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Prevalence and identification of fungi associated with Tinea capitis in school children of Morogoro municipality, Tanzania

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Tinea capitis **is one of the major common skin diseases affecting school-age children in developing countries, whose prevalence and associated fungi have not been fully investigated in these countries. This study investigated the prevalence and fungi associated with *Tinea capitis* infection amongst children attending selected schools in Morogoro Municipality, Tanzania. A descriptive cross-sectional study was conducted involving 72 school children recruited from 10 primary schools in selected class strata in Morogoro Municipality, Tanzania. A multistage sampling technique was used. Skin scrapings were obtained from head lesions of school children followed with fungi isolation. Fungi were identified based on their morphological characteristics and nucleotide sequencing of the 5.8s rRNA gene and flanking internal transcribed spacer regions ITS1 and ITS2. Socio-demographic characteristics of sampled school children were collected using a questionnaire. The fungi associated with tinea capitis in school children were *Trichoderma longibrachiatum*, *Cytobasidium minutum, Aspergillus spp, Ectophoma multirostrata, Aureobasidium pullulans, Aspergillus flavus, Cladosporium tenuissimum, Penicillium flavigenum* and *Fusarium solani.* Out of the 10 primary schools surveyed, Chamwino had an overall higher prevalence with 11 (15.3%) cases of tinea capitis. Overall, 31 (43.1%) of the school children washed their hair at least once a day. It was found that 30 (41.7%) and 39 (54.2%) school children shared combs and had a member in their family with tinea capitis, respectively. Furthermore, only 18 (25%) of the affected school children sought treatment. *Tinea capitis* is associated with multiple dermatophytes amongst school children in Morogoro Municipality. It is recommended that body hygiene education be emphasized in schools and congestion of classrooms be avoided in order to minimize transmission of the disease through contact.**

Key words: *Tinea capitis*, Fungi Identification, dermatomycosis, dermatophytes, school children, Morogoro, Tanzania.

INTRODUCTION

Tinea capitis is a superficial fungal infection of the scalp, eyebrows and eyelashes, with a propensity for attacking hair shafts and follicles. The two most common dermatophytes responsible for tinea capitis infection are *Trichophyton tonsurans* and *Microsporum canis* (Shy, 2019). The infection is the most common dermatophytosis

in children and its transmission is fostered by poor hygiene, overcrowding and sharing of contaminated hats, brushes, pillowcases and other inanimate objects (Kelly, 2012). It is a public health problem that may lead to poor attendance among school children in low and middle-income countries (Chikoi et al., 2018). Studies conducted previously in developing countries have reported a high prevalence of skin infections among school children, the spectrum of which has been highly variable (Ferie et al., 2006; Komba and Mgonda, 2010).

In Tanzania, previous studies have shown that tinea capitis is one of the major skin diseases reported in school children (Andrews and Burns, 2008). Other studies have documented prevalence rates between 12 and 55% of dermatomycosis among school-age children (Ferie et al., 2006; Komba and Mgonda, 2010). However, the prevalence and fungi associated with tinea capitis among children in Tanzania have not been sufficiently investigated. This study isolated and identified diverse fungi associated with tinea capitis in selected school children in Morogoro Municipality, Tanzania. The findings of this study will enhance awareness of skin fungal infections in schools and households and assist the educational and health sectors in formulating appropriate tinea capitis control strategies for school children in Tanzania.

MATERIALS AND METHODS

Study design and setting

The present study was a descriptive cross-sectional study conducted in Morogoro Municipality involving primary school children with clinical presentation suggestive of fungal infections of the head, including ring scalp, baldness and alopecia. Swabs and scrapings were obtained from the head lesions and placed into sterile containers before being transported to the laboratory at Sokoine University of Agriculture for isolation and identification.

Sample and demographic data collection

A multistage sampling technique was used that involved selection of school children from different primary schools and class strata. A total of 72 school children with clinical signs suggestive of tinea capitis infection were recruited into the study from 10 primary schools; Misufini, Mafiga, Msamvu, SUA, Kikundi, Chamwino, Mtawala, Bigwa, Mwere and Mbuyuni. School children aged between 6 and 14 years from classes 1, 3, 5 and 7 were enrolled into the study after obtaining a written consent from parents/guardian. Lesions were physically examined in broad daylight. Afterwards, skin scrapings were obtained from the head lesions using a sterile scalpel blade before being placed into a sterile container. A questionnaire was administered to study participants in order to obtain socio-demographic data. The demographic profile collected from study participants included age, sex, class, residence, hygienic practices and history of infection in order to identify predisposing factors for fungal infections.

