Trichophyton violaceum and *T. soudanense*: re-emerging pathogens in Italy, 2005-2013

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SUMMARY

Dermatomycoses due to *Trichophyton violaceum* are described in Mediterranean Countries, North Africa and in the Horn of Africa where *T. soudanense* is present too, but it was rare until few years ago in Italy.
Aim of the present study was to evaluate an Italian multicenter 9 year (2005-2013) experience concerning these re-emerging pathogens. Fifty three fungal strains were sent from clinical laboratories to the Medical Mycology Committee (CoSM) - Italian Association of Clinical Microbiology (AMCLI) for mycological confirmation. Strains were identified as *T. violaceum* (23) and *T. soudanense* (30) by phenotypic and genotypic methods.
These dermatophytes present epidemiological (high rate of inter-human transmission, high risk among adopted children coming from countries of either the Horn of Africa or Sub-Saharan Africa also in outbreaks of *tinea capitis*) and clinical peculiarities (reduced alopecia, presence of exudative lesions) confirming the originality of these "imported" dermatophyte infections.

KEY WORDS: Tinea, Trichophyton violaceum/soudanense, Epidemiology, Childhood.

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INTRODUCTION

Epidemiology of dermatophytic infections in Europe and Italy has changed rapidly due to the increase in mass tourism, social and economic improvements and immigration (Juncosa *et al.,* 2008; Ameen, 2010). More recently, the development of international child adoption programs has played a significant role in the skin infections' onset in childhood (Mitchell and Jenista, 1997).
Clinical manifestations have also changed over the last three decades because of the appearance of rare agents, like the anthropophilic scalp-infecting *T. violaceum*. Particularly, in the early 20th century *tinea capitis* was the most prevalent

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dermatophytic form in Europe, whereas *tinea pedis* has become the most frequent over the last decades (Borman *et al.,* 2007; Koksal *et al.,* 2009; Tsoumani *et al.,* 2011).
Dermatomycoses due to both *T. violaceum* and *T. soudanense* are usually described in Mediterranean Countries, North Africa and in the Horn of Africa, but their presence is reported also in Europe, mainly among immigrants (Hay *et al.,* 2001; Pereiro and Toribio, 2002; Borman *et al.,* 2007; Neji *et al.,* 2010). *Tinea capitis* was described particularly in children, *tinea corporis* and *tinea unguium* particularly in adults.
Their recovery, which was rare until few years ago, is no longer so unusual in Italy, thus representing a challenge for both the clinician and the microbiologist. Aim of the present retrospective survey is to describe some emerging aspects concerning dermatophyosis by *T. violaceum/T. soudanense* in Italy.

METHODOLOGY

A total of 53 strains isolated over a 9 year period (2005-2013) at different hospitals throughout Northern Italy: Bergamo, Milano; Negrar (Verona); Novara; Pavia; Pinerolo (Torino), and Central Italy: Ancona; L'Aquila; Lucca, Pesaro, Pescara were sent to the Medical Mycology Committee (CoSM) of the Italian Association of Clinical Microbiology (AMCLI) at the 'Papa Giovanni XXIII' hospital in Bergamo to confirm the etiological identification.
All strains were inoculated on Sabouraud Dextrose Agar (bioMérieux sa, La Balme, France) and Potato Dextrose Agar (Becton Dickinson Italia SpA, Milano) agar plates and incubated at 30°C during 10 to 21 days because of their slow growth rates.
Identification of the isolates was achieved by traditional observation of the macroscopic and microscopic features. Particularly, macroscopic observation showed both:
1) slow-growing glabrous, purple-red in color colonies; the reverse was purple or violet; some strains lost the pigmentation and showed white sectors (Figure 1) suggesting *T. violaceum*;
2) slow-growing, glabrous, from yellow to red in color colonies; the reverse was dark yellow even if some strains lost the pigmentation and presented some white sectors, suggesting *T. soudanense* (Figure 2).

