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Identification of the causative dermatophyte of tinea capitis in children attending Mbarara Regional Referral Hospital in Uganda by PCR-ELISA and comparison with conventional mycological diagnostic methods

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Abstract

Tinea capitis is a dermatophyte infection common among prepubertal children in sub-Saharan Africa and mainly caused by *Trichophyton* and *Microsporum* species. Accurate identification is challenging as conventional methods like culture and microscopy are slow and mostly based on morphological characteristics, which make them less sensitive and specific. Modern molecular methods, like polymerase chain reaction (PCR) assays, are gaining acceptance and are quick as well as accurate. The aim of this study was to investigate the clinical patterns of tinea capitis and to accurately identify the most common causative dermatophytes affecting the scalps of children aged 1 to 16 years attending the Skin Clinic at Mbarara University of Science and Technology (MUST), Mbarara, Uganda, East Africa, using both conventional mycological methods and PCR-ELISA for detection of dermatophyte DNA. One hundred fifteen clinical samples from children from Western Uganda attending the MUST Skin Clinic with a clinical diagnosis of tinea capitis were analyzed. *T. violaceum* was identified as the most common causative agent, followed by *M. audouinii*, *T. soudanense*, and *T. rubrum*. The early identification of the causative agent of tinea capitis is a prerequisite for the effective management of the disease, the identification of probable source and the prevention of spreading. Children with tinea

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capitis in Western Uganda should be treated by systemic therapy rather than topical preparations to ensure high cure rates as the most common causative dermatophytes *T. violaceum* exhibits an endothrix rather than ectothrix invasion of the hair follicle.

Key words: Blankophor preparation, fungal culture, dermatophyte, PCR-ELISA, Uganda.

Introduction

Tinea capitis is a cutaneous fungal infection that is common among prepubertal children in sub-Saharan Africa. It is mainly caused by dermatophytes of the *Trichophyton* or *Microsporum* genus; however, causative species may vary according to geographic predilection around the world. In the United States, the antropophilic dermatophyte *T. tonsurans* accounts for more than 95% of the tinea capitis cases,[1] while in Europe zoophilic dermatophytes like *M. canis*[2] or *Trichophyton* anamorph of *Arthroderma benhamiae*[3–4] are predominant. Nonetheless, in recent years a shift toward anthropophilic dermatophytes such as *M. audouinii* has become noticeable.[5] This may be attributed to the increasing number of African immigrants, especially West Africans, where this dermatophyte is known to be endemic.[6] Several studies identified *T. soudanense* as the most common cause of tinea capitis among school children from the Ivory Coast.[7–8] In contrast, *T. violaceum* has been shown to be the main causative dermatophyte of tinea capitis in Kenya,[9] Ethiopia,[10] or Botswana.[11] At the Skin Clinic of Mbarara University of Science and Technology (MUST), a high percentage of children present with superficial fungal infections, particularly affecting the scalp. For instance, in 2010, of a total of 2794 patients attending the Mbarara Skin Clinic, 633 were children aged 1 to 16 years of which 94 (14.8%) could be clinically diagnosed with tinea capitis. According to the MUST records, the diagnosis was based on clinical features and microscopy using potassium hydroxide (KOH) preparation only. These children would then be treated empirically with both topical and systemic antifungal therapy (without any clear treatment protocols). There seems to be a high recurrence rate, since 40% of the children treated reattended the clinic with the same persistent condition (MUST records, 2010). The aim of the study was therefore to accurately determine the most common causative dermatophyte species responsible for tinea capitis in children attending the MUST Skin Clinic. One hundred fifteen clinical samples taken from children of Western Uganda in the period of February to June 2012 with clinical symptoms of tinea capitis were analyzed in two mycology laboratories in Germany (Department of Dermatology, University Hospital Center Jena and Laboratory for Medical Microbiology, Rötha/Mölbis) for dermatophtyes using conventional laboratory methods and PCR-ELISA (polymerase chain reaction–enzyme linked immunosorbent assay).

