiology is crucial not only for ensuring appropriate treatment but also for preventing unnecessary antifungal therapy based solely on clinical evaluation. In this context, PCR plays a vital role in confirming dermatophytosis diagnoses, particularly in patients undergoing antifungal treatment.

Our study, in agreement with the literature, confirms that the real-time multiplex PCR DERMADYN IVD KIT has an excellent performance in the rapid diagnosis of dermatophytoses caused by the main dermatophytes, with high sensitivity, specificity, positive and negative predictive values.

In the future we hope for the introduction of new multiplexes that on the basis of epidemiological data expand the number of identifiable dermatophyte species and the simultaneous search for the main yeast and Non-Dermatophytic Moulds.

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