At microscopic observation both the moulds' aspect types presented distorted hyphae, with very scarce or even completely absent conidia. Conidial production was stimulated by the use of culture media containing thiamine.
Etiological confirmation was performed by a molecular technique (MicroSeq Fungal identification PCR kit, Applied Biosystems) and sequencing (MicroSeq Fungal Identification Sequencing kit, Applied Biosystems) the D2 expansion segment region of the nuclear large-subunit (LSU) ribosomal RNA gene. Sequences from strands were aligned using NCBI BLAST 2 Sequence and the resulting consensus sequence was aligned with sequences stored in GenBank. Sequences producing significant

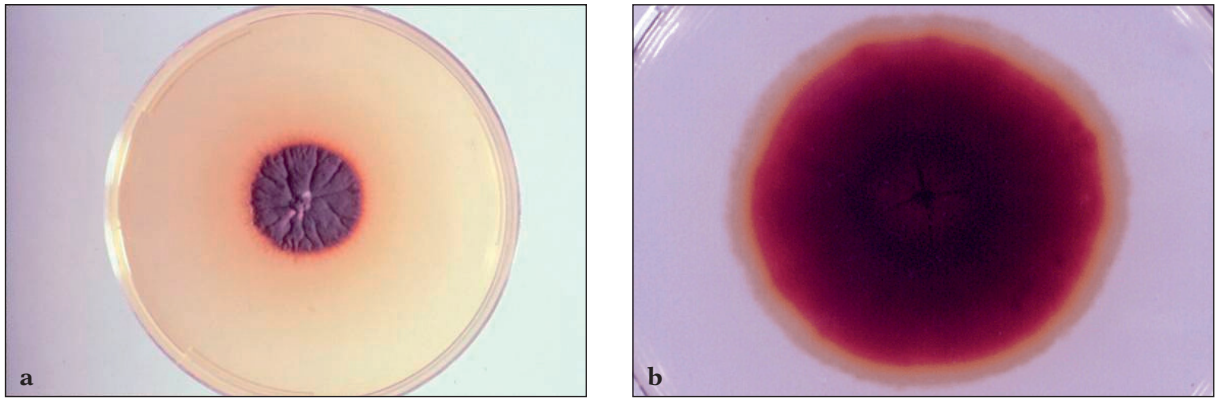

FIGURE 1 - *Trichophyton violaceum: red colony at recto (a) and purple-violet at verso (b).*

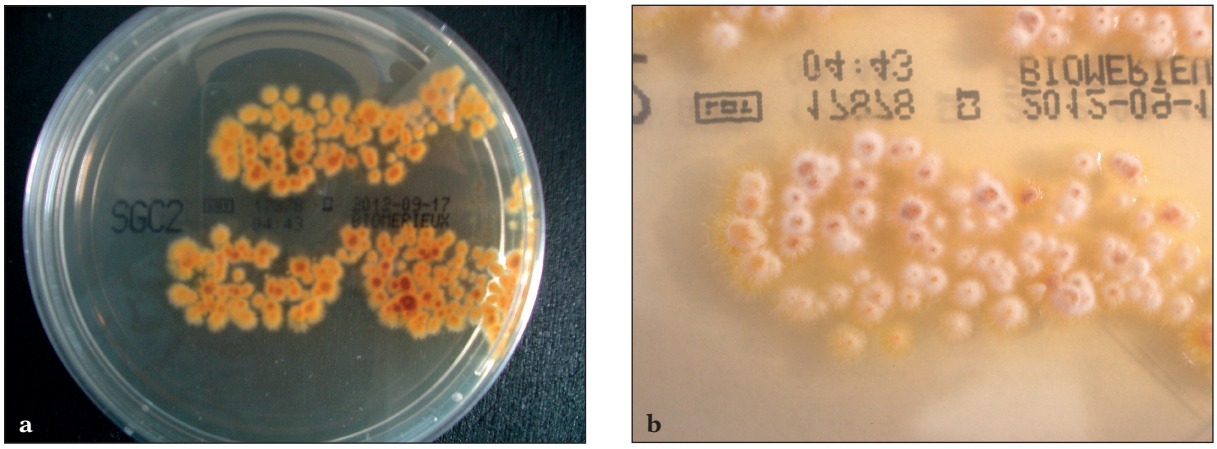

FIGURE 2 - *Trichophyton soudanense: whitish colony at recto (a) and orange-yellonish at verso (b).*

alignments identified the strains with 100% value of identity with the reference strains.