Data analysis

Data obtained from questionnaires were entered cleaned and analyzed using the Statistical Package for Social Sciences (SPSS) version 16.0. (SPSS Inc., Chicago, IL, USA). A chi- square test was used to test the association between infection and other variables. A p-value <0.05 was considered statistically significant.

Ethical consideration

The purposes and benefits of the study were explained to the school children, parents/guardians and teachers. Informed written consent from the parents/guardians of the study subjects was sought before recruitment into the study. Ethical clearance was obtained from the National Health Research Committee of the Tanzania National Institute for Medical Research, certificate number NIMR/HQ/R.8a/Vol. IX/1943.

Laboratory studies

Isolation of tinea capitis

Sabouraud dextrose agar (SDA) was prepared according to the instruction of the manufacturer (Sigma Aldrich, USA). Briefly, after sterilization and cooling of the media, the media was mixed with skin scrapings and poured into 150 mm sterile cell culture dishes (Corning Incorporated, Corning, NY). The skin scrapings in SDA were allowed to solidify and incubated at room temperature for three days and the ensuing colonies were sub-cultured in SDA prepared in 9 mm diameter Petri dishes (MLS, Menen, Belgium). Fungal colonies were passaged four times in order to obtain pure cultures.

Morphological identification

Fungal macro-morphological appearance (color and colony texture) on SDA was appreciated by the naked eye and recorded using a digital camera. For micro-morphological identification, colonies were picked using a cello tape or needle mount technique, stained with lactophenol cotton blue for 1 min followed by observation under a light microscope (Olympus, Tokyo, Japan) at 400X magnification. Photographs of fungal micromorphology were captured using a camera mounted into the microscope.

Fungal DNA extraction

Pure colonies of the fungi were picked using a sterile pipette tip and mixed with 140 µl of phosphate-buffered saline (PBS; Sigma, St. Louis, MO) followed by incubation with 20 µl of 20 mg/ml proteinase K(Macherey-Nagel, Duren, Germany) at 50°C for 2 h. DNA was extracted using a QiaAmp DNA extraction kit(Qiagen, Hilden,

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Germany), following manufacturer's instructions.

Polymerase chain reaction (PCR) and DNA sequencing

The composition of PCR master mix using DNA polymerase consisted of Dream *Taq* polymerase (10 µl), RNase-free water (7 µl), ITS 1F primer 10 µM (1 µl) and ITS 4 primer 10 µM (1 µl) for single reaction. Amplification was done by adding to the reaction mix solution 1 µl of extracted DNA as a template. Amplification of the 5.8 rRNA and flanking ITS1 and ITS2 was done using specific primers ITS 1F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (Balajee et al*.,* 2007; Iwen and Hinrichs, 2002). The amplification conditions consisted of an initial denaturation at 94°C for 10 min followed by 40 cycles of denaturation (45 Sec at 94°C), annealing (45 s at 55°C) and extension (1 min at 72°C), and a final extension at 72°C for 10 min. Amplification was performed in an automated thermal cycler (Applied Biosystems, Foster City, CA). The PCR products were electrophoresed in a 2% agarose gel mixed with Gel Red nucleic acid stain (Phenix Research Products, Candler, NC). Visualization and imaging was done using a GelDoc-EZ Imager (Bio-Rad Laboratories, Hercules, CA). In order to verify the retrieval of fragments of fungi, PCR fragments were purified from the agarose gel using NucleoSpin gel and PCR clean-up kit (Macherey-Nagel, Duren, Germany) and subjected to dideoxynucleotide cycle sequencing using Big Dye Terminator Cycle Sequencing Kit Version 3.1 (Applied Biosystems, Foster City, CA). Products from dideoxynucleotide cycle sequencing reaction were purified by ethanol precipitation and separated on a 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). The obtained sequences were deposited at GenBank and assigned with accession numbers. In order to identify the isolated tinea capitis*,* ITS1-5.8S-ITS2 nucleotide sequences were aligned with sequences deposited at GenBank database using a BLASTn tool (www.ncbi.nlm.nih.gov/blast).

RESULTS

Morphological fungi identification

Fungal cultures on SDA were considered positive if there was growth after 3 days of incubation or negative if there was no growth following 7 days of incubation. Fungi with varying structures and color on primary SDA culture presented mixed colonies of varying texture and color, including white, cotton-like, and green crenated ones. Others appeared with radial furrows, grey cotton-like and creamy shinning colonies. Sub-cultivation of the isolates achieved pure colonies with different presentations as shown in Figure 1.

