

Risk Factors Associated with *Tinea capitis* among Pupils in Lagos, Ekiti and Ogun States, Nigeria

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Abstract: Dermatophytes, the aetiological factor of *Tinea capitis* are pathogens causing infections of various courses. Limited studies of *Tinea capitis* have been carried out in Nigeria. This study therefore is aimed at determining the risk factors associated with this infection in Ado-odo Ota (Ogun State), Somolu, (Lagos State) and Oye-Ekiti, (Ekiti State). Scalp lesions of 102 primary school children were aseptically collected, after which questionnaires were used to collect their bio/socio-demographic data. The samples were analysed using microscopic, cultural, biochemical and molecular techniques, while the data was statistically analysed. In a total of one hundred and two (102) children aged between 1-14 years consisting of 73 (71.6 %) males and 29 (28.4 %) females, 75 (73.5 %) were infected with dermatophytes. The dermatophytes consist of 29.3% *Trichophyton*, 65.3% *Microsporum* and 5.3 % *Epidermophyton*. Blasting of the sequenced Intraspecific ('ITS') genes confirmed the presence of the isolates. Males were most affected with socio-economic factors such as employment status of the parents, influencing infections ($P < 0.001$). Other factors that facilitated transmission include: knowledge of mode of transmission, sharing of combs and towels, place of hair shaving-barbing, and age (5-10 most vulnerable, 4 and 11-14 less vulnerable). Number of children in the family and school location were discovered not to be risk factors. Health education is paramount in eradicating this infection, hence the introduction of hygiene policy into educational curricular, will ultimately help to reduce the menace of *Tinea capitis*.

Keywords: *Tinea capitis*, Dermatophytes, 'ITS' genes, Blasting, Molecular techniques

INTRODUCTION

T*inea capitis* is predominantly a disease of pre-adolescent children, being less common in adults (Robertson and Wright, 2000). Typical age of onset is between 5-10 years, the infection accounting for 92.5% of dermatophytosis in children younger than 10 years. The aetiological agents include *Trichophyton*, *Microsporum*, and *Epidermophyton* (Sarabi, 2008). The most prevalent dermatophyte causing *Tinea capitis* infection is *Microsporum canis* followed by *Trichophyton mentagrophytes*, *Trichophyton violaceum*, *Trichophyton verrucosum* and *Trichophyton rubrum* (Anosike et al., 2006). Transmission is enhanced by poor standard of living and hygiene, climatic conditions, and over-crowding (Oyeka and Okoli, 2003). Occasionally fungi and their spores can remain alive on combs, brushes, unwashed towels, furniture, bed-sheets, and other fomites for long period (Seema et al., 2011). Moreover, asymptomatic carriers transmit anthropophilic dermatophytes making *Tinea capitis* difficult to eradicate. *Tinea capitis* is associated with clinical features and symptoms like Favus (Macit, 2010),

Keroin (Kemal et al., 2013), blackdot (Singal et al., 2001), grey patches, scaling, erythema, itching, hair loss and lesions similar to impetigo. In tropical countries, a warm and humid climate, crowded environment and poor sanitary conditions promote the spread of the infection (Nwogu et al., 2008). Pathogenesis is enhanced by the ability of dermatophyte to utilize casein and keratin for its growth (Mendez-Tovar, 2010). Over the past two decades, the incidence of this infection has increased, and the population at risk has increased dramatically, with prevalence rate of about 7.4% being reported in Nigeria (Ayanbimpe et al., 2008). Young age, poor ventilation, overcrowded environment, male gender and poor hygiene increases susceptibility to this infection. The country suffer massive financial loss through health expenses on this infection that could easily be prevented. This study is therefore aimed at identifying the risk factors associated with *Tinea capitis* among pupils in Oye-Ekiti, Ado-odo Ota and Somolu local government of Ekiti, Ogun, and Lagos state respectively. The study highlights the need for infection control measures and strict health policy which will keep the promise of meeting the millennium development goals of reduction in child mortality and morbidity.