RESULTS

The strains were identified as *T. violaceum* (23) and *T. soudanense* (30), respectively. Table 1 summarizes the epidemiological characteristics (sex, age, predisposing conditions, geographical origin and Italian region of residence, clinical localization, and etiology).
These isolates had been recovered from various cases of dermatophyte indections (12 *tinea corporis*, 2 *tinea unguium*, 36 endothrix *tinea capitis* and 3 mixed infections) (Figure 3). The cases were observed: 3 in 2005, 1 in 2006, 3 in 2008, 13 in 2009, 9 in 2010, 9 in 2011, 14 in 2012 and 1 in 2013. Thirty-eight patients were from Africa (Ethiopia: 17, Senegal: 17, Congo: 1, Burkina Faso: 1, Morocco: 1 and Nigeria: 1) whereas four were from Asia (2 from Pakistan, and 1 each from India and Philippines) and one from Mediterranean Europe (Albania). Eleven patients were Italian: they were either foster parents (4) of adopted children coming from

TABLE 1 - *Case reports (2005-2013).*

	Age/Sex	*Predisposing conditions*	*Origin*	*Localization*	*Etiology*	*Italian Region*	*Year*
1	2/F	Long-term hospital stay	Ethiopia	*tinea capitis*	*T. violaceum*	Veneto	2005
2	38/F	Nurse (case 1)	Italy	*tinea corporis*	*T. violaceum*	Veneto	2005
3	43/F	Nurse (case 1)	Italy	*tinea corporis*	*T. violaceum*	Veneto	2005
4	7/M		Congo	*tinea corporis*	*T. soudanense*	Lombardy	2006
5	4/M		Nigeria	*tinea capitis*	*T. soudanense*	Lombardy	2008
6	4/F		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2008
7	10/F		Senegal	*tinea corporis*	*T. soudanense*	Lombardy	2008
8	6/M	International Adoption Programme	Ethiopia	*tinea capitis*	*T. violaceum*	Lombardy	2009
9	2/M	International Adoption Programme	Ethiopia	*tinea capitis*	*T. violaceum*	Lombardy	2009
10	4/M	International Adoption Programme	Ethiopia	*tinea capitis*	*T. violaceum*	Lombardy	2009
11	3/F	International Adoption Programme	Ethiopia	*t. corporis/ capitis*	*T. violaceum*	Marche	2009
12	38/F	Mother (case 11)	Italy	*tinea corporis*	*T. soudanense*	Lombardy	2009
13	4/F		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2009