Following sequencing of the PCR products and conducting BLASTn at GenBank, the fungal isolates that were found to be associated with tinea capitis were identified as *Trichoderma longibrachiatum, Cytobasidium minutum, Aspergillus spp, Ectophoma multirostrata, Aureobasidium pullulans, Aspergillus flavus, Cladosporium tenuissimum, Penicillium flavigenum,* and *Fusarium solani* (Table 1).

Socio-demographic characteristics and prevalence of *Tinea capitis*

A total of 72 school children were recruited for the study subjects, 12 (16.67%) had tinea capitis*.* Out of the 10 primary schools surveyed, Chamino had 11 (15.3%) cases which was the highest overall prevalence and the difference was statistically significant ($p < 0.05$). The age range of the school children was between 6 and 14 years and boys, n=39 (54.2%), were more affected than girls, n=33 (45.8%). However, there was no statistical significant difference ($p > 0.05$) between the genders. The majority of children belonged to the age group of 9 to 14 years 62 (86.1%) and among school children of class five, 28 (38.9%), this difference was statistically significant ($p<0.05$).

Awareness of risk factors for Tinea capitis

Upon assessment of the habits and behaviors of school children regarding hair hygiene, of the 72 school children interviewed, 31 (43.1%) reported to wash their hair at least once a day and 61 (84.7%) regularly visited the barber for haircut. Only 18 (25%) of the infected school children did seek treatment at a health facility while 47 (65.3%) children used drugs to self-treat the infection. Thirty (41.7%) school children commonly shared combs at home and 39 (54.2%) children had an infected member in the family (Tables 2 and 3).

DISCUSSION

The present study investigated the prevalence, socio-demographic characteristics and identification of fungi associated with *Tinea capitis* in school children in primary schools of Morogoro. This study has shown an overall prevalence of tinea capitis in 10 primary school children in Morogoro region to be 16.67%. This prevalence is slightly lower than in previous reports, 48.86% in the Tanzania (Chikoi et al., 2018; Komba and Mgonda, 2010). The lower prevalence observed might be due to differences in the study populations of the studies with respect to, their socio-demographic and behavioral characteristics.

Identification of fungi isolates by morphological and molecular techniques has indicated that the following fungi are associated with tinea capitis: *Trichoderma longibrachiatum, Cytobasidium minutum. Aspergillus* spp*, Ectophoma multirostrata, Aureobasidium pullulans, Pichia terricola, Aspergillus flavus, Cladosporium tenuissimum, Penicillium flavigenum* and *Fusarium solani.* The two most common fungal agents of tinea capitis infection have been reported to be *Trichophyton tonsurans* and *Microsporum canis* (Komba and Mgonda*,*