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Educating most children on preventive measures will make a great impact because most children are eager to learn, and can therefore instill and promote positive behavioural change in their environment.

MATERIALS AND METHODS

Study Size/Areas/Duration: The study was conducted among primary school children in Somolu Local Government Area of Lagos state, Oye-Ekiti Local Government Area of Ekiti state, and Ado-odo Ota Local Government Area of Ogun State Nigeria, within a period of six months (March to August, 2017).

Advocacy/Ethical Consideration: Approval was sought and obtained from the health research ethics committee for this research work at, Lagos State University Teaching Hospital (LASUTH), with reference number; REF.NO. LREC.06/10/852.

Inclusion/ Criteria: All pupils (suspected to be infected with *Tinea capitis*) aged between 1-14 years in the selected primary schools, who were present in school at the period of study, and whose parents consented, were included for the study.

Sample Collection: Infected scalp was first sterilized with 70% alcohol; after which the scrapings and infected hair stub were eventually collected aseptically on a sterile brown paper, using a sterile tooth brush. The collected samples were transported to the laboratory for analysis.

Tool for Data Collection: Well-structured questionnaires were administered to the parents/guardians of the children, in order to obtain the socio-economic and bio-demographic data of the children (Menan *et al.*, 2002).

Sample Processing: Samples were processed using standard microbiological procedures. The isolates were examined and identified using microscopic, colonial morphology, biochemical and molecular characteristics (Robert and Pihet, 2008).

Direct Microscopic Examination: Scalp lesion was placed on a slide, after which two drops of 20% potassium hydroxide (KOH) were placed on the slide, warmed and examined within 30 minutes for the presence of fungal hyphae and/or spores under a light microscope (x10, x20 and x40) magnification. Each scraping was aseptically inoculated in Sabouraud dextrose

agar (SDA) containing Chloramphenicol and Cycloheximide, in order to inhibit the growth of bacteria and saprobic fungi respectively (Margill *et al.*, 2007). The plates were incubated at 28°C for one to two weeks and examined for growth (Rebel and Taplin, 1979). Isolates were then, sub-cultured in SDA to obtain a pure culture, and then characterized by duration of growth, surface morphology and colony pigmentation (Ellis *et al.*, 2007).

Indirect Microscopic Examination: Isolates were examined microscopically for morphological features (formation of macroconidia, microconidia, and hyphae) of fungi using lactophenol cotton blue mounts of the pure culture.

Biochemical Tests

Rice grain test: Isolates were transferred to a vial containing cooked sterile rice grains, incubated and then inspected for growth at 30°C for 5 days (Ayanbimpe *et al.*, 2008).

In vitro Hair Perforation Test: Sterilized short strands of hair were deposited in Petri dishes containing 25 ml of sterile distilled water and three drops of 10% dermatophyte extract (Enany *et al.*, 2013). After one week of incubation at 25°C, the hair strands covered by mycelium, were examined under microscope by mounting in lactophenol cotton blue stain; perpendicular to the long axis of the hair indicates a positive test (Padhye *et al.*, 1980).

Urea Utilization Test: Isolates were inoculated and incubated in Christensen's urea medium, at 28°C for five days, after which they were observed for growth (Philpot, 1967).

Casein Hydrolysis Test: Isolates were inoculated and incubated in casein dextrose yeast extract agar, at 28°C for 5 days.

Molecular Identification

This Involved DNA extraction and amplification of 'ITS' genes using 'ITS' primers. The ' genes were sequenced, after which the aligned sequences were blasted (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Analysis of Data

Data entry and analysis were performed using microsoft and spread sheet software. The results were then presented in descriptive statistics using frequency table. Significance of associations was based on P-values < 5%.

