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Characterization of Dermatophytes Isolated from Primary School Children in Parts of Some Selected South West States of Nigeria

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Abstract
Tinea capitis, is predominantly a disease of pre-adolescent children, being less common in adults. This study was aimed at isolating and characterizing dermatophytes recovered from scalp lesions of primary school children in parts of Ekiti, Lagos and Ogun states. Lesions were aseptically collected from 102 children suspected of being infected by dermatophytes, after which their bio/socio-demographic data were collected. Out of the 102 samples, 95 were potassium hydroxide (KOH) positive, while 75 dermatophytes were isolated and confirmed by their physical and biochemical characteristics. The most prevalent dermatophyte was found to be Microsporum canis(n=21; 28%), followed by Microsporum audounii (n=18; 24%,), Trichophyton tonsurans (n=10; 13.3%), Microsporum gypseum (n=10; 13.3%), Trichophyton mentagrophyte (n=8; 10.7%), Epidermophyton flocossum (n=4; 5.3%), Trichophyton verrucosum (n=2; 2.7%), and Trichophyton rubrum (n=2; 2.7%,). Comprehensive microbiological analyses facilitated the identification of dermatophytes. Health education is paramount in eradicating this infection, hence the implementation of personal hygiene policy into educational curricular, will ultimately help to reduce the menace of dermatophyte infection.

Keywords: Dermatophytes, Hygiene, Lesion, Microbiological, Synergism, Education, *Tinea capitis*

INTRODUCTION
Tinea 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, and the infection accounts for 92.5% of dermatophytosis in children younger than 10 years. The incidence may also vary by sex, depending on the causative fungal organism of which species of *Trichophyton*, *Microsporum* and *Epidermopyton* are the sole aetiological agents (Sarabi, 2008).

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The most prevalent dermatophyte causing *Tinea capitis* is *Microsporum canis* followed by *Trichophyton mentagrophytes*, *Trichophyton violaceum*, *Trichophyton verrucosum* and *Trichophyton rubrum* (Anosike *et al*., 2006).*Microsporum canis* is five times more common in boys than in girls; after puberty however, the reverse is the case, due to hormonal factors (Likit & Demirhindi 2008). Girls and boys are equally affected by *Trichophyton*, the reverse being the case in adults where women are more frequently infected (Ayyildiz *et al*.,2008).

Transmission is enhanced by poor standard of living and hygiene, climatic conditions, and over-crowding through human, animal or soil sources (Oyeka & Okoli 2003). *Tinea capitis* is associated with clinical features and symptoms like 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 (Ngwogu & Otokunefor, 2008).

The ability of dermatophytes to invade non-living cutaneous layer and its appendages such as hair and nails, and digest the proteinaceous substrate including keratin, for its growth and multiplication, contributes to its pathogenesis (Mendez-Tovar, 2010). They also secrete a variety of extracellular proteinases which help them to invade and penetrate host tissues. Treatment regime for *Tinea capitis* involves the use of oral therapy such as Griseofulvin (Fulvicin), Itraconazole (Sporanox), Terbinafine (Lamisil), and Fluconazole, in addition to topical agents such as 2% Ketoconazole. *Tinea capitis* is causing a lot of deaths in other partsf the country. In Nigeria, the extent to which it is a problem has not been well documented (Emele & Oyeka, 2008). *Tinea capitis* is widespread in some urban areas, particularly in children of Afro-Caribbean origin in North America, Germany, Hungary, Central America, and South America (Szepictowski & Baran, 2005). It is common in parts of Africa and India (Avasn *et al*., 2005). In Ethiopia, the incidence of *Tinea capitis* is 8.7% among children aged 4-14 years (Woldeamanuel *et al.,* 2005); while in Southeast Asia, the rate of infection has been reported to have decreased dramatically from 14% (average of male and female children) to 1.2% in the last 50 years because of improved general sanitary conditions and personal hygiene. A lot of work has been done on the prevalence of Tinea capitis, but little work has been done on characterization of diverse dermatophytes associated with *Tinea* in Nigeria; consequently; as a result of that, there is paucity of data on their level of pathogenecity .

Therefore, there is need for extensive work that will be used to determine the variations of these pathogens, and the data obtained used in health policy formulation. This work is therefore aimed at detecting dermatophytes from school children in order to assess the various dermatophytes; this will strategically and ultimately mitigate the menace of these organisms.