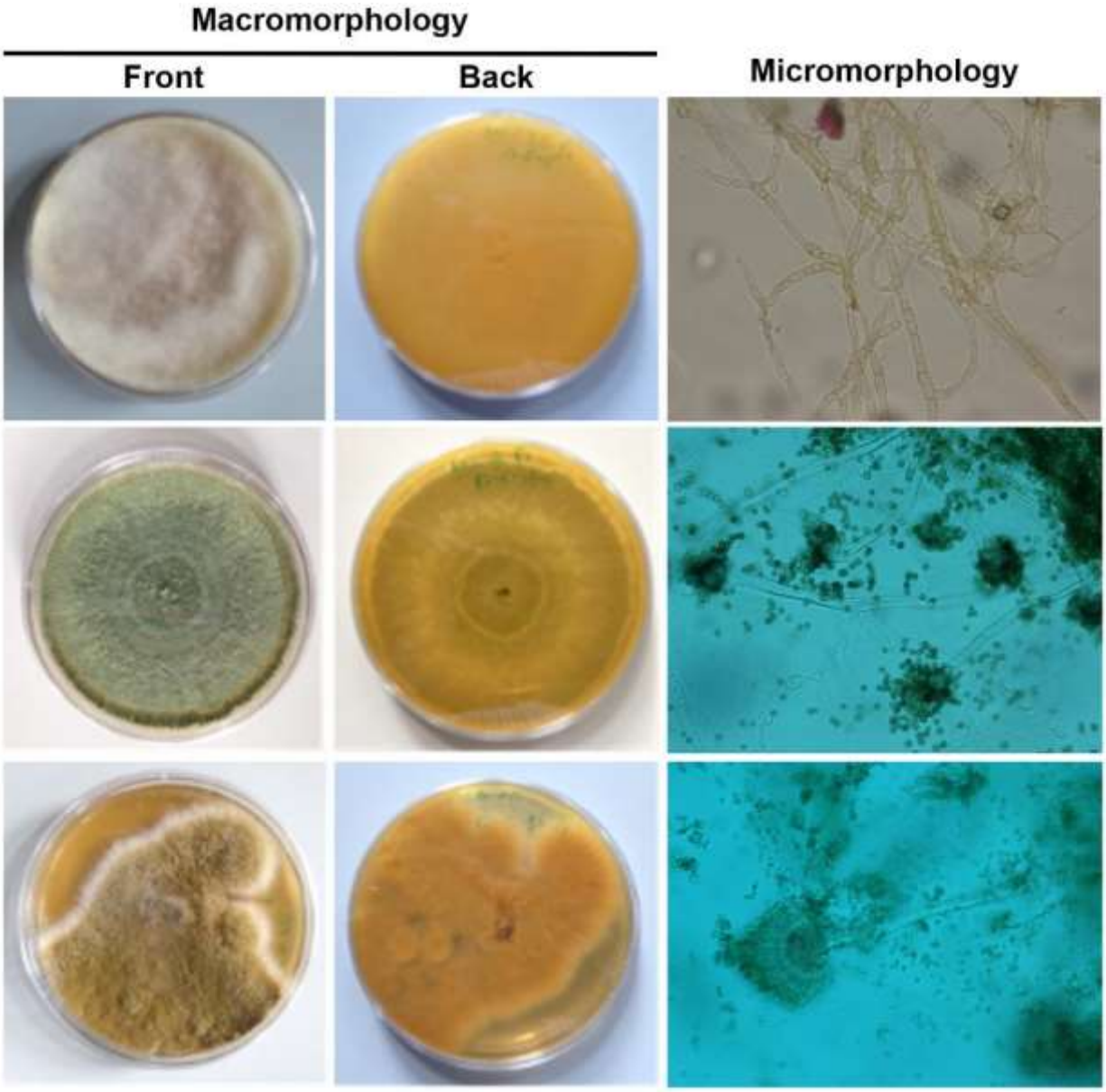

Figure 1. Morphology of fungi associated with Tinea capitis in school children of Morogoro Municipality. Macromorphology of fungi was obtained by visualization of fungi using a naked eye while micromorphology was viewed under the light microscope at 400X magnification.

2010). Some of the dermatophytes identified in the study such as *Aspergillus* spp have been reported elsewhere to be causative agents of the tinea capitis in children (Jia et al., 2019; Wiegand et al., 2017). The study findings are in agreement with studies conducted in Uganda in which reported moulds *A. niger, F. oxysporum,* and *S. brevicaulis* in 12 cases by culture methods. The Uganda studies raised the question whether these moulds were potential agents of tinea capitis in the children or if were mere contaminants. So far there is no confirmatory evidence that moulds are capable of causing tinea capitis (Wiegand et al., 2017). Recently, (Chokeva et al., 2016) discussed the possible role of *A. niger* in tinea capitis infection and concluded that pathogenic moulds could be 'new' etiologic agents of *Tinea capitis* in children, especially in areas of social deprivation and poor living environments.

This study has revealed that tinea capitis is particularly prevalent in children between the ages of 9 and 14 years. This is in agreement with other studies, that children

Table 1. Fungal isolates associated with tinea capitis after BLASTn.

Sample	BLASTn	Accession number
KIK 02	*Trichoderma longibrachiatum*	MN700636
KIK 04	*Cytobasidium minutum*	MN700637
MSU 03	*Aspergillus sp*	MN700638
MSU 03	*Ectophoma multirostrata*	MN700639
MSU 04	*Fusarium solani*	MN700640
SUA 07	*Aureobasidium pullulans*	MN700641
MSU 06	*Pichia terricola*	MN700642
KIK 06	*Cladosporium tenuissimum*	MN700643
CHA 03	*Penicillium flavigenum*	MN700644
KIK 06	*Aspergillus flavus*	MN700645

KIK= Kikundi, MSU= Msufini, CHA= Chamwino.

Table 2. Socio-demographic characteristics of study subjects.