It is common practice that the diagnosis of dermatophytes is verified in mycological laboratories with conventional diagnostic methods including the direct examination, ideally using fluorescence staining with Calcofluor or Blankophor and fungal culture.[12] The fluorescent whiteners mentioned bind to chitin and cellulose components of the fungal cell wall and fluorescence when exposed to UV light. This makes detection of fungal elements faster and more accurate compared to using potassium hydroxide, in fact, fluorescence staining has a sensitivity and specificity of 100% and 86%. Furthermore, fluorescence staining improves visibility, is simple to use, inexpensive, and rapid.[13] However, no identification up to the species level is possible.[12] Conventional culture methods, though specific and sensitive, have two significant drawbacks. Cultivation requires a long incubation period (4 to 6 weeks),[14] and dermatophyte strains may develop atypical characteristics (different colony morphologies, pleomorphism, etc.). In addition, nonvital fungi can lead to false negative results.[15] Misdiagnosis may lead to harmful or inadequate treatment.[16] Advances in the molecular diagnostics of dermatophytosis have improved the speed, specificities, and sensitivities. Methods such as gene-specific PCR,[17–18] Southern blotting,[19–20] sequencing,[21–22], and real-time PCR[23–24] are therefore promising techniques for providing rapid and accurate diagnosis of dermatophytes. Recently, an effective PCR-ELISA method has been developed for rapid detection of dermatophyte species within 24 hours directly from clinical specimens.[25–27] This method is easy and reproducible. After direct extraction, the isolated genomic DNA of skin scrapings, hair and nail samples from patients with suspected dermatophyte infections is amplified with species-specific digoxigenin-labelled primers targeting the topoisomerase II gene. The subsequent ELISA procedure with biotin-labelled probes allows a sensitive and specific identification of the five most common dermatophytes—*Trichophyton rubrum*, *T. interdigitale*, *T. violaceum*, *Microsporum canis*, and *Epidermophyton floccosum*.[25]

Materials and methods

Study design

The study was a descriptive cross-sectional study in which samples of scalp scrapings and hair fragments were collected at one point in time from a homogeneous

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population. The study site was the MUST Skin Clinic, which is the only dermatological referral centre for the people living in Southwestern Uganda. The skin clinic caters for both outpatients and inpatients who either came directly or as result of consultations by medical personnel from different hospital departments including ward, outpatient department, medical ward, and surgery ward of Mbarara Regional Referral Hospital.

Hair roots and skin scrapings (samples) were collected from 115 children, aged 1 to 16 years that were clinically diagnosed with tinea capitis (see Table S1 in the supplement for patient information). Samples were packed in Myco-Trans kits (Doenitz ProLab, Augsburg, Germany) to avoid contamination. The samples were then transferred to two laboratories in Germany for evaluation, that is, 65 samples were analyzed at the Department of Dermatology, University Hospital Center Jena, and 50 samples were analyzed at the Laboratory for Medical Microbiology Rötha/Mölbis. However, all samples were evaluated according to the same protocols.

The Ethics Commission of the Mbarara University School of Graduate Studies had agreed to the study. Written informed consent was obtained from parents or guardians of the children identified.

Materials

Blankophor stain was obtained from Bayer (Leverkusen, Germany), and lactophenol cotton blue staining solution was purchased from Merck (Germany). Sabouraud´s dextrose agar plates and Dermasel agar plates were from bioMérieux (Nürtingen, Germany). QIAamp DNA Mini Kit 250 was purchased from Qiagen (Germany), the PCR mastermix was used from bioBudget (Germany), and the PCR-ELISA Dig Detection Kit was from Roche (Germany). All other chemicals were obtained from ROTH (Germany).

Detection of fungal material in patient samples—Blankophor preparation

For direct identification of fungi in the patient´s samples (scales, hairs), a drop of the fluorescent whitener solution (contains 0.1% (m/v) Blankophor, 10% (v/v) DMSO, and 1.8% NaOH (m/v) in distilled water) was put onto an object slide. A prewetted inoculation loop was used to transfer a few scales or hairs from the patient material into the staining solution. The sample was covered with a cover slip and incubated for at least 20 minutes. For microscopic evaluation, a fluorescence microscope (Olympus Deutschland GmbH, Germany) with the filter set WU (400–450 nm) was used. Fungal material can be distinguished by a bright blue fluorescence and the characteristic fungal features.