MATERIALS AND METHODS

Study Areas: The study was conducted among primary school children in Somolu Local government area (Latitude: 6° 31' 58.6344" N; Longitude: 3° 22' 2.604" E) of Lagos state, Oye-Ekiti local government area (latitude: 7^{o} 47′59′′ N; longitude 5^{o} 19′ 57′′ E) of Ekiti state, and Ado-odo Ota local government area (latitude: 6^{o} 40′59.99″ N; longitude: 3^{o} 40′59.99″ E) of Ogun state Nigeria, within a period of six months (March to August, 2017). The sanitary infrastructure and the environmental condition in the primary schools and the communities as a whole is poor; being characterized by overcrowded class rooms, poor ventilation and sewage disposal, water contamination due to vandalized pipes, and majority of residents lacking access to in-home piped water.

Ethical Clearance

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.

Study Design

This was a non-probability study; the study subjects conveniently selected from the three primary schools in Somolu, Oye, and Ado-odo/Ota local government area using convenience sampling method (Etikan, 2016). The desired sample size (N) was 102 while the study population (pi) was 667.

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. This included the children with severe clinical symptoms (symptomatic), and the ones with mild symptoms (asymptomatic) of *Tinea capitis*.

Exclusion Criteria

Pupils who were suspected not to be infected with *Tinea capitis*, and those whose parents did not give consent to their children's participation in the study were excluded from the study.

Sample Size Determination

One hundred and two samples were collected from clinically diagnosed children, based on a non-probability sampling method known as convenience sampling method(Kirby *et al*,, 2002), which is represented by the formula;

$$N = Z^2 \frac{p(1-p)}{D^2}$$

Where N=required sample size;

P=Expected prevalence of the infection which is 7.4% in this study;

Z=Normal standard deviation for required confidence level of 95% =1.96; D=Tolerance sampling error=5% (0.05).

N=1.96X1.96X0.05 (1-0.05) /0.05X0.05=102

Sample and Data collection

Scrapings of scalp lesions were collected aseptically on a sterile brown paper using a sterile tooth brush, after sterilization of scalp with 70% alcohol, after which structured questionnaire were used to collect socio/bio demographic data of the children (Menan*et al*., 2002). The collected samples were transported to the laboratory for microbiological analysis.

Sample Processing

Direct Microscopic Examination

Samples were placed and examined on a slide containing 20% potassium hydroxide(Hardy diagnostic, USA) without vigorous squashing of the specimen on the slide , and covered with a slip (Cobo *et al*., 2010). Scalp lesions and broken-off hair-stub were placed on slide with one or two drops of 20% potassium hydroxide (KOH) without vigorous squashing of the specimen on the slide, and the slide covered with a slip. The samples were warmed intermittently for 5 minutes over a flame and examined within 30 minutes to allow softening and digestion of keratin, and the slides were later evaluated for the presence of fungal hyphae and/or spores

under a light microscope (x10, x20 and x40) magnification. The presence of fungal hyphae and/or spores within (endothrix) and/or around (ectothrix) hair shafts was considered to be a positive test.

Dermatophyte Isolation

The samples were processed using standard microbiological procedures. Samples were inoculated in prepared Sabouraud dextrose agar- 32.5gm/500ml, (containing Cyclohexemide-2.5gm/500ml and Chloramphenicol-3.025gm), incubated at 28^0 C for 14 days. Dermatophyte culture were sub-cultured to obtain pure culture, which were then observed for morphological properties such as colony pigmentation, texture and color Macroscopic examination of dermatophytes was characterized by duration of growth, surface morphology and change in colony pigmentation (Mendez-Tovar, 2010).

Microcopy of Isolates

The isolates were examined microscopically for morphological features (formation of macro conidia, microconidia, and hyphae) of fungi using lactophenol cotton blue mounts of the pure culture (Bose *et al*., 2011).