Variable	Total number n=72, (%)	Infected, n (%)	p-value
Sex			
Male	39(54.2)	9(12.5)	0.480
Female	33 (45.8)	3(4.16)	
Age (years)			
6-8	10 (13.9)	4(5.55)	0.001
9-14	62 (86.1)	8(11.11)	
School			
Chamwino	11(15.3)	5 (6.94)	0.001
Bigwa	4 (5.6)	0 (0.00)	
SUA	5(6.94)	0(0.00)	
Mafiga	7(9.72)	0(0.00)	
Msamvu	8(11.1)	2 (2.78)	
Kikundi	9(12.5)	2(2.78)	
Mtawala	6(8.33)	0 (0.00)	
Mwere	8(11.1)	0(0.00)	
Misufini	7(9.72)	1 (1.39)	
Mbuyuni	7(9.72)	2(2.78)	
Class			
One	9 (12.5)	2(2.78)	0.001
Three	11(15.3)	3 (4.17)	
Five	28 (38.9)	5 (6.94)	
Seven	4 (5.61)	2(2.78)	

below the age of 10 years were highly infected (George and Altraide, 2008), suggesting that dermatophytosis, especially tinea capitis, is predominantly a pre-pubertal disease (Moto et al., 2015). The infection was more prevalent in male school children (54.2%), possibly because parents/guardians are more insistent on the hygiene of female than their male children (Falahati et al., 2003). Furthermore, frequency of hair wash among the children could reduce tinea capitis because children who washed more frequently presented lower infestation rates

Table 3. Personal hygiene and habits among study participants.

Risk factor	Number, N	Percentage)	p-value
Frequency of hair wash			
Once a day	31	43.10	0.001
Five times a day	2	2.80	
Do not wash	39	54.10	
Sharing of pillows			
Share	20	27.80	0.001
Do not share	52	72.20	
Hair cutting by barbers			
visit the barber	61	84.70	0.001
Do not visit barber	11	15.30	
Hygiene of the barber's tools			
Barbers clean the shavers	26	36.10	0.001
Barbers do not clean the shavers	46	63.90	
Antifungal use			
Use antifungal for treatment	47	65.30	0.010
Don't use antifungal	25	34.70	
Hospital visits for treatment			
Attend hospital	18	25	0.001
Don't attend hospital	54	75	
Contact with domestic animals			
Played with domestic animal	8	11.10	0.001
Did not play	64	88.90	
Sharing of hair combs			
Share	30	41.70	0.157
Don't share	42	58.30	
Sharing of hats			
Share	19	26.40	0.001
Don't share	53	73.60	
Infection in the family			
Infected member	39	54.20	0.480
Uninfected member	33	45.80	

(Afolabi et al., 2018; Chikoi et al., 2018). It was also reported that 84.7% of the barber's shops did not clean the shaving devices regularly after a haircut.

Hospital attendance was reported by only 25% of the infected children with tinea capitis, possibly due to lack of resources to pay for treatment by parents/guardians, who resorted to applying herbs or other cheaper remedies, with questionable efficacy (Chikoi et al., 2018; Ferie et al., 2006). This study further, showed a significant association between tinea capitis infection and contact with domestic animals such as cats and dogs, ($p<0.05$). It is suspected that the infection may be shared between children and domestic animals (Ameh and Olkolo, 2004). Studies elsewhere have reported that sharing of clothes,

towels, and combs facilitate transmission of tinea capitis (Chikoi et al., 2018). In this study, there was no significant association between infection and sharing of hair combs, beds or possible contact with an infected family member (p> 0.05). This is in line with previous findings in the southern part of Tanzania (Chikoi et al., 2018).

CONCLUSION AND RECOMMENDATIONS

The findings of this study indicate a high level of tinea capitis infection among children attending government-owned public primary schools in the Morogoro Municipality, Tanzania. Apart from *T. tonsurans* and *M. canis*, diverse dermatophytes are potentially opportunistic etiologic agents of tinea capitis in children, especially those living in environment with poor hygienic practice. The severity of tinea capitis among school going children is moderately high and that socio-demographic and behavioral factors profoundly influence the development and occurrence of the infection in children. There was a significant association between tinea capitis and certain unhygienic practices, and contact with domestic animals. This suggests an animal–human transmission of tinea capitis is likely in the study area; however, this study did not carry out a detailed investigation to confirm this transmission route. Majority of the affected children did not seek for medical assistance; hence, school health programs must emphasize on the importance of skin and hair hygiene to enable early detection, prevention and eventual treatment of dermatophytosis among school children. Since the study population was small and this being a point prevalence cross-section study, the tinea capitis infection is most likely underestimated.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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