

Dermatophytes Species Isolated of HIV-Infected Patients Identified by ITS-RFLP and ITS Region Sequencing from Triângulo Mineiro, Minas Gerais State of Brazil

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Abstract

Dermatomycoses are a group of diseases worldwide distributed and affect skin, hair and nails. Several fungal species can be involved and keratinophilic fungi belonging to the genera *Trichophyton*, *Microsporum* and *Epidermophyton* are very common. These infections are globally distributed and their incidence has progressively increased. Despite their high incidence, few studies have been carried out in HIV-infected patients, regarding frequency, clinical presentations, and species identification. This study aimed to evaluate some epidemiological and clinical aspects of dermatophytosis in HIV-infected patients and to attempt phenotypical and molecular characteristics of their agents. Of 398 patients included, 306 were HIV-infected. Clinical data and samples of skin, hair and nail lesions were simultaneously obtained. The dermatophytes yielded in culture were phenotypically identified and evaluated by ITS-RFLP and ITS (Internal Transcribed Space) sequencing. Dermatophytes frequency in HIV-infected patients with cutaneous lesions was 11.76% similar to the 15.22% observed in non-HIV individuals. *Trichophyton rubrum* was the most common isolated species in both groups. Through ITS-RFLP, 29/32 (90.63%) of dermatophytes isolates were identified at the species level. From the 10 isolates randomly selected for ITS sequencing, seven confirmed the ITS-RFLP results while three *Trichophyton* sp. were not identified as dermatophytes. The identification of these species in HIV-infected patients is highly desirable in order to improve the knowledge of their fre-

quency, geographical distribution and relation to clinical presentation.

Keywords

Dermatophytosis, Dermatophytes, ITS-RFLP, ITS-Sequencing, HIV-Infected Patients, *Trichophyton rubrum*

1. Introduction

Dermatomycoses are common cutaneous infections of keratinized tissues, caused by several fungal species which invade the corneous layer of skin, hair and nails. Among these, keratinophilic fungi belonging to the genera *Trichophyton*, *Microsporum* and *Epidermophyton* are very common. These infections are globally distributed and their incidence has progressively increased [1]. According to the World Health Organization (WHO), approximately 20% - 25% of the world population has some kind of cutaneous fungal infection [1] [2] [3].

These infections can have acute or chronic clinical presentation, depending on the degree of inflammation and it is hypothesized that these mycoses trend to be more frequent and severe in immunocompromised individuals [4] [5] [6]. The HIV infection is one of the most important risk factors to the occurrence of fungal infections and due to the scarcity of data, the prevalence, clinical features, diagnosis and outcome of the dermatomycosis in HIV-infected individuals are still unclear [7]. Moreover, cellular immunity is pivotal in the control of these infections and Th1 and Th2 responses balance can determinate the progression of the infection in such patients [8] [9].

The taxonomy of dermatophytes remained confused for a long time, until the discovery of the sexual reproduction in keratinophilic species, which led to the introduction of the biological concept of species [10]. With the advent of molecular biology, conventional morphological taxonomy was influenced by data obtained DNA sequencing which improved the taxonomic system [11]. One of the sequencing targets is the Internal Transcribed Spacer (ITS) region of ribosomal DNA, which is considered the main barcode of the fungal kingdom [12] [13]. Furthermore, the sequencing of different genes, specially using the genealogical concordance phylogenetic species recognition (GCPSR) can be useful to improve fungal diagnosis, biodiversity range, species conservation and taxonomy accuracy [11].

The present report aimed to evaluate some epidemiological and clinical aspects of dermatophytosis in HIV-infected patients and to attempt characterizes phenotypic and molecular aspects of their etiological agents in a teaching hospital from Brazil.

