

# Prevalence of dermatophytes infections at three elementary schools in Cape Verde

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## Research Article

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# Abstract

Dermatophytoses are infectious skin diseases of public health importance because of their transmissibility and high prevalence, especially among school-age children. This is the first study aiming to estimate and report the burden of dermatophytoses on school-age children in Cape Verde. Children attending the afternoon shift of three elementary schools in the city of Achada Igreja, Cape Verde were observed; samples were collected from 60 students with suspected lesions, including hair, nails, and skin scraping. A total of 19 dermatophyte isolates were obtained, corresponding to a point prevalence of 7.63%. Morphological species identification demonstrated 3 different species *Trichophyton soudanense*, *Trichophyton rubrum*, and *Trichophyton violaceum*; re-identification by sequencing the (Internal transcribed spacer) ITS-5.8S rDNA region revealed *T. soudanense* as the most prevalent species, with only one case of *T. rubrum*. We document *T. soudanense* infections in the skin, nails, and scalp, and not only as an agent of tinea capitis as described before. This study reinforces the need of using culture and accurate identification methodologies for gathering epidemiological trends of dermatophytoses throughout the archipelago, especially in school-age students.

## Introduction

Dermatophytoses, are common contagious skin diseases caused by fungi, known as dermatophytes [1, 2]. They represent an important global public health problem despite being more prevalent in hot and humid regions [3, 4]. In Africa, it has always been and continues to be frequent to diagnose dermatophytoses, mainly in school-age children living in poor areas with poor environmental hygiene [5]. These are multifactorial skin infectious diseases, including geographic, climatic, socio-economic (6), also with strong evidences of genetic predisposition (7,8). Due to its non-lethality, it has earned poor attention compared to invasive fungal infections, despite the emotional and psychological marks imposed by its clinical aspects on patients together with its economic impact [9–11].

The causative agents, dermatophytes, belonging to the family Arthrodermataceae, are ubiquitous fungi, comprising the genera *Trichophyton*, *Microsporum*, *Epidermophyton*, *Arthroderma*, *Lopophyton*, *Nannizia*, *Ctenomyces*, *Guarromyces*, and *Paraphyton* [12.]. Although not all species are pathogenic, all are keratin hydrolyzers, initially, soil saprophytes (geophilic) that have adapted to animals (zoophilic) and humans (anthropophilic), making them their reservoirs [3, 13, 14]. The group of anthropophilic, among which *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton violaceum*, *Trichophyton interdigitale*, *Trichophyton soudanense*, *Trichophyton schoenleinii*, *Microsporum audouinii*, and *Epidermophyton floccosum* stand out as important in public health, due to high interpersonal transmission, ability to inhibit or evade the immune system, especially in chronic infections [15], and *in vitro* resistance or clinical recalcitrance to treatment [11, 16]. While some of these agents are globally distributed, others are restricted to certain regions and described as being eradicated in others [17]. Their prevalence can change over time, and one of the main factors contributing to this change is population displacement (migration, mass tourism, and refugees reception) [3, 18, 19, 20]. The introduction of disease-causing agents has led to changes in the epidemiology of dermatophytes, while effective treatments and/or measures to prevent

exposure also changed the epidemiology of these agents with some species becoming less common [2, 13, 18, 19].

In Cape Verde, an African archipelago, there are no studies regarding epidemiological data on dermatophytoses, although clinicians informal reports describe high number of suspected cases especially among school-age children. This, together with the changing trends of dermatophytoses throughout the world, took us to this study, with the objective of gathering data on the profile and prevalence of infections by dermatophytes at three primary schools in Cape Verde.

## **Materials And Methods**

### **Selection of Patients and Ethical Issues**

This study was designed as a cross-sectional study carried out in three rural schools, with low socioeconomic status, in the city of Achada Igreja, a small town located in the central region of the island of Santiago, Cape Verde, 23 km northwest of the capital city of Praia. Population data were obtained by the Cabo Verde National Institute of Statistics and students' habits and living conditions through a questionnaire. The municipality consists of one city and 21 villages where 8608 residents live; of which 528 are students at three elementary schools. All ethical and legal requirements were fully met, with previous submission and agreement by the Ethics Committee of the participating institutions (Cape Verde Comissão Nacional de Proteção de Dados authorization number 237/2019 and the Faculty of Medicine of the University of Coimbra, Portugal, Ethics Committee authorization number 014-CE-2020). Informed consent was obtained from the guardians together with the children's assent to participate.