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	Age/Sex	*Predisposing conditions*	*Origin*	*Localization*	*Etiology*	*Italian Region*	*Year*
14	2/F		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2009
15	3/M		Albania	*tinea capitis*	*T. soudanense*	Lombardy	2009
16	28 / F		Senegal	*tinea unguium*	*T. soudanense*	Lombardy	2009
17	6/M		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2009
18	4/M		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2009
19	10/M		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2009
20	8/M		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2009
21	3/F	International Adoption Programme	India	*tinea capitis*	*T. violaceum*	Piedmont	2010
22	2/F		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2010
23	5/M		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2010
24	1/F		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2010
25	6/M		Pakistan	*tinea corporis*	*T. soudanense*	Lombardy	2010
26	3/M	International Adoption Programme	Ethiopia	*t. corporis/ capitis*	*T. soudanense*	Lombardy	2010
27	32/F	Mother (case 26)	Italy	*tinea corporis*	*T. soudanense*	Lombardy	2010
28	4/F	Playmate (case 26)	Italy	*tinea corporis*	*T. soudanense*	Piedmont	2010
29	4/F	Playmate (case 26)	Italy	*tinea corporis*	*T. soudanense*	Marche	2010
30	7/M		Senegal	*tinea corporis*	*T. soudanense*	Lombardy	2011
31	7/M		Ethiopia	*tinea corporis*	*T. violaceum*	Lombardy	2011
32	33/F	Mother (case 31)	Italy	*tinea capitis*	*T. violaceum*	Lombardy	2011
33	4/F	International Adoption Programme	Ethiopia	*tinea capitis*	*T. violaceum*	Lombardy	2011
34	43/F	Physiotherapist (case 33)	Italia	*tinea corporis*	*T. violaceum*	Lombardy	2011
35	17/F		Morocco	*tinea capitis*	*T. violaceum*	Lombardy	2011
36	4/M	International Adoption Programme	Ethiopia	*tinea capitis*	*T. soudanense*	Lombardy	2011
37	4/F	Playmate (case 36)	Italy	*tinea capitis*	*T. soudanense*	Lombardy	2011
38	13/M		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2012
39	2/M	International Adoption Programme	Ethiopia	*tinea capitis*	*T. violaceum*	Lombardy	2012
40	36/M	Nurse (case 39)	Italy	*tinea capitis*	*T. soudanense*	Lombardy	2012
41	4/M		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2012
42	7/M		Senegal	*tinea capitis*	*T. soudanense*	Lombardy	2012
43			Ethiopia	*t. corporis/ capitis*	*T. violaceum*	Lombardy	2012
44	5/M	International Adoption Programme	Ethiopia	*tinea capitis*	*T. violaceum*	Lombardy	2012
45	40/F	Mother (case 44)	Italy	*tinea corporis*	*T. violaceum*	Marche	2011
46	22/M		Philippines	*tinea unguium*	*T. soudanense*	Lombardy	2012
47	19/F		Ethiopia	*tinea capitis*	*T. violaceum*	Lombardy	2012
48	14/M		Burkina Faso	*tinea capitis*	*T. violaceum*	Lombardy	2012
49	6/M		Ethiopia	*tinea capitis*	*T. violaceum*	Lombardy	2012
50	9/M		Pakistan	*tinea capitis*	*T. soudanense*	Lombardy	2012
51	4/M	International Adoption Programme	Ethiopia	*tinea capitis*	*T. violaceum*	Lombardy	2012
52	9/M		Ethiopia	*tinea capitis*	*T. violaceum*	Lombardy	2012
53	6/M		Ethiopia	*tinea capitis*	*T. violaceum*	Lombardy	2013

FIGURE 3 - *Clinical aspects of dermatophyosis by T. violaceum: in a family outbreak: tinea capitis in an adopted child from Ethiopia (a); tinea corporis: ringworm at cheek (b), arm (c) and foot (d), and tinea unguium (e) in his foster mother.*

Ethiopia, nursing personnel (3), physiotherapist (1) of non-profit organizations or children (3) attending different kindergartens where African playmates were present.

Four Ethiopian children included in the same international adoptees' program resulted affected by *tinea capitis* by *T. violaceum* even if they were diagnosed at different times by different laboratories in Italy.

All patients were cured after standard therapy based on griseofulvin or terbinafine administration.

DISCUSSION

The molecular taxonomy of the *T. rubrum* complex recently reclassified or synonymized as *T. rubrum* or *T. violaceum* fifteen species and varieties belonging to the complex: *T. circonvolu-*

tum, *T. fisheri*, *T. fluviomuniense*, *T. glabrum*, *T. gourvilii*, *T. kanei*, *T. kuryangei*, *T. megninii*, *T. pedis*, *T. raubitscekii*, *T. rodhaini*, *T. rubrum* var *nigricans*, *T. soudanense*, *T. violaceum* var *indicum*, and *T. yaoundei* (Graser *et al.,* 2000).