2. Population and Methods

2.1. Population

This study was carried out at the Infectious Diseases Outpatient Unit in a Bra-

zilian Teaching Hospital from August 2014 to December 2015. A total of 398 adult patients of both genders were randomly recruited and divided into three groups: I composed by 153 HIV-infected individuals with skin, hair or nail lesions, II composed by 153 HIV-infected without apparent clinical lesions in these sites and III composed by 92 non-HIV-patients with clinical lesions similar to group I seen at the Dermatology Outpatient Clinic of the same hospital. The inclusion criteria were: both genders, age over 18 years, that was receiving antiretroviral therapy and agree to participate of the study. Those who referred antifungal use within 30 days prior to the recruitment or disagreed to participate were excluded.

Epidemiological and clinical data of the HIV-infected patients with dermatomycosis were registered and a clinical exam was performed. Nail and cutaneous samples were obtained by scraping while hair fragments were obtained by removing a small amount of hair containing their bulb. Concomitantly, nail scrapings samples of the 153 HIV-infected without clinical lesions were obtained.

These clinical samples were examined by direct microscopic using potassium hydroxide 20% (KOH) in order to identify fungal structures. Then, they were seeded in Sabouraud Dextrose Agar added with 0.05 mg·mL⁻¹ chloramphenicol and Mycosel Agar, incubated at 28°C ± 2°C and observed for up to 30 days. Identification of the yielded filamentous fungi was carried out using the microculture technique in Potato Dextrose Agar for seven days at room temperature.

2.2. Molecular Identification of Isolates

The DNA extraction was performed by the phenol-chloroform method [14]. Then, a PCR reaction of the ITS region of ribosomal DNA was performed using ITS-1 and ITS-4 primers as previously described with modifications [15]. Briefly, each reaction contained a final volume of 50 µL with 50 ng of template DNA, 5 µL of the 1X PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.1%, 1.5 mM MgCl₂), 0.25 mM dNTP's (dATP, dCTP, dGTPe dTTP), 1.25 U Taq DNA polymerase (Invitrogen, Brazil) and 70 pmol of each primer. The PCR was performed under the following conditions: initial denaturation at 94°C (3 min) followed by 29 cycles with denaturation at 94°C (30 s), association at 57°C (30 s), extension at 72°C (45 s) and one cycle 10 minutes at 72°C.

Next, the DNA was digested using the restriction enzyme *MvaI* Fast Digest (Thermo Scientific, USA) according to the manufacturer's instructions. The fragments were detected by electrophoresis in 2% agarose gel at 80 V for 2 hours. The profiles were visually identified by comparison with the restriction patterns already described [16] [17]. The strain ATCC MYA4439 of *Trichophyton interdigitale* was used as a positive control.

The amplified DNA of ten randomly selected isolates was submitted to the sequencing reaction using the BigDye terminator 3.1 reagent kit (Applied Biosystems, Foster City, CA, USA). The sequences obtained were analyzed in the software Chromas-pro 1.5 and then compared with the available data in the National Center of Biotechnological Information (NCBI—National Center for Biotechnology Information), including sequences of reference strains. Multiple align-

ments were performed in the Clustal W software [18]. The best model used in the phylogenetic tree was chosen from the software JModel test using the Bayesian Information Criterion (BIC). The Tamura Nei substitution model with Gamma distribution was the best model for the aligned dataset and used to construct the phylogenetic tree. The phylogenetic analysis was performed in the MEGA 7.0 software with the Maximum likelihood method [19]. The Bootstrap analysis with 1000 replicates was used to support the tree nodes.

2.3. Statistical Analysis

Data analysis was performed using Epi Info software (Statistical Package for the Social Sciences, version 7.0, USA) and GraphPad Prism (version 5.0, USA). The verification of normal distribution of the quantitative variables was done by the Shapiro-Wilk test. Continuous variables with normal distribution were expressed as mean \pm standard deviation and those with ab-normal distribution were expressed in median, with maximum, minimum and percentiles values. Mann-Whitney and Fisher exact tests were used to evaluate the differences and frequencies among groups. Values of $p < 0.05$ were considered statistically significant.