### **Data and Sample Collection**

Of the total 528 students, only 249 students on the afternoon shift were enrolled in this study. In the clinical examination of the students' signs and symptoms such as itching, spots, scaling, hair loss, kerion, scab, pustules, and family history regarding the presence of dermatophytoses were taken into consideration for the clinical diagnosis of dermatophytoses. Sociodemographic issues and hygiene habits were obtained in the form of a questionnaire. The students with clinically suspected dermatophytoses were examined and skin, hair, or nail samples were collected according to standard collection procedures and kept in sterilized containers until laboratory processing. Before collection, the lesions were documented with photos.

### **Culture and Identification of Dermatophytes**

The clinical samples were cultivated in Sabouraud dextrose agar (SDA) added with chloramphenicol (50 µg/ml) and cycloheximide (300 µg/ml). After culture with up to thirty days of incubation at 30°C, the isolated dermatophytes were identified first based on the macroscopic and microscopic characteristics of the isolates, in SDA and in PDA (Potato Dextrose agar). The identification was further confirmed using molecular tools by sequencing the ITS region of the rDNA.

For that, DNA was extracted from cultures of 7 days on Potato dextrose Agar (PDA) medium, using the Instagene Matrix extraction kit (Bio-Rad Laboratories, Hercules, CA, USA). The extracted DNA concentration and purity were analyzed using the Nanodrop (Version: Manufacturer, Origin). Universal primers ITS1 (3'- TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) were used to amplify the ITS-5.8S regions of the rDNA gene cluster.[21] The amplified DNA was purified using the commercial NucleoSpin®Extract II kit. Purified DNA samples were sent for sequencing by LGC Genomics (Germany, Berlin). The identification was obtained by comparing the sequences in the NCBI database using a Blast Search.

## Results

The clinical informal observation of a considerable number of skin infections with presentations suspected of being dermatophytoses among the students, drew the attention of the health authorities to the need of proceeding to an observational study. Attention was focused on the clinical signs of fungal infection. In the three schools selected for this study, a total of 249 students attended the 1st until the 6th school years, in the afternoon shift. Among these 249 students, 134 (53.8%) had skin diseases, among which 60 students were suspected or had a clinical diagnosis of dermatophytoses; these were selected to be included in this study. The 60 students, corresponding to 44.8% of the children with suspected skin diseases of the three schools, and to 24.1% of the total school population in the afternoon shift, 26 (43.3%) were girls, and 34 (56.7%) were boys, aged between 7 and 14 years (Table 1; Supplemental material). All students were African Cape Verde born, living in villages next to the schools, without closer contact with foreign people. The students from the three schools had similar socio-economic settings; most of them had fair hygiene practices, with 3 to 7 showers per week but with daily contact with animals husbandries and farming activities with no hygiene procedures between these activities; 46.6% (28) were usually barefoot and 61.6% (37) shared clothes with other members of the family (including sharing clothes, shoes, bath towel, bed, and sheets) (Table 1).

Table 1  
Sociodemographic data of the studied school-age population

		Number/percentage*
Mean age		10.9 ±4.5
Sex	Female	26 (43.3%)
	Male	34 (56.7%)
Lesion localization	Scalp	5 (8.33%)
	Ear	1 (1.66%)
	Hand	4 (6.66%)
	Toenail	1 (1.66%)
	Leg	6 (10%)
	Arm	6 (10%)
	Body	12 (20%)
	Foot	1 (1.66%)
	Interdigital	4 (6.66%)
	Nail	16 (26.66%)
	Face	4 (6.66%)
	Barefoot	
Shared clothes		37 (61.6%)
Shower/week	3	12 (20%)
	4	20 (33.33)
	5	16 (26.66%)
	6	4 (6.66%)
	7	8 (13.33)
*Percentage of the total of students selected based on the observation of suspected skin lesions(60)		