Biochemical characterization

Isolates were futher identified using urease test by evaluating the utilization of urea media by dermatophytes at 25°C for a maximum of 5 days accompanied by a corresponding change of the media to pink in a positive test (Kanbe, 2008). Sodium chloride tolerance analysis and stimulation of macroconidia formation was tested by assessing the growth of dermatophytes on SDA containing 3% Nacl at 25°C for a maximum of 5days. Rice grain utilization assay was performed by culturing the isolates in cooked rice grain in vial at 25°C for a maximum of 5days (Ayanbimpe *et al*., 2003). Casein hydrolysis assay was performed by incubation of isolates in casein agar at 25°C for a maximum of 5days, after which it is assayed for breakdown of casein by caseinase enzyme of dermatophytes. Invitro hair perforation evaluation was performed by depositing autoclaved short hair strands in Petri dishes containing 25 ml of sterile distilled water and three drops of 10% dermatophyte extract and incubating for) one week at 25°C (Enany *et al*., 2013). The hair strands covered by mycelium, were examined under microscope by mounting in lactophenol; the perpendicular to the long axis of the hair was a special character for hair perforation test.

RESULTS

A total of 102 samples collected from pupils aged 1-14 years were analyzed for the presence of *Tinea capitis*; Ninety five samples were potassium hydroxide positive represented by different types of hyphae (Figure 1a), while lactophenol staining of dermatophytes revealed septate hyphae and spores (Figure 1b). Seventy (75) isolates of dermatophytes were recovered in the following decreasing order of size - *Microsporum canis* (21; 28%), *Microsporum audounii* (18; 24.0%), *Trichophyton tonsurans* (10; 13.3%), *Microsporum gypsem* (10;13.3%)), *Trichophyton mentagrophyte* (8; 10.7%), *Epidermophyton flocossum* (4; 5.3%) (Table 2), *Trichophyton rubrum* (2; 9.1%) (Table2). *Trichophyton tonsurans* produced a whitish cottony colony at the front, and pitch coloured colony at the reverse side of the plate after 4 days. Microscopy of the culture revealed numerous microconidia of varying sizes and shapes formed at right angle to the hyphae and the sporangium. *Trichophyton rubrum* produced yellow-brown colony at the front, and dark yellow-brown colony at the reverse side of the plate, after five days. Microscopy revealed numerous small micro and macroconidia of various shapes and sizes.

Trichophyton mentagrophytes grew to produce flat, yellow to orange pigmented colony at the front, and a light yellow colony at the reverse side. Microscopy of the culture revealed numerous clustered grape like, circular microconidia, which are arranged in single, double and in clusters.

Trichophyton verrucosum was identified by its slow growth and appearance as a white- blue, small, button or disc-shaped, cerebriform, having a suede-like surface, a raised centre, and a flat periphery. The reverse side of the media turned mikish yellow after 7 days of growth. Microscopy of the culture revealed several branches of hyphae with few microconidia.
Microsporum gypseum was identified by production of a creamish colony at the front of the plate, and red-yellow colony at the reverse side of the plate.

Microsporum canis produced golden yellow- cream pigmented colony at the front of the plate, and a sphaerical creamish yellow surface at the reverse side of the plate. Microscopic appearance revealed short numerous non-septate and septate hyphae surrounded by numerous microconidia.

Epidermophyton floccosum grew to produce a white-pink colony at the front of the plate, and white brown colony at the reverse side of the plate. Microscopy of the culture revealed, large thick-walled, multicellular, club-shaped macroconidia.
Microsporum audounii produced white grey cottony colony characterized by a mouse-fur texture at the front of the media in the plate, with milkish pigmentation at the reverse side of the media in the plate. Microcopy revealed a long septate hyphae, with few microconidia around the hyphae.

Zoophilic and geophilic dermatophytes (*M. canis, T.mentagrophyte* and *Epidermophyton flocossum*)*and geophilic* ones (*M.gypseum*) destroyed or perforated hair strands as a result of deposition of spores inside and outside the hair strands, while anthrophilic dermatophytes (*T.rubrum, T.tonsurans, M.audounii*) did not destroy or perforate the hair strands. The biochemical characteristics of the isolates is summarized in Table 3. The prevalence of *Tinea capitis* is highest among age group 5-10, seconded by 11-14 and then 1-4 (Table 1). Family size of greater than 3 is more at a risk of contracting dermatophytes; likewise male gender (Table 1). School 'B' has the highest infection rate, followed by school 'A' and then school 'C' (Table 1).