The project was approved by the Research Ethics Board of the Triângulo Mineiro Federal University in Uberaba, Minas Gerais State, Brazil under protocol number: 1716.

3. Results

Of 306 HIV-infected patients, 178 (58.17%) were male (mean age: 43.86 ± 12.24 years) (Table 1). Of 153 HIV patients with cutaneous, hair or nails lesions, in 61 (39.87%) at least one fungal structure was observed by direct examination whereas in 72 (47.06%) of cases a positive fungal culture was obtained. The presence of skin, hair or nail lesions was significantly associated with the chance to obtain a positive fungal direct exam or culture ($p < 0.001$) (Table 2).

Of 92 non-HIV patients evaluated, 55 (59.78%) were female (mean age: 52.64 ± 13.63 years). Of these, 43 (46.74%) and 48 (52.17%) presented a fungal positive direct exam and culture, respectively (Table 2).

Among the recovered fungal isolates, 32 were phenotypically identified as dermatophytes, of which 18 (56.25%) were obtained from HIV-infected patients while the remaining 14 (43.75%) were from non-HIV patients (Table S1).

The frequency of dermatophytes in HIV-infected patients with clinical lesions (11.76%) was similar to that observed in non-HIV patients (15.22%) ($p = 0.4406$). *Trichophyton rubrum* was predominant followed by *Trichophyton interdigitale* in both groups (Table S1).

Of the 18 HIV-infected patients with dermatophytes isolation, 14 (77.78%) were males (mean age: 48.22 years) while the 9 (64.29%) of the 14 non-HIV patients with isolated dermatophytes were female (mean age: 50.93 years). Most individuals were from urban areas and had a low scholar level. Other epidemiological and clinical data are described in Table 3 and Table S1.

Table 1. Main demographic, epidemiological and clinical data of the HIV-infected and Non-HIV patient groups.

		HIV-infected patients (n = 306)	Non-HIV patients (n = 92)
Age (years)	Average (\pm SD ^{**})	43.86 (\pm 12.24)	52.64 (\pm 13.63)
Gender	Male	178 (58.17%)	37 (40.22%)
Educational level	Complete elementary school	46 (15.06%)	4 (4.35%)
	Incomplete elementary school	168 (54.90%)	32 (34.78%)
	Complete high school	44 (14.38%)	32 (34.78%)
	Incomplete high school	20 (6.54%)	4 (4.35%)
	Complete college	8 (2.61%)	9 (9.78%)
	Incomplete college	6 (1.96%)	5 (5.43%)
	None	14 (4.58%)	6 (6.52%)
	Profession	Retired	68 (22.22%)
Housewife		53 (17.32%)	21 (22.83%)
Farmer		31 (10.31%)	3 (3.26%)
Student		5 (1.63%)	1 (1.09%)
Others		111 (36.27%)	45 (48.91%)
None		38 (12.42%)	2 (2.17%)
Domicile	Urban	275 (89.87%)	85 (92.39%)
	Rural	31 (10.13%)	7 (7.61%)
	Swimming/pool practices*	-	42 (13.86%)
Skin/nail lesions	-	153 (50%)	92 (100%)
Previous history of skin/nail lesions*	Yes	134 (44.23%)	62 (67.39%)
	No	159 (52.47%)	28 (30.43%)
	Unknown	10 (3.3%)	2 (2.17%)
Underlying diseases	-	130 (42.48%)	50 (54.35%)
Frequency of the contact with animals*	Common	157 (51.82%)	35 (38.04%)
	Often	26 (8.58%)	10 (10.87%)
	Occasional	13 (4.29%)	8 (8.70%)
	None	107 (35.31%)	39 (42.39%)

*Three patients from the HIV-infected group were excluded due to unavailable data; **SD: standard deviation.

Table 2. Mycological diagnosis according with the presence or absence of skin, hair or nail lesions in HIV-infected patients.