After culture, several fungi were isolated, including yeasts and molds (results not shown). Since this study's focus was on dermatophytes, the first approach was to make a presumptive identification of the species by its morphological characteristics. The isolates macroscopically resembling dermatophytes were kept in Sabouraud and in PDA and were identified, using macroscopic colony morphology, in three species. Phenotypic traits such as rapid growth (one week) of compact and glabrous colonies, with

irregularly and radially ridged surfaces, beige (Fig. 1a, e, g), and yellowish (Fig. 1c, f, h), and darker or yellowish on the reverse (Fig. 1b, d), with a flat to the folded surface and a velvety texture (Fig. 1a, c, e-h), were suggestive of *T. soudanense*. Whereas colonies with a suede surface, dark red pigmentation, surrounded by white, reverse pigmentation yellow-brown or wine-red was suggestive of *T. rubrum* (Fig. 2a-d). Colonies with a flat spiral relief, with a silvery purple center with a violet edge or purple colonies with a white outline (Fig. 2g-h), including the reverse (Fig. 2e-f), and a suede-like surface texture (Fig. 2g-h), were suggestive of *T. violaceum*.

The identification was also performed using the amplification and sequencing of the ITS-5.8S region of the rDNA, with no total match (Table 2). In fact, based on the sequences of ITS-5.8S rDNA all the isolated dermatophytes, *T. soudanense* was identified as the main cause of dermatophytoses at the three elementary schools, presenting different infection patterns, as shown in Fig. 3. The group of fungi with colony morphology resembling dermatophytes (total of four isolates), in which this morphological identification was not possible, the nucleotide sequence revealed non-dermatophyte filamentous fungi, *Chaetomium convolutum* (two isolates), *Arthrinium marii* (one isolate), and *Aureobasidium pullulans* (one isolate) (Table 2). The rest of the cultures were positive for *Candida* spp., *Rhodotorula* spp., and other filamentous fungi (results not shown). In one of the clinical cases, *Rhodotorula* spp. and *T. rubrum* were isolated from the same clinical sample. Among the identified isolates of *T. soudanense*, 77.78% (14/18) were from glabrous skin samples (body, hand, leg, arm, and face), nails (3/18; 16,67%), and (1/18; 5.55%) of the scalp. Only one student had a positive culture for *T. rubrum*, in a nail lesion. Although all selected students have daily contact with animals, pets, and cattle, no zoophilic species was isolated. Of these children infected with *T. soudanense*, 10/18 (55.6%) were boys, aged between 7 and 14 years and 8/18 (44.4%) were girls aged between 8 and 11 years. In Fig. 3 it can be observed the lesions due to *T. soudanense*, either in the skin, nails, or scalp.

Table 2  
Isolation and identification of dermatophytes from the lesions clinically suspected as dermatophytoses.

Sample ID	Body Site	Fungal identification		
		Antifungal Treatment	Morphology	ITS-5.8S sequencing
CV3	Body	Ketoconazole	<i>T. violaceum</i>	<i>T. soudanense</i>
CV4	Hand	Ketoconazole	<i>T. soudanense</i>	<i>T. soudanense</i>
CV8	Nail	None	<i>T. soudanense</i>	<i>T. soudanense</i>
CV10	Leg	None	<i>T. soudanense</i>	<i>T. soudanense</i>
CV11	Body	Ketoconazole	<i>T. rubrum</i>	<i>T. soudanense</i>
CV12	Arm	None	<i>T. violaceum</i>	<i>T. soudanense</i>
CV15	Face	Ketoconazole	<i>T. violaceum</i>	<i>T. soudanense</i>
CV20	Body	None	<i>T. violaceum</i>	<i>T. soudanense</i>
CV24	Leg	None	<i>T. rubrum</i>	<i>T. soudanense</i>
CV30	Body	None	<i>T. violaceum</i>	<i>T. soudanense</i>
CV42	Arm	Ketoconazole	<i>T. soudanense</i>	<i>T. soudanense</i>
CV45	Body	Ketoconazole	<i>T. soudanense</i>	<i>T. soudanense</i>
CV47	Body	None	<i>T. soudanense</i>	<i>T. soudanense</i>
CV50	Toenail	None	<i>T. rubrum</i>	<i>T. soudanense</i>
CV52	Hand	Clotrimazole	<i>T. rubrum</i>	<i>T. soudanense</i>
CV53	Skin	Econazole	N.i.	<i>Aureobasidium pullulans</i>
CV54	Nail	None	<i>T. soudanense</i>	<i>T. soudanense</i>
CV55	Nail	None	<i>T. rubrum</i>	<i>T. rubrum</i>
CV56	Nail	None	N.i.	<i>Chaetomium convolutum</i>
CV57	Face	None	<i>T. soudanense</i>	<i>T. soudanense</i>
CV59	Skin	Ketoconazole	N.i.	<i>Arthrimum marii</i>
CV60	Scalp	Griseofulvin	<i>T. soudanense</i>	<i>T. soudanense</i>
CV61	Face	None	N.i.	<i>Chaetomium convolutum</i>