Table 1: Prevalence of *Tinea capitis* in the study centres

Characteristics	Category	N	Frequency with *Tinea* infection (n)		% infected (C)	(X^2) Chi square value	p-value	Tri	Mic	Epi
			Infected (A)	Not infected (B)						
Age group (years)	1 – 4	12	9	3	75	13.0040	75	9	15	1
	5 – 10	54	47	7	87		87	8	23	2
	11 – 14	36	19	17	52.8		52.8	5	11	1
No of children in a family	≤ 3	34	20	14	58.8	5.667	58.8	7	12	0
	> 3	68	55	13	80.9		80.9	15	35	4
Gender	Female	29	22	7	75.9	0.113	75.9	10	11	1
	Male	73	53	20	72.6		72.6	12	38	3
School	A	24	17	7	70.8	0.572	70.8	5	11	1
	B	59	45	14	76.3		76.3	13	29	3
	C	19	13	6	68.4		68.4	4	9	0

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

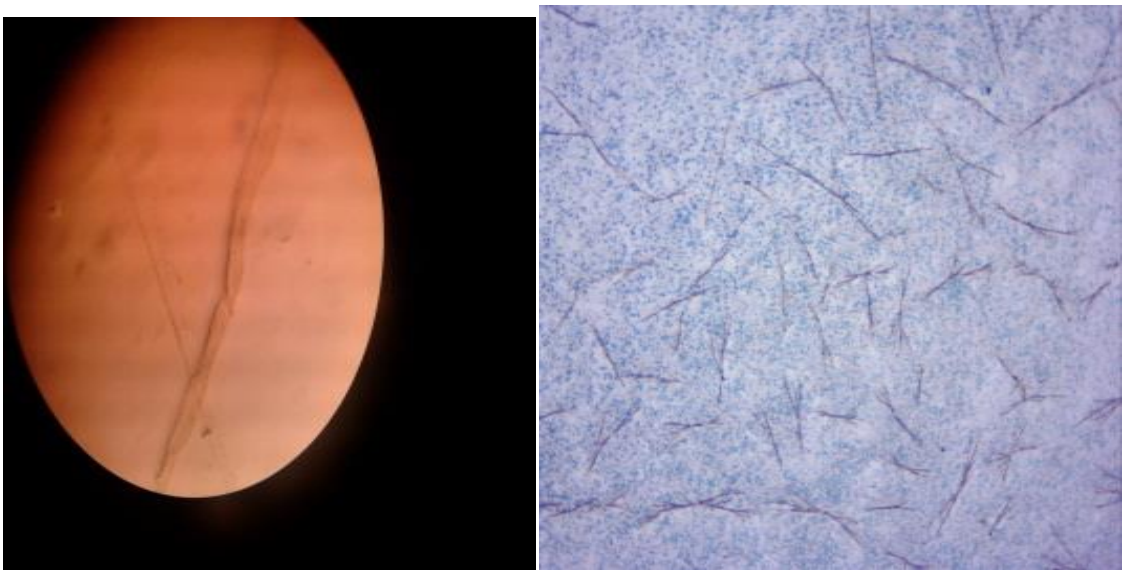
Table 2: Prevalence of the different species of dermatophytes isolated

Species	No. of isolates (n)	Percentage (n/N*100) %
Microsporum canis	21	28
Microsporum audounii	18	24
Trichophyton tonsurans	10	13.3
Microsporum gypseum	10	13.3
Trichophyton mentagrophtye	8	10.7
Epidermophyton flocossum	4	5.3
Trichophyton rubrum	2	2.7
Trichophyton verrucossum	2	2.7
Total	**75**	**100**

Table 3: Biochemical characteristics of isolates

Isolate	CHT	3% NACL TT	UUT	IVHPT	GROWTH AT 37^0C	RGT
M. gypseum	Partial hydrolysis	Negative	Positive	Positive	Positive at day 5	Positive
M.audounii	Partial hydrolysis	Positive	Negative	Negative	Positive at day 4	Positive
M. canis	Partial hydrolysis	Positive	Positive	Positive	Positive at day 4	Positive
T. mentagrophyte	Partial hydrolysis	Positive	Positive	Positive	Positive at day 3	Positive
T. tonsurans	Slightly partial hydrolysis	Positive	Positive	Negative	Positive at day 6	Positive
T. verrucossum	Complete hydrolysis	Positive	Positive	Positive	Positive at day 7	Positive
T. rubrum	Partial hydrolysis	Positive	Negative	Negative	Positive at day 6	Positive
E. flocossum	Partial hydrolysis	Positive	Positive	Positive	Positive at day 3	Positive