Identification of dermatophytes by culture

A primary culture was prepared under sterile conditions from the patient material by plating scales or hairs onto Dermasel and Sabouraud´s dextrose agar plates. Plates were closed with duct tape to avoid desiccation and incubated for at least 4 weeks at room temperature. Macroscopic assessment of the plates was performed weekly and any noticeable fungal growth was recorded. Macroscopically, cultures were evaluated by colour of the colony's top and bottom side, surface characteristics, and margin shape. After 4 weeks, samples were prepared for microscopic appraisal. A drop of lactophenol cotton blue staining solution was put onto an object slide. An adhesive tape (4–5 cm in length) was carefully pressed onto the colony surface to transfer fungal material to the adhesive side of the tape. Afterwards, the tape was placed on the object slide with the lactophenol cotton blue. Any air bubbles were carefully removed by wiping with a paper towel. For microscopic evaluation, a light microscope (Olympus Deutschland GmbH, Germany) was used. Microscopically, culture samples were assessed by hyphae formation, type and number of micro conidia, existence and shape of macro conidia, as well as appearance of chlamydospores.[28]

Differentiation of dermatophytes by PCR-ELISA

Dermatophytes were identified by PCR-ELISA using primer combinations specific for *Trichophyton rubrum* (TR), *Trichophyton interdigitale* (TI), *Trichophyton violaceum* (TV), and *Microsporum canis* (MC) as well as *T.* anamorph *Arthroderma benhamiae* (AB).[25,27] Recognition of the first four species is based on the amplification of a gene sequence of the topoisomerase II gene by PCR, while the identification of *T.* anamorph *A. benhamiae* is based on the amplification of a gene sequence of the ITS 1 gene. Subsequently, the PCR products were detected using a biotin-labelled hybridization probe. The binding of PCR product to hybridization probe was verified by ELISA (enzyme-linked immunosorbent assay).

The QIAamp DNA Mini Kit 250 was used for DNA isolation from the patient material. Scales or hairs were transferred into a tube containing 180 μl ATL buffer using a pre-wetted inoculation loop, thoroughly vortexed for 20 s before 20 μl proteinase K solution was added, and, subsequently, incubated over night at 56°C. Afterward, 200 μl AL buffer were added, each tube is thoroughly vortexed for 20 s, and incubated for 10 min at 66°C. After addition of 210 μl ethanol, the contents of each tube was carefully mixed and loaded onto a provided spin column. Tubes were centrifuged for 1 min at 16.000 g, the spin column was transferred to a new tube, and the filtrate was discarded. In

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Table 1. Primer and hybridization probe sequences for the PCR-ELISA.

Species	Designation	Sequence
Trichophyton rubrum (TR)	forward	5′-GCC TGT TGT TCC GCT CAT TCT T-3′
	reverse	5′-Dig-CGG CTA GGA GGG CGT GGT AGA A-3′
	hybridization	5′-Biotin-CAT ATG ATT ACC TTC TGA GCG TAA G-3′
Trichophyton interdigitale (TI)	forward	5′-GCA TGA TTT AGA AGT GTA ATG CTG-3′
	reverse	5′-Dig-GGT GCC AGC CAT GTC GTA GAC-3′
	hybridization	5′-Biotin-TCG AAG CCT TGG TTA AAA GAA GG-3′
Trichophyton violaceum (TV)	forward	5′-GAT CCA CAA GGT ATG TAT TAG TTA-3′
	reverse	5′-Dig-GGT GCC AGC CAT GTC GTA GAC-3′
	hybridization	5′-Biotin-TCG AAG CCT TGG TTA AAA GAA GG-3′
Microsporum canis (MC)	forward	5′-GCT GGT AAA TAA CAC CGA TGA TGG-3′
	reverse	5′-Dig-TGT ATC TGA TAT GCA TAC CTT CC-3′
	hybridization	5′-Biotin-CCG TAT CAA GCT ATA TGA GCG AC-3′
Arthroderma benhamiae (AB)	forward	5′-TCC GTA GGT GAA CCT GCG G-3′
	reverse	5′-Dig-GCT GCG TTC TTC ATC GAT GC-3′
	hybridization	5′-Biotin-TCT TGG AAA GCT GTC AGT- 3′