N.i.: Morphologically not identifiable

Among the students with confirmed dermatophytosis, 11/19 (57.9%) were not receiving any treatment, 6/19 (31.57%) received topical therapy, essentially ketoconazole; one child aged 7, diagnosed with tinea capitis was receiving griseofulvin (Table 1).

In what regards the prevalence, it can be considered that in the total population of students of the three schools (249 students attending the afternoon shift), the point prevalence of dermatophyte infection was 19/249 (7.63%).

## Discussion

The incidence of dermatophytoses is becoming increasingly common in developed economies [18, 22, 23] but, in several African countries, this incidence is very high [5, 24–26]. In Cape Verde, despite the clinical empirical awareness of the same tendency towards a high incidence of dermatophytoses, especially among school-age children in rural areas, there are no reports on dermatophytoses and on which species are more frequent. To our knowledge, this is the first report on dermatophytoses and implicated species ever carried out in Cape Verde. This study was conducted in school-age children, and, at the three elementary schools (total of 249 students in the afternoon shift), 134 children were first selected presenting suspicious signs of dermatophytosis. Among these, only 60 children were clinically considered as having lesions compatible with dermatophytoses, and were selected for this study. Of the 60 suspected cases, it was possible to confirm dermatophyte infections in 19 of these children, with a point prevalence of 7.63%, a value lower than that described by several other studies [5]. In the clinical examination of the students, several clinical signs and symptoms were observed, mainly non-inflammatory conditions, scales, and spots similar to seborrheic dermatitis, desquamation of the gray spot, kerion, crust, pustules, hair breakage and loss, nail destruction, and itching in most cases. Dermatophytoses are usually reddish inflammatory lesions, centrifugal, with scaling and occasionally blistering on the edge between the lesion and healthy skin [27]. However, similar lesions can be caused by other organisms, making the clinical diagnosis merely presumptive [27–30]. Most of the selected children, with suspicious dermatophytoses, although with average habits of the daily shower (Table 1), had inadequate hygiene after activities in which could be exposed to fungi such as walking long distances to school, agricultural or animal husbandry activities, usually in their barefoot. Children with such a lifestyle were previously described as having a higher susceptibility of getting dermatophytoses [3, 31]. We also report insufficient health-seeking practices, since even if they attended to a family practitioner and an antifungal was prescribed, there is no monitoring and follow-up of the outcome of the infection/lesion; some were under topical treatment with ethnomedical products (results not shown).

Two different identification methodologies were used, the morphological classical identification of dermatophytes and molecular biology, by ITS-5.8S rDNA sequencing, and several fungal species were isolated and identified out of the lesions. Based on the observation of macroscopic and microscopic features, three species were identified, *T. soudanense*, *T. violaceum*, and *T. rubrum*. However, re-identification using molecular biology, showed only one *T. rubrum*, with the remaining isolates confirmed to be *T. soudanense* after sequencing of the ITS region and comparison to the NCBI database. *T.*