Key: CHT = casein hydrolysis test, NACLTT= sodium chloride tolerance test, UUT =urea utilization test. IVHPT = in-vitro hair perforation test, RGT represents rice grain test



(a) (b)

Figure 1a: potassium hydroxide stain of sample reveals Non septate fungal hyphae without cross walls
Figure 1b: lactophenol stain of isolate reveals spores and septate fungal hyphae with cross walls

DISCUSSION

This study which revealed that most of the infections were due to *M. canis*, seconded by *M. audounii*, showed that contact with infected domestic animals and man were probably the most important host that transmitted these agents (http://blast.ncbi.nlm.nih.gov/Blast, 2017). The increased incidence of these organisms as the cause of *Tinea capitis* in Nigeria had also been reported in Western Kenya, Libya and Nigeria (Enemour & Emendu, 2009).

. However, in another study in North-east India, *T. tonsurans* was isolated as the most common dermatophyte. *Tinea capitis* caused by *T. rubrum* is a rare event worldwide (Betatancourt, 2009). The low incidence of *Trichophyton verrucosum* among the children could be associated with low rates of interaction with cattle which served as reservoir for the dermatophytes. Although in a particular study, *T. verrucossum* recorded the highest number of dermatophytes isolated, suggesting that prevailing pathogens vary among different regions and change with time in accordance with the existing living and hygiene conditions. *Epidermophyton flocossum* was the only geophilic specie and the second least number of dermatophyte isolated, as a result of sporadic nature of the disease caused by this specie (Ziemerand Kohl, 2005). However, this was different from a study where *E. flocossum* was the most common dermatophyte isolated. Hair perforation was a result of deposition of spores inside and outside the hair strands. Growth of *T. rubrum* on 3% sodium chloride was as result of the tolerance of that percentage (3%) of sodium and chlorine ion by the isolated dermatophytes. Isolated dermatophytes grew at 37^{o}C as a result of possession of enzyme (Keratinase and Caseinase) that metabolized the dermatophytes' substrate at that particular temperature. Isolates of *T. rubrum* and *M. audounii* were urease negative while the other isolates were positive as a result of breakdown of urea to ammonia resulting to a P_h change of the medium to alkaline, with a corresponding color change of phenol red from straw yellow to pink (Panasiti *et al.,* 2006). Hydrolysis of casein was as a result of enzymatic breakdown of casein agar by caseinase which resulted to zones of inhibition. Invitro hair perforation test confirmed that isolates of zoophilic (*M. canis, T. mentagrophyte, T. rubrum*and*T. verrucosum*) and geophilic dermatophytes (*E. floccosum*) destroyed or perforated hair strands resulting from deposition

of spores inside and outside the hair strands, while anthrophilic dermatophytes (*T.tonsurans* and *M. audounii*) did not destroy or perforate hair strands (Davide *et al.,* 2011).

The highest incidence of infection occurred in younger children aged 5-9 years, (probably because of their immature immunity, poor knowledge and less concern for hygiene), unlike those greater than 10 years (Faithi & Al Samarai, 2000). This finding is also in co-relation 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, prefer to plait their hair rather than barbing (David *et al.,* 2010).

Additionally, other factors that contributed to the transmission of this infection included overcrowding, poor personal and environmental hygiene, which are common practices in most primary schools in Nigeria (Al samarai, 2007).

Microscopy (use of potassium hydroxide) was the cheapest and fastest method of diagnosis, considering the fact that analyses with the use of KOH was performed within two days and costed not more than #3000. However, analyses with KOH is insensitive and unspecific since it can only identify the organism to the kingdom (Fungi), but not to the genus or species level. Cultural and lactophenol staining of the isolates and biochemical characterisation was confirmatory of the different species of dermatophytes isolated (Uchida, 2009). Isolates of different species present different morphological characteristics like pigmentation, colour, texture and elevation at the front and reverse side of the plate. Microscopy of the culture revealed numerous microconidia and macroconidia of varying sizes and shapes formed at right angle to the hyphae (Fig 1b).

CONCLUSION

There was a high prevalence (73.5%) of *Tinea capitis* in the study area; *M. canis* and *M. audounii* being the main causative agents. The present study also revealed that dermatophytes are heterogeneous, and therefore new genomic regions need to be analyzed to elucidate the taxonomic relationship of a large group that show differences in phenotype but are similar in ecology.

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CONFLICTS OF INTEREST

There are no conflicts of interest noted. No financial support.

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