sum, 500 μl AW 1 buffer was loaded on each spin column. Tubes were centrifuged for 1 min at 16.500 *g*, the spin column was transferred to a new tube, and the filtrate was discarded; 500 μl AW 2 buffer was loaded on each spin column. Tubes were centrifuged for 1 min at 16.500 *g*, the spin column was transferred to a new tube, and the filtrate was discarded; 400 μl AW 2 buffers were loaded on each spin column. Tubes were centrifuged for 1 min at 16.500 *g*, the spin column was transferred to a new tube, and the filtrate was discarded. To dry the spin columns completely, the tubes were again centrifuged for 1 min at 19.000 *g*. Subsequently, the dried spin column was transferred to a new tube; 70 μl AE buffer were loaded onto the spin column, left for 2 min to allow complete wetting of the filter material, and then tubes are centrifuged for 1 min at 16.500 *g* to elute the DNA. The spin column was discarded and the eluted DNA was then stored at −20°C.

For the dermatophyte-specific PCR, 24 μl working solution were suspended into the corresponding wells of a 96-well PCR plate and 6 μl DNA sample (approx. 6 ng), 6 μl ddH_2O (negative control) or 1 μl positive control + 5 μl dH_2O are added. Per reaction, the working solution contains 6 μl 5* PCR mastermix, 16.5 μl dH_2O, 0.75 μl forward primer, and 0.75 μl digoxigenin-labelled reverse primer. The primer sequences for each dermatophyte accounted are presented in Table 1. As positive controls, DNA isolated from cultures of the specific dermatophytes was used. All working steps had to be performed on ice to avoid unwanted reactions. Before the PCR plate with the samples was placed into the thermocycler (Mastercyler gradien, Eppendorf AG, Germany), it was meticulously sealed with a PCR foil (Sarstedt AG & Co KG, Germany). Following PCR programs were used: PCR - TR-TI -TV-MC: primary denaturation at 95°C for 5.3 min; 42 cycles of denaturation at 94°C for 15 s, annealing at 60°C for 20 s, and extension at 72°C for 90 s; and terminal extension at 72°C for 7.7 min. PCR-AB: primary denaturation at 96°C for 10 min; 42 cycles of denaturation at 95°C for 60 s, annealing at 60°C for 60 s, and extension at 72°C for 60 s; and terminal extension at 72°C for 10 min.

The digoxigenin-labelled PCR products were incubated with species-specific biotin-labelled hybridization probe for detection of PCR products by ELISA. Thus, a working solution of 0.75 μM was prepared for each specific dermatophyte hybridization probe (Table 1). In sum, 10 μl denaturation solutions (PCR-ELISA Dig Detection, Roche, Germany) were added to each well of an uncoated 96-well plate and carefully mixed with 25 μl PCR product before incubation for 10 min at room temperature. To each well, 100 μl hybridization solution (hybridization buffer [PCR-ELISA Dig Detection, Roche, Germany] + specific hybridization probe) was added and carefully mixed. The complex of PCR product and hybridization probe was detected by binding of biotin to streptavidin. Therefore, 100 μl were transferred to the corresponding wells of a streptavidin-coated 96-well plate (Roche, Germany). Plates were covered with an adhesive foil and incubated at 56°C for 1.5 hours. To remove any unbound items, plates were washed six times with 150 μl washing solution (PCR-ELISA Dig Detection, Roche, Germany) per well. Afterward, the PCR product was detected by incubation with HRP (horse radish peroxidase)-labelled anti-digoxigenin antibodies; 100 μl anti-Dig-Pod solution (PCR-ELISA Dig Detection, Roche, Germany) is added to each well and the plates are incubated at 37°C for 30 to 40 min in the dark. Subsequently, plates were washed

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Table 2. Comparison of results for Blankophor preparation versus culture (A), Blankophor preparation versus PCR-ELISA (B), and culture versus PCR-ELISA (C).