	Presence of lesions n (%)	Absence of lesions n (%)	p
KOH+	61 (39.87)	12 (7.84)	<0.0001
KOH-	92 (60.13)	141 (92.16)	-
Culture+	72 (47.06)	31 (20.26)	<0.0001
Culture-	81 (52.94)	122 (79.74)	-
Total	153 (100)	153 (100)	-

*KOH: potassium hydroxide 20%.

Table 3. Epidemiological and clinical data of patients with positive culture for dermatophytes.

		HIV-infected with cutaneous lesions n = 18 (%)	Non-HIV patient n = 14 (%)
Age (years)	Average (range)	48.22 (32 - 63)	50.93 (37 - 71)
Gender	Male	14 (77.78)	5 (35.71)
History of contact with animals	-	10 (55.56)	7 (50)
Underlying diseases	-	7 (38.89)	9 (64.29)
Previous skin/nail lesions	-	13 (72.22)	10 (71.43)
Previous antifungal therapy	Yes	8 (44.44)	5 (35.71)
	No	8 (44.44)	8 (57.14)
	Not reported	2 (11.11)	1 (7.14)
T CD4⁺ cells/mm³	<200	8 (44.44)	NA
	200 to 499	2 (11.11)	NA
	≥500	8 (44.44)	NA
Viral load (copies of RNA/mL)	<50	8 (44.44)	NA
	50 to 100,000	7 (38.89)	NA
	>100.000	3 (16.67)	NA

NA: not available.

3.1. ITS-RFLP

Of 32 isolated dermatophytes, 29 (90.63%) were identified at the species level by the ITS-RFLP profiles. All 18 isolates phenotypically characterized as *T. rubrum* were confirmed by ITS-RFLP. Of the six isolates previously identified as *T. interdigitale*, four presented the typical profile of this species while two were reclassified as *T. rubrum* (Figure 1). Two isolates previously identified as *Microsporum canis* and *T. verrucosum* were confirmed as well (data not shown).

From the six isolates (D04, D05, D07, D22, D30 and D32) previously identified as *Trichophyton* sp., three (D04, D30 and D32) were confirmed as *T. rubrum* by the ITS-RFLP (Figure 1). The remaining isolates did not present any pattern of digestion by this technique.

3.2. Sequencing of the ITS Region and Phylogenetic Analysis

Ten (31.25%) of isolates were randomly selected for ITS sequencing. The generated ITS fragments presented approximately 630 bp. Of these, seven (70%) were adequately confirmed being five *T. rubrum* and two *T. interdigitale*. The sequences presented 97% to 100% of identity in relation to clinical isolates from USA, Australia, India, China, Russia and Japan.

The comparison of these sequences with the 29 reference sequences from GenBank, demonstrated that the *T. rubrum* isolates herein identified appear to have a greater phylogenetic homogeneity in comparison to the CBS 392.58 reference