In particular, the *T. rubrum* complex is distinguished in two monophyletic clades based on ITS sequences, the first constituted by *T. violaceum* and its conspecific strains (*T. gourvilii*, and *T. yaoundei*), the second by *T. rubrum* with its conspecific taxons like *T. fisheri, T. fluviomuniense, T. kanei,*. *Trichophyton soudanense* and *T. glabrum* are now considered synonyms of *T. violaceum* (Graser *et al.,* 2000). However, *T. violaceum* is characterized by a very slow growth (2 to 4 weeks) and yields tiny colonies, whereas *T. soudanense* is not slow growing (one week) and yields larger colonies (de Hoog *et al.,* 2000).

Dermatophytosis due to *T. violaceum/T. soudanense*, which are genetically indistinguishable, are frequent in Sub-Saharan Africa and in Maghreb (Bendjaballah-Laliam and Djazer, 2014; Ellabib *et al.,* 2002; Morar *et al.,* 2004; Ali *et al.,* 2009; Sagrhrouni *et al.,* 2011). Both were identified in 24 patients in Baltimore in a six year period (2000-2006) possibly associated with changes in immigration to the Maryland area (Magill *et al.,* 2007).

They have been more frequently observed in Italy over the last 25 years as reported by Albanese *et al.* (1995) in Lombardy, and by Flemmia *et al.* (1995) in the Florence area, even if this species was believed to have disappeared from Italy until 2002 when Romano *et al.* described two *T. soudanense* cases in Italian children who had had contact with African people.

Until the first cases observed in 2005 at the Hospital of Negrar (North-eastern Italy) when three people presented *tinea corporis* and *tinea capitis* by *T. violaceum,* their incidence was very low over the past decades in Italy. An increase in *tinea capitis/corporis* cases caused by *T. violaceum* and *T. soudanense* in Italy is currently observed. On the contrary, it must be noted that no *T. langeroni* or *T. tonsurans* have been isolated in the same period at the Italian centers belonging to this survey.

Even if this survey refers only the limited number of clinical cases reported to the Mycological Committee of the Italian Association of Clinical Microbiologists (AMCLI) for etiological confirmation, it must be noted that 79.6% of the observed *T. violaceum/T. soudanense* dermatophytosis occurred in immigrants, particularly those coming from Africa because of belonging to international adoptees' programs, but not in autochtonous people. Furthermore, it must be demphasized that many children coming from abroad presented *tinea* only after a journey in the parents' native country, confirming that the epidemiological reservoir of dermatophytes was not in Italy.

It is well known that international adoptees are exposed to difficult living conditions prior to being adopted. Even if malnourished states, substandard medical assessment and unreliable immunizations are frequently present, children from Ethiopia and other Horn of Africa regions differ from other groups of internationally adoptees because of better behavioural problems at arrival (Miller *et al.,* 2008). However, skin infections are not uncommon, and dermatophytes have to be carefully investigated (Ampofo 2013).

These infections showed some peculiar characteristics:

1) a high rate of inter-human transmission (confirmed by 12 cases in foster parents or in nurses);
2) clinical (*tinea capitis* particularly in children: often reduced alopecia with black dots due to broken and curled hairs, sometimes in the presence of exudative and aching lesions, possibly evolving to evil-smelling favus-like crusts; *tinea corporis* and *tinea unguium* particularly in adults);
3) microbiological difficulties in diagnosis because of their infrequency.

International adoption programs have played a significant role in the onset of uncommon clinical dermatophyte infections in adoptees particularly from the Horn of Africa or of the Sub-Saharan Africa where a greater incidence of *T. violaceum/T. soudanense* was first observed.

REFERENCES

Albanese G., Di Cintio R., Crippa D., Galbiati G. (1995). *Trichophyton soudanense* in Italy. *Mycoses*. **38**, 229-230.

Ali J, Yifru S.B., Woldeamanuel B. (2009). Prevalence of tinea capitis and the causative agent among

school children in Gondar, North West Ethiopia. *Ethiop. Med. J.* **47**, 261-269.

Ameen M. (2010). Epidemiology of superficial fungal infections. *Clin. Dermatol.* **2**8, 197-201.

Ampofo K. (2013). Infectious disease issues in adoption of young children. *Curr. Opin. Pediatr.* **25**, 78-87.