A		Blankophor preparation		
		Positive	**Negative**	Total
culture	**positive**	87	14	**101**
	negative	8	6	**14**
	total	**95**	**20**	115
B		Blankophor Preparation		
		Positive	**Negative**	Total
PCR-ELISA	**positive**	78	9	87
	negative	17	11	28
	total	**95**	**20**	115
C		Culture		
		Positive	**Negative**	Total
PCR-ELISA	**positive**	85	2	87
	negative	16	12	28
	total	**101**	**14**	115

six times with 150 μl washing solution (PCR-ELISA Dig Detection, Roche, Germany) per well. Also, 100 μl substrate solution (ABTS, PCR-ELISA Dig Detection, Roche, Germany) was added to each well and the plates are incubated at 37°C for 30 min in the dark. A colour development indicates a binding of the specific hybridization probe by which the dermatophyte species would be identified. Absorbance was measured at 405 nm with a reference wavelength of 495 nm using a plate photometer (Polarstar Galaxy, BMG Labtech, Germany). An optical density (OD) of 0.2 was designated as cutoff value.

Statistical analyses

Quantitative data analysis was done using Microsoft Excel (Microsoft Office Professional Plus 2010) and involved performance of descriptive statistics, determination of frequency distribution and cross-tabulation of key variables. Sensitivity and specificity were calculated for Blankophor preparation, culture, and PCR-ELISA according to Diepgen.[29]

Results

Of the 115 cases, 62 were male and 47 were female. In six cases the sex was not recorded. The average patient age was 6 years. Blankophor-staining revealed 95 samples positive for fungal material (82.6%), while 20 samples were negative (17.4%). More frequently an endothrix invasion of the hair shaft was observed in the positive samples as opposed to an ectothrix infection of the hair.

All 115 samples were subjected to culture evaluation regardless of the results obtained by Blankophor-preparations. Dermatophytes were cultured for a period of 4 weeks both, on Dermasel and Sabouraud´s dextrose agar. In 101 samples fungi grew (87.8% positive), while no growth was observed in 14 samples (12.2% negative). Comparison of results for culture versus Blancophor preparations revealed a 0.91 sensitivity and 0.3 specificity (Table 2A). Accuracy was determined to be 0.81 with $\alpha = 0.12$ (false positive results) and $\beta = 0.07$ (false negative results).

Afterward, the fungi cultured were assessed macroscopically for morphological characteristics as well as for microscopic features using lactophenol cotton blue staining. The most common dermatophytes isolated were *T. violaceum* (73/115), *M. audouinii* (19/115), *T. soudanense* (5/115), and *T. rubrum* (3/115) (Fig. 1). In few cases, moulds like *Scopulariopsis brevicaulis* (7/115), *Fusarium oxysporum* (3/115), or *Aspergillus niger* (2/115) were isolated. In several samples mixed infections were found, for example, *M. audouinii* and *T. violaceum* (3/115), *T. violaceum* and *S. brevicaulis* (4/115), *T. soudanense* and *M. audouinii* (1/115), as well as *T. violaceum, T. soudanense*, and *T. rubrum* (1/115). Table 3 summarizes the species identified by culture. Due to the nine double and one triple infections, a total number of 112 isolates were found in the 101 positive samples.

The PCR-ELISA method also identified *T. violaceum* as the main causative agent for tinea capitis in children attending MUSC (77/115, 67.0%) followed by *T. rubrum* (7/115, 6.1%) (Table 3). Double infections were found for *T. violaceum* and *T. rubrum* (1/115) as well as *T. interdigitale* and *T. rubrum* (2/115). *M. canis* or *Trichophyton* anamorph of *Arthroderma benhamiae* were not observed. A high amount of 28 cases out of 115 were found to be negative by PCR (24.3%). However, it is important to note that only the