*soudanense* and *T. violaceum* were considered synonyms by some authors [32]. Recent insights, using MALDI-TOF MS, recognized these as different species of the *T. rubrum* complex [33, 34]. Moreover, a recent phylogenomic approach revealed ITS sequencing as appropriate to distinguish between the three main species of the *T. rubrum* complex, *T. soudanense*, *T. violaceum*, and *T. rubrum* [35]. Besides these dermatophytes, we were initially misled by the appearance of the clinical presentation of the lesions and of the macroscopic characteristics of the agar cultures, resembling dermatophytes, but identified as *Aureobasidium pullulans*, *Chaetomium convolutum*, and *Arthrinium marii*, saprophytic and human opportunistic fungi. [36–38]. These results reinforce that dermatophytoses agents cannot be accurately diagnosed based solely on clinical manifestations and on morphological characteristics, with molecular biology techniques assuming an important role in increasing the accuracy, especially in species or strains that do not present typical morphological characteristics, as reported by others [21, 30].

*T. soudanense* is an anthropophilic dermatophyte endemic in Africa [5], primarily responsible for tinea capitis and tinea corporis, in children, and more rarely in adults. It is also sporadically isolated in countries with cultural, social, or economic relations with endemic areas, across Europe and North America [22, 23]. A high prevalence of *T. soudanense* has been reported as a result of growing racial mixing linked to migratory movements, particularly among African immigrants, which confirms the endemic nature of this agent in Africa. Although the number of subjects involved in this study was small, the prevalence of *T. soudanense* corroborates data from several other studies reporting that *T. soudanense* is the most prevalent species of *Trichophyton* in Central and Western regions of Africa. However, we note that these studies were focused on cases of tinea capitis [24, 39–43], whereas we report *T. soudanense* as the infecting agent in skin, nails, and scalp.

Considering the non-inflammatory nature of most of the lesions observed and the context of these children's lifestyle, family history, and sociodemographic issues, a presumptive diagnosis of infection by anthropophilic dermatophyte species was raised. However, taking into account some cases of inflammatory lesions, the child's lifestyle consisting of activities exposing them to contact with the ground and animals, it was expected to have a high prevalence of geophilic and zoophilic species. This trend of the switch between zoophylic and anthropophilic dermatophytes was reported by others in India [31]. It is well established that people with dermatophytoses are at risk of spreading the infection to others by direct or indirect contact through shared surfaces and equipment and that the persistence within the same physical environment may represent opportunities for reinfection and disease spread [44, 45]. *T. soudanense* has a high epidemiological rate of inter-human transmission. In this study, the sources of infection were unknown. However, most patients from the same school had direct contact with each other, suggesting the contagious nature of these infections. This emphasizes the importance of carrying out periodic epidemiological studies and molecular typing that would allow answering the question of the origin of the isolated strains.

An epidemiological study with accurate identification of dermatophytes is essential for the efficient management of this public health problem [5, 46], because it may 1) help local health providers to differentiate between dermatophytic and non-dermatophytic superficial infections; 2) identify the

potential sources of infection by comparing the intra-specific variation; 3) to draw strategies to prevent transmission; 4) to implement the appropriate antifungal therapies [47–49] and 5) to help public health authorities to draw an epidemiological map (i.e., which dermatophyte species are found in the population's understudy).

Due to the lack of reports or studies on dermatophytes in Cape Verde, it is not known whether the isolated strains were endemic or imported. However, as all the participants were nationals, with no contact with immigrants, it is legitimate to conclude that the dermatophytes species found in this study are endemic. It was left to elucidate whether, in each school, the infections were caused by a single strain that persisted in the population and was transmitted between students or by different strains. Molecular typing of strains could indicate these important epidemiological issues [50–52].

Although our study is limited by its small size, it is a clinical and public health asset in Cape Verde, providing complementary information to the available epidemiological data on the prevalence of dermatophytes infection in Africa, more specifically in an archipelago. Future studies need to be carried out to assess the incidence and trends of change at the national level.

## **Declarations**

### **Acknowledgments**

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### **Conflict of interest**

The authors have no conflict of interest to declare.

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### **Financial Interests**

All authors declare that they have no financial interests in this study.