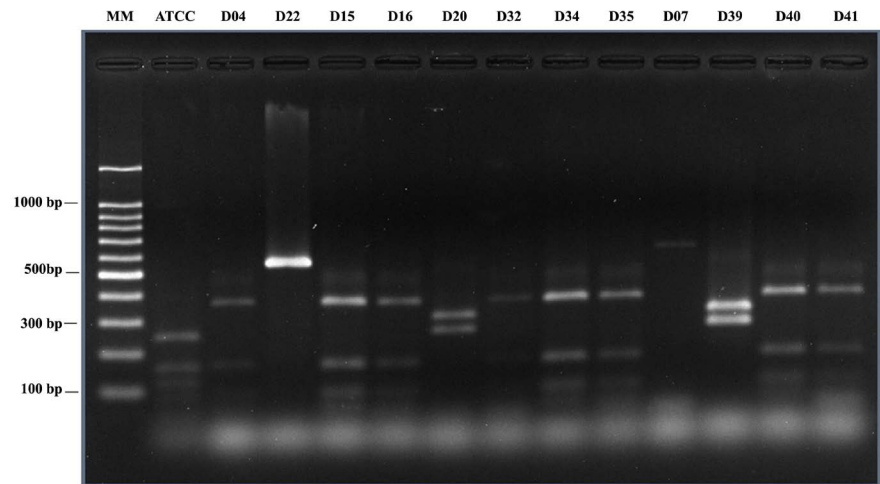


Figure 1. Agarose gel (2%) representative of the band pattern after digestion with *Mva I*. ATCC: MYA4439 *Trichophyton interdigitale*; D04, D15, D16, D32, D34, D35, D40, D41: *Trichophyton rubrum*; D22, D07: not digest samples; D20, D39: excluded isolates. MM: LowRanger 100 bp DNA Ladder (NorGen Biotek, Canada). Color: Ethidium Bromide (0.5 µg/mL).

strain. Otherwise, the isolates D33 and D38 were grouped together with the anthropophilic strains of *T. mentagrophytes* complex (**Figure 2**).

The remaining three isolates formerly identified as *Trichophyton* sp. by their phenotypic aspects were identified within *Ascomycetes*: *Lecanicillium aphanocladii* (MH346472), *Preussia* sp. (MH346502) and *Montagnulaceae* sp. (MH348207).

4. Discussion

Dermatophytes comprise a group of pathogenic filamentous fungi that cause cutaneous mycoses and due to their ubiquity in nature and high transmissibility, the infections caused by these agents are often considered a neglected problem [20] [21]. Currently, HIV infection is considered one of the major global public health problems and a wide gamut of diseases with cutaneous involvement in HIV-infected patients has been already reported. Due to the immunosuppression, atypical skin lesions can lead to clinical misdiagnosis and treatment difficulties [22] [23].

The relationship between dermatophytosis and HIV infection remains poorly understood, regarding their frequency, causative species and therapeutical outcome [9] [24]. Of 306 HIV-infected patients herein evaluated, 50% presented cutaneous, hair or nail lesions, often with atypical and heterogeneous appearance. Despite fungal etiology had been suspected, other causes should be excluded and for this direct exam, culture and molecular methods are pivotal to improve the diagnosis accuracy and therapeutical decision [25].

In the present report, 39.87% and 47.06% of HIV-infected patients with skin, hair or nail lesions presented direct exam and positive fungal culture respectively. Whereas in non-HIV individuals, 46.74% and 52.17% of cases presented direct exam and culture positive respectively. These figures were similar with those