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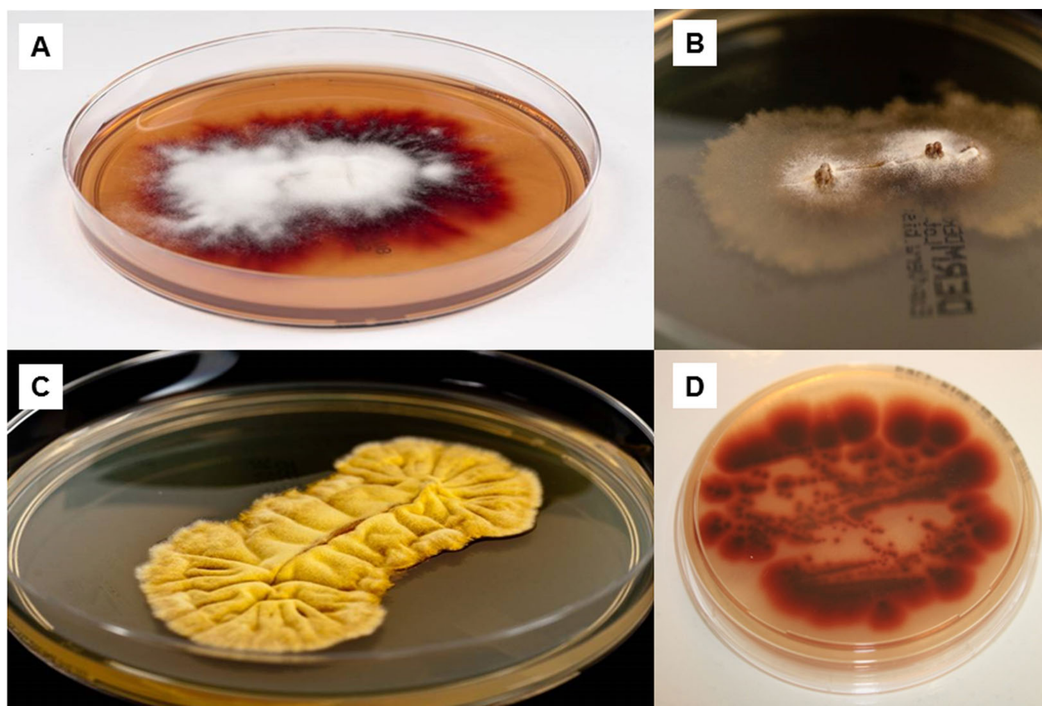

Figure 1. Cultures of the most common dermatophytes grown from the 115 samples obtained from children with clinically suspected tinea capitis: (A) *Trichophyton violaceum*, (B) *Microsporum audouinii*, (C) *Trichophyton soudanense*, and (D) *Trichophyton rubrum*.

Table 3. Species identified in the 115 samples obtained from children with clinically suspected tinea capitis by culture evaluation and PCR-ELISA.

	% (no./total no. isolates)	
	Culture[1]	PCR-ELISA[2]
Trichophyton rubrum	2.7 (3/112)	8.9 (8/90)
Trichophyton interdigitale	…	2.2 (2/90)
Trichophyton soudanense	4.5 (5/112)	…
Trichophyton violaceum	65.2 (73/112)	88.9 (80/90)
Microsporum audouinii	17.0 (19/112)	…
Microsporum canis	…	…
Arthroderma benhamiae	…	…
Aspergillus niger	1.8 (2/112)	…
Fusarium oxysporum	2.7 (3/112)	…
Scorpolariopsis brevicaulis	6.3 (7/112)	…

[1]including nine double infections and one triple infection.
[2]including three double infections.

five species for which primer pairs were included can be identified using this method, while other species will yield negative results. Comparison of results for PCR-ELISA versus Blancophor preparations revealed a 0.82 sensitivity and 0.55 specificity (Table 2B). Accuracy was determined to be 0.77 with $\alpha = 0.08$ (false positive results) and $\beta = 0.15$ (false negative results). However, comparison of results for PCR-ELISA versus culture revealed a 0.84 sensitivity and 0.85 specificity (Table 2C). Accuracy was determined to be 0.84 with $\alpha = 0.02$ (false positive results) and $\beta = 0.14$ (false negative results).