### **Author Contributions**

Conceptualization, methodology, writing—original draft preparation, review and editing were performed by Edmilson Emanuel Monteiro Correia and Teresa Gonçalves; sample, data collection and analysis, were performed by Edmilson Emanuel Monteiro Correia and Teresa Gonçalves, Marta Mota and Luciano Vagner Ascensão de Melo Veiga; supervision of the research, Teresa Gonçalves. All authors have read and agreed to the published version of the manuscript.

## Ethical Approval

All ethical and legal requirements were fully met, with previous submission and agreement by the Ethics Committee of the participating institutions (Cape Verde Comissão Nacional de Proteção de Dados authorization number 237/2019 and the Faculty of Medicine of the University of Coimbra Ethics Committee authorization number 014-CE-2020). Informed consent was obtained from the guardians together with the children's assent to participate.

## Consent to participate and Publish

The authors affirm that written informed consent to collect the samples and publish the results, protecting the identity of the children, was obtained both from the parents and the government institution responsible for data protection.

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## Figures

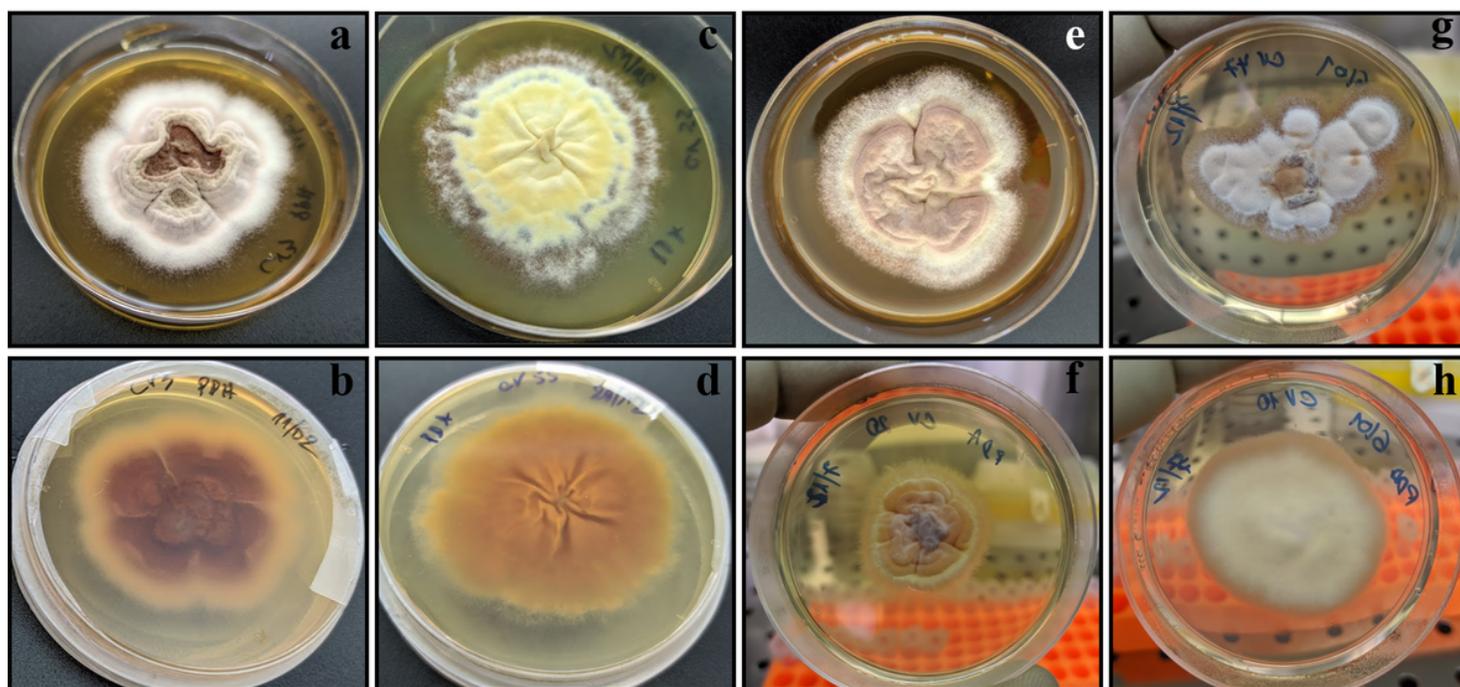
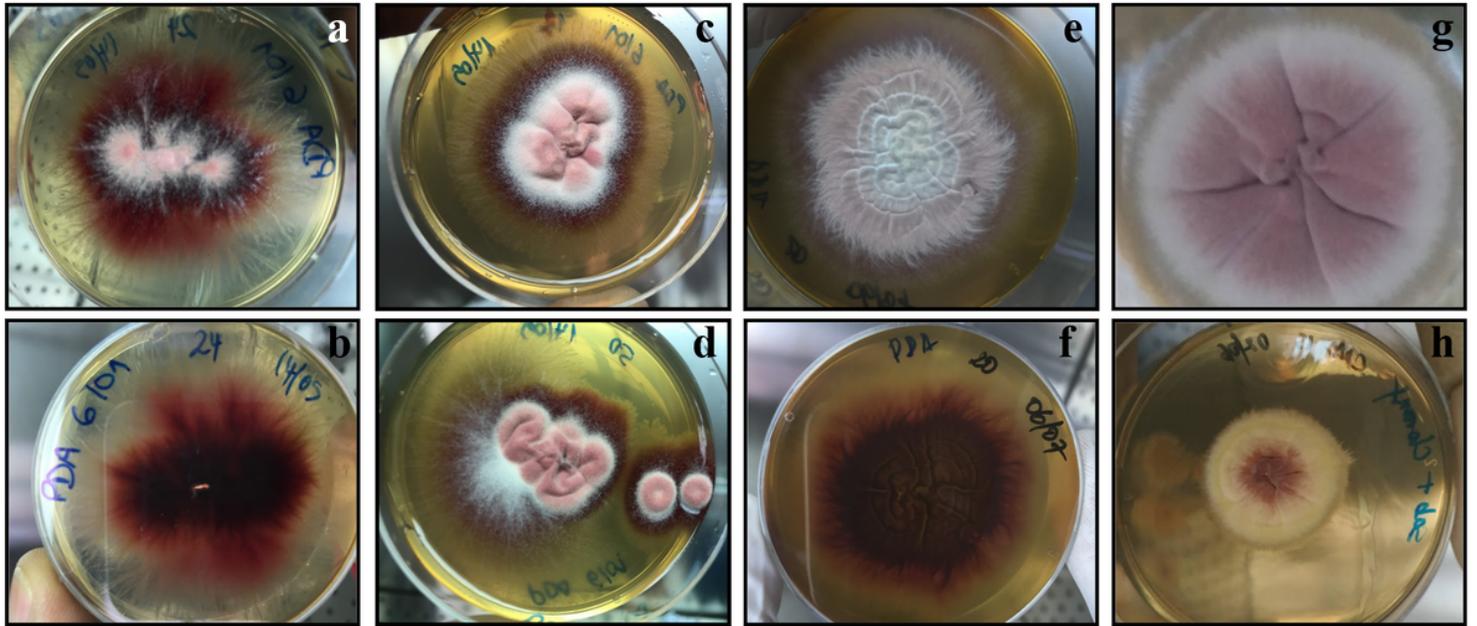


Figure 1

Macroscopic presentation of isolates suggestive of *Trichophyton soudanense*, confluent, velvety, yellow, and beige colonies (in potato dextrose agar-PDA).



**Figure 2**

Macroscopic presentation of isolates suggestive of *T. rubrum* (a - d), white to reddish colonies, mostly flat to slightly raised, suede-like, reverse appearing red, and suggestive of *T. violaceum* (e - h), glabrous or serous colonies, folded and violet with white sectors and deep violet on the reverse (in potato dextrose agar - PDA)



**Figure 3**

Clinical aspects of dermatophytoses by *T. soudanense* in elementary school children in Achada Igreja, Picos, Santiago Island, Cape Verde. From top to bottom, left to right: fungal infections of nails, onychomycosis (a, b, c); arm and leg, tinea corporis(d, g, h); face, tinea faciei (e, f); and scalp, tinea capitis (i, j).

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