Bendiaballah-Laliam H., Diazer H. (2014). Epidemiology of Tinea capitis in the suburbs of Tipasa, Algeria. *J. Mycol. Med.* **24**, 141-143.

Campbell C.K., Fraser M., Johnson E.M. (2007). Analysis of the dermatophyte species isolated in the British Isles between 1980 and 2005 andreview of worldwide dermatophyte trends over the last three decades. *Med. Mycol*. **45**, 131-141.

De Hoog G.S., Guarro J., Gené J., Figueras M.J. (2000). Atlas of clinical fungi. 2nd edn. Centraalbureau voor Schimmelcultures/Universitat Rovira i Virgili. 992-994.

Ellabib M.S., Khalifa Z., Kavanagh K. (2002). Dermatophytes and other fungi associated with skin mycoses in Tripoli, Libya. *Mycoses*. **45**, 101-104.

Flemmia M., Vannini P., Difonzo E.M. (1995). *Tinea capitis* in the Florence area between 1985 and 1993. *Mycoses*. **38**, 325-328.

Graser Y., Kuijpers A.F.A., Presber W., De Hoog G.S. (2000). Molecular taxonomy of the *Trichophyton rubrum* Complex. *J. Clin. Microbiol.* **38**, 3329-3336.

Hay R.J., Robles W., Midgley G., Moore M.K., European Confederation of Medical Mycology Working Party on *Tinea Capitis*. (2001). *Tinea capitis* in Europe: new perspective on an old problem. *J. Eur. Acad. Dematol. Venearol.* **15**, 229-233.

Juncosa T., Aguilera P., Jaen A., Vicente A., Aguilar A.C., Fumado V. (2008). *Trichophyton violaceum*: an emerging pathogen. *Enferm. Infecc. Microbiol. Clin.* **26**, 502-504.

Koksal F., Er E., Samasti M. (2009). Causative agents of superficial mycoses in Istanbul, Turkey: retrospective study. *Mycopathologia*. **68**, 117-123.

Tsoumani M., Jelastopulu E., Bartzavali C., Varnvakopoulou S., Dimitracopoulos G., Anastassiou E.D., Chistofidou M. (2011). Changes of dermatophytoses in southwestern Greece: an 18-year survey. *Mycopathologia*. **172**, 63-67.

Magill S.S., Manfredi L., Swiderski A., Cohen B., Merz W.G. (2007). Isolation of *Trichophyton violaceum* and *Trichophyton soudanense* in Baltimore, Maryland. *J. Clin. Microbiol.* **45**, 461-465.

Miller L.C., Tseng B., Tirella L.G., Chan W., Feig E. (2008). Health of children adopted from Ethiopia. *Matern Child Health J.* **12**, 599-605.

Mitchell M.A., Jenista J.A. (1997). Health care of the internationally adopted child part 1. Before and at arrival into the adoptive home. *J. Pediatr. Health Care*. **11**, 51-60.

Morar N., Dlova N.C., Gupta A.K., Aboobaker J. (2004). Tinea capitis in Kwa-Zulu Natal, South Africa. *Pediatr. Dermatol.* **21**, 444-447.

Neji S., Makni F., Cheikhrouhou F., Sellami H., Boudaya S., Turki H., Ayadi A. (2010). First case of *Trichophyton soudanense* isolated in Tunisia. *Mycopathologia*. **170**, 353-355.

Pereiro M. Jr, Toribio J. (2002). Tinea caused by *T. soudanense* in non african subjects in Spain. *Acta Derm. Venereol.* **82**, 141-142.

Romano C., De Aloe G., Calcaterra R., Gianni C. (2002). Case reports. Tinea capitis due to *Trichophyton soudanense* and *Trichophyton schoenleinii*. *Mycoses*. **45**, 518-521.

Saghrrouni F., Bougmiza I., Gheith S., Yaakoub A., Gaied-Meksi S., Fathallah A., Mtiraoui A., Ben Said M. (2011). Mycological and epidemiological aspects of *tinea capitis* in the Sousse region of Tunisia. *Ann. Dermatol. Venereol.* **138**, 557-563.