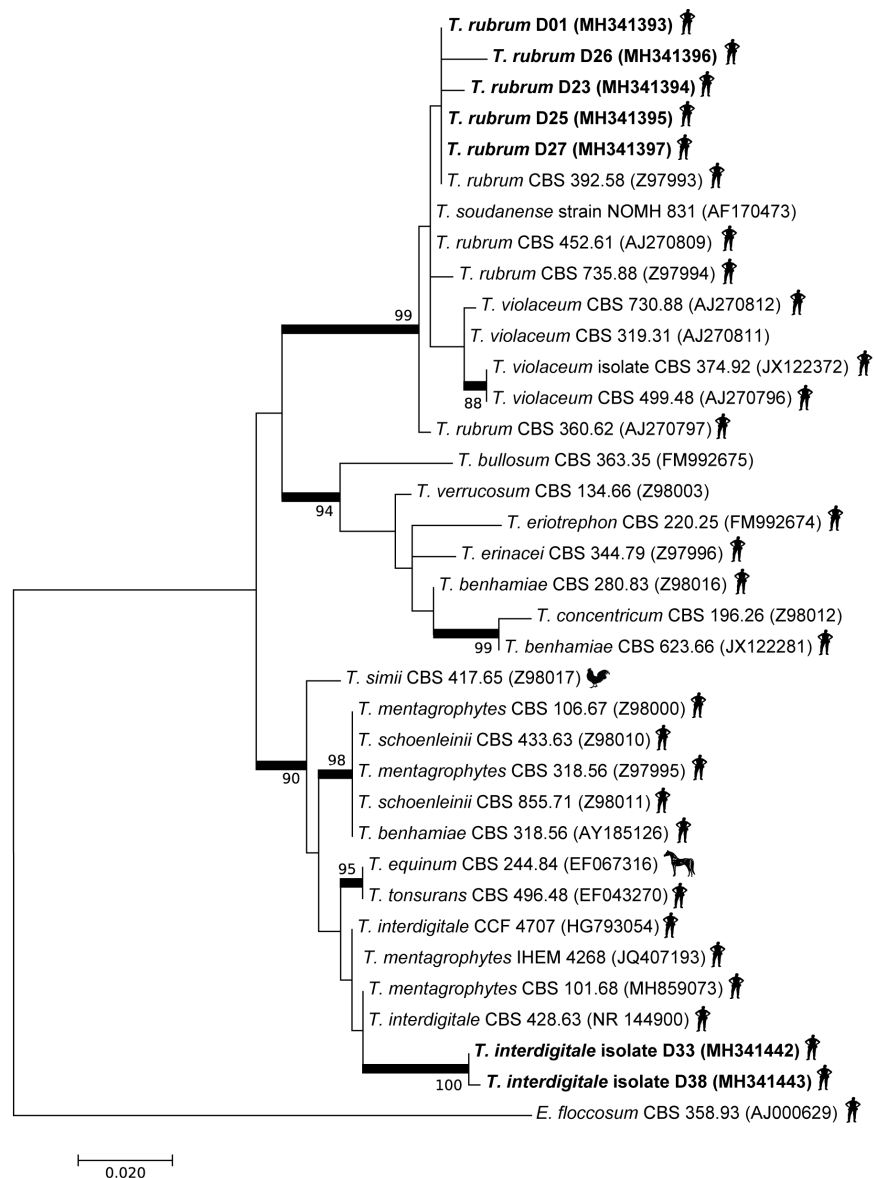


Figure 2. The evolutionary history of the phylogenetic tree was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-1415.8245) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3745)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 437 positions in the final dataset and 36 isolates were tested. The isolates are described according species of *Trichophyton*, strain ID, GenBank number, and the host from where they were recovered (e.g. human, horse, or chicken). The isolates described in bold are described in this study. The reference strain *Epidermophyton floccosum* CBS 358.93 was used as outgroup. The bootstrap values above 80% are described next to the branches.

reported from India, where the positivity rate of direct microscopy and culture were respectively 49% and 51% in 100 non-HIV patients with clinical suspicious of dermatophytosis [26].

The frequency of dermatophytes isolation between HIV-infected patients and

non-HIV individuals was similar, a finding in line with another report where 105 HIV-infected patients and 63 non-HIV individuals were evaluated. According to the authors, there was no correlation between the T CD4⁺ cell count and the prevalence of dermatophytosis in HIV patients [7].

Although the relation of dermatophytosis and HIV-infected patients is still unclear, some authors pointed out an inverse relationship between T CD4⁺ cells count and the frequency and extension of cutaneous lesions in these individuals [9]. Most patients herein evaluated exhibited high T CD4⁺ cell counts and undetectable viral load values, which reinforce that they presented adequate adhesion to antiretroviral therapy and partially could explain the low frequency of dermatophytes recovered despite 306 patients were evaluated. Herein and as described for other mycosis, the HIV infection can change the clinical presentation without alters the rate incidence. Maybe, the stratification of CD4⁺ values and the inclusion of patients with advanced immunodeficiency could identify different risk categories for dermatophytes infection.

Regarding the anatomic site, the toenails and feet were the sites where dermatophytes were more frequently isolated. The severity of the nail involvement with chronic and dystrophic changes and a poor antifungal response is a common clinical observation in these patients. This finding agrees with literature data and confirms the results from a recent Brazilian survey where onychomycosis and *Tinea pedis* were the most common clinical pictures in 84 HIV-infected patients evaluated [27]. In contrast, another study from the same country described a high frequency (70%) of *Tinea corporis* in 20 HIV-infected patients [28].