Differences in dermatophyte identification between culture and PCR-ELISA methods were prevalent (Table 3). *M. audounii* was identified in 15 samples as single organism by culture. However, PCR-ELISA identified *T. violaceum* in three samples, one as a double infection of *T. rubrum* and *T. violaceum* and eleven samples were negative. In the three double infections of *M. audouinii* and *T. violaceum* distinguished by culture, PCR-ELISA yielded only *T. violaceum* as causative agent. This is due to the lack of primers for detection of *M. audouinii* with the PCR-ELISA. The double infection of *M. audounii* and *T. soudanense* was classified as *T. rubrum* by PCR-ELISA, which can be explained by cross reactivity of *T. rubrum* and *T. soudanense*. In addition, three out of seven identifications of *T. violaceum* in culture were classified as *T. rubrum* by PCR-ELISA. In two cases a double infection with *T. interdigitale* was found, while the other two cases were negative in the PCR-ELISA. Twice *T. rubrum* was identified by culture, but in the same samples *T. violaceum* was identified by PCR-ELISA. *T. soudanense* was identified in three samples as sole causative agent according to culture evaluation. Here, PCR-ELISA detected *T. rubrum* instead of *T. soudanense*. The mixed infection of *T. soudanense* with *T. violaceum* and *T. rubrum* found in one culture sample yielded only *T. violaceum* as causative

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agent by PCR- ELISA. *S. brevicaulis* was found in two cultured samples as sole causative agent, but PCR-ELISA classified one of them as *T. violaceum* and the other as negative. Moreover, the four double infections of *S. brevicaulis* and *T. violaceum* observed in culture were identified as *T. violaceum* by PCR- ELISA, while the double infection of *S. brevicaulis* and *F. oxysporum* yielded a negative result by PCR-ELISA. *F. oxysporum* was further recognized in two samples by culture evaluation, while PCR-ELISA positively identified one as *T. violaceum* and the other two were negative. Additionally, two negative cultures samples were identified as *T. violaceum* by PCR-ELISA, and *T. interdigitale* was only detected by PCR-ELISA and not by culture evaluation.

Discussion

According to MUST records, the prevalence of clinically diagnosed tinea capitis among children aged 1 to 16 years presenting at Mbarara University Skin Clinic was estimated to be 14.8% in 2010. This makes tinea capitis one of the common dermatologic presentations among children in East Africa.[30] In 2005, the incidence of tinea in sub-Saharan Africa was estimated to be over 78 million.[31] This reveals the magnitude of the problem, which may be accounted for by interplay of several factors, namely, age, sex, race, low social economic status, and probably the warm humid conditions which favor germination of the arthrocodinia on the keratinocytes and the corneus layer.

Direct microscopic examination of KOH-macerated samples is still the gold standard for the diagnosis of dermatophytosis.[25] Direct microscopy with Blankophor stain had a positive yield of 95/115 (82.61%). Although direct microscopy is fast and simple, false-negative results can be observed in 5–15% of cases.[32] However, this method has its drawbacks as it is less specific and cannot identify the dermatophyte species and genera, which is critical in deciding treatment options. Fungal cultures require long incubation periods up to 6 weeks,[33] yet it enables species identification of the fungal pathogen based on morphological and physiological features. The most common identified dermatophyte by culture evaluation was *T. violaceum* followed by *M. audouinii*, *T. soudanense,* and *T. rubrum*. However, its major drawbacks are the long duration for the dermatophyte growths, which can go up to 4 weeks.[34] Moreover, there is a considerable morphological diversity among dermatophytes which poses a challenge and requires great expertise and technical experience in order to accurately identify the species. Even with experience, some biotypes or variations have been considered unambiguous species in the past, based on profound differences in morphology and pattern of infection.