RESULTS

A total of 102 samples collected from pupils aged 1-14 years were analysed for the presence of dermatophytes implicated in *Tinea capitis*, and the risk factors associated with the infection. Ninety five (95) samples tested positive to microscopy using potassium hydroxide (KOH), while 75 isolates of dermatophytes were identified. Among the isolates, *M.canis* (42.9%) was mostly isolated, while *T. verrucosum* (2.7%) and *T. rubrum* (2.7%) were the least species isolated. Although other species like *M. audounii* (24%), *T. tonsurans* (13.3%), *M.*

gypseum (13.3%), *T. mentagrophyte* (10.7%) and *E. floccosum* (5.3%) were also isolated.

Biochemical characteristics of the isolates is summarized in table 1. Incidence of *Tinea capitis* in correlation to the demographic characteristics of the pupils such as age, parents' employment status, knowledge of mode of transmission, place of hair shaving, and sharing of combs/towels was significant, where as the incidence of the infection by family size and school was not significant (Table 2). Distribution of causative agents according to the gender, school and number of children in the family, was also insignificant (Table 3).

Table 1: Biochemical characteristics of isolates

Isolate	CHT	3% NACL TT	UUT	IVHPT	GROWTH AT 37°C	RGT
<i>M. gypseum</i>	Partial hydrolysis	negative	positive	Positive	Positive at day 5	Positive
<i>M. audounii</i>	Partial hydrolysis	positive	negative	Negative	Positive at day 4	Positive
<i>M. canis</i>	Partial hydrolysis	positive	positive	Positive	Positive at day 4	Positive
<i>T. mentagrophyte</i>	Partial hydrolysis	positive	positive	Positive	Positive at day 3	Positive
<i>T. tonsurans</i>	Slightly partial hydrolysis	positive	positive	Negative	Positive at day 6	Positive
<i>T. verrucosum</i>	Complete hydrolysis	positive	positive	Positive	Positive at day 7	Positive
<i>T. rubrum</i>	Partial hydrolysis	positive	negative	Negative	Positive at day 6	Positive
<i>E. floccosum</i>	Partial hydrolysis	positive	positive	Positive	Positive at day 3	Positive

Key: CHT represents casein hydrolysis test

NACLTT represents sodium chloride tolerance test

UUT represents urea utilization test

IVHPT represents in-vitro hair perforation test

RGT represents rice grain test

Table 2: Incidence of *Tinea capitis* in correlation to the demographic characteristics of pupils

Characteristics	Category	N Frequency with Tinea infection (n)		% infected (C)	(X ²) Chi square value	pvalue	Tri	Mic	Epi
		Infected (A)	Not infected (B)						
Father's employment status	Civil servant						1	7	0
	Self-employed			68.6		<0.001	8	18	1
	Unemployed		1	96.3			13	24	3
Mother's employment status	Civil servant	0	1	0	17.979	<0.001	0	0	0
	Self-employed	56	33	58.9			8	18	1
	Unemployed	45	42	93.3			14	31	3
Age group (years)	- 4	12	9		13.0040		9	15	1
	- 10	54	47	87		<0.001	8	23	2
	- 14	36	19	52.8			5	11	1
No of children in a family	≤ 3	34	20		5.667		7	12	0
	> 3	68	55	80.9		0.017	15	35	4
Knowledge of mode transmission	Have knowledge	12	1		29.700		3	12	4
	Have no knowledge	90	74	82.2		<0.001	19	37	0
Sharing of combs and towels	Yes	88	73		26.905	<0.001	18	33	3
Place of hair place of hair shaving	No	14	2	14.3	44.946		4	16	0
	Home	24	6			<0.001	3	6	0
	Home	24	6	25.0			3	6	0
	Barbing salon	62	59	95.2			11	31	4
	Home & barbing salon	16	10	62.5			8	12	0
Exposure to animals	Yes	83	71		33.036	< 0.001	16	34	3
	No	19	4	21.1			6	15	1
Gender	Female	29	22		0.113		10	11	1
	Male	73	53	72.6		<0.001	12	38	3
School	A	24	17		0.572	0.751	5	11	1
	B			76.3			13	29	3
	C			68.4			4	9	0