Most dermatophytes species herein identified belong to the *Trichophyton* genus which is in line with other reports that point out 90% of the dermatophytosis around the world are caused by species of this genus [29] [30]. Although *T. rubrum* is found as the main agent of dermatophytosis in HIV-infected patients, it presents global distribution and also is the most common agent recovered from clinical specimens [7] [27].

The *T. verrucosum* isolate was confirmed by ITS-RFLP and recovered from a patient who referred frequent contact with domestic animals. Despite this zoonophilic species is an uncommon agent in humans, its isolation could be clinically relevant since it was recovered from an HIV-infected individual. This species has been commonly associated with farm activities and cattle are known as the main infection source. However, accidental transmission can also occur by contaminated objects [31] [32].

The therapy to superficial mycoses in HIV/AIDS patients is usually started with topical antifungals, whereas the use of systemic drugs is recommended in cases where there is no improvement or when lesions are extensive, deep or widespread. Several reports have pointed out that oral medicaments such as terbinafine, itraconazole or fluconazole are the most appropriate therapeutic options for these individuals. According with other authors, they must be prescribed from two to six weeks except those who present onychomycosis and re-

quire more time of treatment [9] [24]. Patients with dermatomycosis herein described received topical or oral itraconazole with clinical remission of their lesions except those who presented nail involvement and exhibited a poor response.

Despite, culture continues to be one of the most used methods for mycological diagnosis, the ITS-RFLP allowed the identification of 90.63% of the isolated dermatophytes at the species level. The obtained band patterns were variable in size among the different species, which allowed the visual differentiation among *T. rubrum*, *T. interdigitale*, *M. canis* and *T. verrucosum* [17]. The *T. rubrum* complex includes species with different phenotypic characteristics, however this variability has not been homogeneously observed in molecular studies. In these cases, the restriction pattern from other DNA regions or the use of additional enzymes could increase the accuracy of the method [16] [33]. Recently, a whole-genome analysis showed a clonal population profile of this species, which may explain the genetic homogeneity of the complex [34].

In accordance to the current taxonomy of dermatophytes, the anthropophilic isolates are separated from the zoophilic ones constituting the *T. mentagrophytes* species complex [11]. The *T. interdigitale* isolates herein identified presented the same restriction pattern on ITS-RFLP and were grouped together with the anthropophilic ones in the phylogenetic analysis. A recent report with 60 clinical isolates of dermatophytes showed that the most of them belonged to the anthropophilic variant of *T. mentagrophytes* complex [35].

Despite, the DNA sequencing is a reliable technique for fungal identification species and the ITS region is relatively conserved in dermatophytes species, the combination of different genes sequencing of these species by MLST (Multilocus Sequence Typing) undoubtedly is pivotal to improve their identification [11].

Despite the low number of isolates evaluated, the results herein showed that identification based on morphological characteristics of dermatophytes species presents high level of complexity and accuracy limitations, although it is still one of the most used method in the context of clinical mycological diagnosis. Otherwise the ITS-RFLP appears to be useful and reliable tool to identify and to differentiate dermatophytes at the species level. In addition, this technique is still cheaper and simpler than any sequencing method, especially when taking into account limited-resource settings.

Moreover, the sequencing is considered the ideal method to identify the dermatophytes with more precision at the species level and to observe their intraspecific variability. However, logistical and economical issues around the world remain as the main obstacle for its wide implementation as well.

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script.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Authors' Contributions

T.B.F. and M.L.S.V. conceived the study; T.B.F., L.S.L.J. and B.S.P. collected the data; T.B.F. and L.B.S. performed experiments; T.B.F., L.B.S., L.E.A.S., K.F.P. and M.L.S.V. analyzed data; F.A.A.S. and L.R.G. contributed new methods; T.B.F., D.J.M., K.F.P. and M.L.S.V. led the writing.