T. violaceum was also identified as the most common dermatophyte by PCR-ELISA. The PCR-ELISA method offers the advantage of being directly applicable to clinical samples that are adequately extracted, discriminating five common dermatophyte species within 24 h.[30] However, *M. audouinii* and *T. soudanense* could not be confirmed by PCR-ELISA as primers for these dermatophytes were not available. Yet these dermatophytes are known to be endemic in Africa.[35] *T. soudanense* has been consistently found inseparable from *T. rubrum* by molecular studies.[36] Indeed, recent taxonomy does not list the species *T. soudanense*.[36] Hence, it is not surprising that within this study four dermatophytes, identified as *T. soudanense* by culture evaluation, were positively detected as *T. rubrum* by PCR-ELISA. *M. audouinii* was isolated 19 times in culture, 15 times as pure isolates, and in 4 cases as mixed cultures. Using the PCR-ELISA, these specimens were identified as *T. violaceum* (mixed infection of *M. audouinii* and *T. violaceum*), *T. rubrum,* or negative. This had to be expected, as only *M. canis* probes were used and none specific for *M. audouinii*. That *M. audouinii* could not be detected directly by PCR-ELISA is a limitation of this method. However, a study by Roque et al. stressed how difficult and time consuming it is to identify *M. audouinii* and further differentiate it from *M. canis*.[16] For that reason, the MA1-F and MA1-R primers were developed and tested. The MA1 primer proved to be very specific for identification of *M. audouinii* in diagnosis of tinea capitis and could be applied directly to skin and hair samples.[16] However, this method has the setback that it takes 1 to 4 days to get results. Hence, it was not used in this study. Moreover, 'white strains' of *T. violaceum* might be misdiagnosed as *T. rubrum* by culture.[37] The reasons for the two culture positive samples (*T. violaceum*) and PCR negative are unclear. However, this was also experienced by Beifuss et al., who attributed it to different sampling conditions but could not rule out insufficient reactions of the PCR-ELISA.[25] In addition, the faster growth of the moulds *S. brevicaulis* or *Fusarium sp.* may suppress the slowly growing *T. violaceum* in fungal cultures. Hence, identification in the respective samples by PCR-ELISA was possible but not achievable by culture.

In the study presented, moulds such as *A. niger*, *F. oxysporum*, and *S. brevicaulis* were identified in 12 cases by culture. It is dubious whether these moulds could be the causative agent for the tinea capitis in the children or are simply contaminants. However, in the study presented, any mould isolated should be considered a contamination as samples were taken only once and not repeatedly from the patients in this study. Recognition of a mould as causative

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agent would require a repeated isolation from the same lesion of the patient for diagnostic certainty. So far, there is no serious evidence that moulds are capable of causing a tinea capitis–like dermatomycosis. Yet, there is a growing amount of contradictory results from etiological and epidemiological studies from all over the world.[38] Recently, a paper written by colleagues from Bulgaria discusses the possible role of *A. niger* in tinea capitis, following the incidence and clinical course of nondermatophytic tinea capitis cases. They concluded that pathogenic moulds could be a new etiologic agent in tinea capitis in children, especially in patients with poor living conditions or social deprivation.[38] To this date, it is not possible to establish if this might be also the case in Uganda. Hence, it could be of interest to investigate this further, using repeated sampling from the patients included in the study to confirm possible infections with moulds and distinguish between contaminants.

Molecular-biologic methods, like the PCR-ELISA method used in this study, are a promising tool in clinical science proven by their high success rate at accurately diagnosing bacteria and viruses. For this reason, their application in mycology has been highly embraced, as these methods will alleviate the problems of time consumption and technical issues, encountered by conventional methods like dermatophyte culture. It was therefore concluded that implementation of the PCR-ELISA alongside Blankophor staining as routine methods for diagnosis of dermatophytes at MUSC is recommendable as they improve sensitivity and specificity of the diagnosis. The early identification of the causative agent of tinea capitis is a prerequisite for the effective management of the disease, the identification of probable source and the prevention of spreading. Children with tinea capitis in Western Uganda should be treated by systemic therapy rather than topical remedies to ensure high cure rates as the most common causative dermatophyte *T. violaceum* exhibits an endothrix rather than ectothrix invasion of the hair follicle.

Supplementary material

Supplementary data are available at *MMYCOL* online.

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Declaration of interest

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