Key: % infected (C) = A/N*100, Tri=Trichophyton, Mic= Microsporum, Epi=Epidermophyton

Table 3: Distribution of causative agents in correlation to school, gender and number of children in the family

Characteristics	<i>Microsporum</i>	<i>Trichophyton</i>	<i>Epidermophyton</i>	Total	Chi Square	p-value
Sex						
Male	38 (71.7%)	12 (22.6%)	3 (5.7%)	53	3.915	0.141
Female	11 (50.0%)	10 (45.5%)	1 (4.5%)	22		
Total	49	22	4	75		
School						
A	11 (64.7%)	5 (29.4%)	1 (5.9%)	17	0.902	0.924
B	29 (64.4%)	13 (28.9%)	3 (6.7%)	45		
C	9 (69.2%)	4 (30.8%)	0 (0%)	13		
Total	49	22	4	75		
Number of children in a family						
≤ 3	35 (64.8%)	15 (27.8%)	0 (0%)	22	1.723	0.423

DISCUSSION

To the best of our knowledge, this is the first study aiming to evaluate *Tinea capitis* and its risk factors among pupils in school 'A'- Somolu, 'B'- Ado-odo Ota, and C- Oye- Ekiti. This study which showed that most of infections were due to *M. canis*, seconded by *M. audouinii*, revealed that infected domestic animals (Betatancourt et al., 2009) and man were the most important host that transmitted these agents. The high incidence of *Tinea capitis* caused by *M. canis* and *M. audouinii* was due to frequent contact of these children (especially those in rural areas) with animals (such as Rat, Cat, Dog, Goat) and human respectively (Mentintas et al., 2004). The increased occurrence of these organisms as the cause of *Tinea capitis* in Nigeria had also been reported in other studies; however, in another study in north-east India, *T. tonsurans* was isolated as the most common dermatophyte. *Tinea capitis* caused by *Trichophyton rubrum* is a rare event worldwide (Ziemer et al., 2005). Apart from *M. canis* and *M. audouinii*, which were the predominant species isolated in this study, two anthropophilic species (*T. rubrum* and *T. tonsurans*), three

zoophilic species (*T. mentagrophyte*, *T. verrucosum* and *M. gypseum*) and one geophilic specie (*Epidermophyton floccosum*) were also isolated. The low occurrence of *Trichophyton verrucosum* among the children could be associated with low rates of interaction with cattle which served as reservoir for the dermatophyte. In a particular study, *T. verrucosum* recorded the highest number of dermatophyte isolated, suggesting that prevailing endemic pathogens vary among different regions and change with time in accordance with the existing living and hygiene conditions.

Epidermophyton floccosum was the only geophilic species and the second least number of causative agent isolated, as a result of sporadic nature of the disease caused by this species. However, this was different from a study, where *E. floccosum* was the most common dermatophyte isolated. Statistical analysis gave a brilliant elucidation of the relationship between the causative agents and the bio/socio demographic characteristics of pupils. In others words, statistics is a very important tool which clearly highlights the risk factors associated with *Tinea capitis*.

The highest incidence of infection occurred in younger children aged 5-9 years (Faithi and Al Samarai, 2000). This finding is also in correlation with the findings of Ekanem, which revealed that old age reduces susceptibility to this infection, as reflected in the low incidence of this infection in children aged 11-14; although children with *Tinea capitis* may improve spontaneously at puberty (Emele and Oyeka, 2008). The highest incidence of *Tinea capitis* in males could be due to greater physical activity and increased sweating, while the low incidence rate in girls could be associated with the fact that

most of the girls practice better personal hygiene.

CONCLUSION

M. canis, seconded by *M. audouinii* were the main causative agents. Findings from this study also revealed that age, sex, place of hair shaving, housing type, bathing hygiene, sharing of combs and towels and employment status of parents were risk factors associated with this infection. Therefore personal hygiene, accurate and prompt assessment, diagnosis and treatment of this infection will help to prevent further transmission, and as such stop the menace of this infection.

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