Conflicts of Interest

There are no conflicts of interest to report.

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Supplementary Material

Table S1. List of the of the patients and isolates identified in the study containing the anatomical site, HIV status, CD4+ count, direct microscopy, phenotypic identification, ITS-RFLP and ITS sequencing.

Patient Strain ID	Anatomical site	Gender	HIV status	CD4+ T-cell count	Direct microscopy	Phenotypic identification	ITS-RFLP	Sequencing	
P23	D01	toenails	M	HIV-infected	104	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	<i>T. rubrum</i>
P27	D04	toenails	M	HIV-infected	94	positive	<i>Trichophyton</i> sp.	<i>T. rubrum</i>	not sequenced
P201	D09	feet	F	HIV-infected	542	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced
P85	D10	toenails	F	HIV-infected	171	negative	<i>T. verrucosum</i>	<i>T. verrucosum</i>	not sequenced
P182	D11	toenails	M	HIV-infected	1585	positive	<i>T. interdigitale</i>	<i>T. interdigitale</i>	not sequenced
P168	D12	feet	M	HIV-infected	160	positive	<i>T. interdigitale</i>	<i>T. interdigitale</i>	not sequenced
P164	D13	arms	M	HIV-infected	529	negative	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced
P188	D14	inguinal	M	HIV-infected	123	positive	<i>T. interdigitale</i>	<i>T. rubrum</i>	not sequenced
P194	D16	trunk	M	HIV-infected	118	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced
P184	D17	toenails	F	HIV-infected	1008	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced
P247	D21	toenails	M	HIV-infected	427	negative	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced
P202	D22	fingermails	M	HIV-infected	876	negative	<i>Trichophyton</i> sp.	not digested	<i>Montagnulaceae</i> sp.
P109	D23	toenails	M	HIV-infected	1105	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	<i>T. rubrum</i>
P238	D24	toenails	M	HIV-infected	74	negative	<i>T. interdigitale</i>	<i>T. rubrum</i>	not sequenced
P381	D25	feet	M	HIV-infected	95	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	<i>T. rubrum</i>
P235	D26	toenails	M	HIV-infected	930	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	<i>T. rubrum</i>
P405	D34	feet	F	HIV-infected	730	negative	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced
P15	D41	toenails	M	HIV-infected	388	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced
P16	D05	hands	M	non-HIV	NA	positive	<i>Trichophyton</i> sp.	not digested	<i>Lecanicillium aphanocladii</i>
P71	D06	arms	F	non-HIV	NA	negative	<i>M. canis</i>	<i>M. canis</i>	not sequenced
P103	D07	trunk	F	non-HIV	NA	positive	<i>Trichophyton</i> sp.	not digested	<i>Preussia</i> sp.
P123	D15	feet	F	non-HIV	NA	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced
P185	D18	toenails	F	non-HIV	NA	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced
P372	D27	fingermails	M	non-HIV	NA	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	<i>T. rubrum</i>
P390	D28	feet	F	non-HIV	NA	negative	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced
P384	D29	toenails	M	non-HIV	NA	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced
P397	D30	feet	F	non-HIV	NA	positive	<i>Trichophyton</i> sp.	<i>T. rubrum</i>	not sequenced
P382	D32	toenails	M	non-HIV	NA	positive	<i>Trichophyton</i> sp.	<i>T. rubrum</i>	not sequenced
P403	D33	feet	F	non-HIV	NA	positive	<i>T. interdigitale</i>	<i>T. interdigitale</i>	<i>T. interdigitale</i>
P222	D35	toenails	F	non-HIV	NA	negative	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced
P64	D38	feet	F	non-HIV	NA	positive	<i>T. interdigitale</i>	<i>T. interdigitale</i>	<i>T. interdigitale</i>
P175	D40	toenails	M	non-HIV	NA	positive	<i>T. rubrum</i>	<i>T. rubrum</i>	not sequenced

NA: Not available.