

Isolation of Dermatophytes among Primary School Pupils in Akungba Akoko, Ondo State

Dr. (Mrs) Folorunso V. T

Department of Microbiology, Adekunle Ajasin University,
Akungba Akoko, Ondo state, Nigeria.

ABSTRACT

Dermatophytic infection remain a public health problem among children globally and particularly in Nigeria. Hence, A cross sectional study was conducted to investigate the prevalence of dermatophytic infection among primary school children in Akungba Akoko, a part of Ondo State, Nigeria. A total of one hundred and forty-five (145) children were included in the study. Sixty-five (65) of the study population (145 pupils) was infected with dermatophytosis. The nails, skin scrappings and hair samples of participant were collected aseptically randomly. The samples were appropriately cultured, examined microscopically, and characterized using molecular methods. The study revealed that the prevalence of dermatophytic infection among school children was 44.82%. Furthermore, the study showed higher prevalence and distribution of dermatophytosis in boys (69.23%) than in girls (30.77%). Among the isolated dermatophytes, *M. audouinii* had the highest frequency of 32.20% while *M. gypseum* had the least frequency of 4.61%. The study also revealed that school children of age group 1-5 and 6-10 years appeared more susceptible to dermatophytic infection. In respect to infection site, majority of the identified dermatophytes were isolated from the scalp (93.85%) compared with those that occurred as skin lesions (6.15%). From these findings, the prevalence of dermatophytes among school children in Akungba akoko area of Ondo state demands an urgent public health measure from concerned personnels. Meanwhile, a survey of larger population size is recommended to validate the findings from this study.

Keywords: Dermatophytes, Prevalence, Akungba, School children

INTRODUCTION

Dermatophytes are special group of fungi that infect the stratum corneum of the epidermis and keratinized tissues such as skin, hair and nails of humans and animals (23, 42). Medically, dermatophytes are causative agent of dermatophytosis, a term used for fungal infection of the superficial layer of the skin, nail, hair and scalp. The infections are caused by 40 identified specific fungal species of *Trichophyton*, *Microsporum* and *Epidermophyton* genera (10). Dermatophytosis has diversity in cutaneous manifestations and the severity of the disease depends on various factors such as strain or species of infecting dermatophyte, the sensitivity of the host and the site of infection (45).

Dermatophytoses are named after the body site involved; these include *Tinea pedis* (athlete's foot), *Tinea corporis* (ringworm of the body), *Tinea capitis* (ringworm of the scalp), *Tinea cruris* (ringworm of the groin) and *Tinea unguium* (ringworm of the nails) (48). Furthermore,

dermatophytes are categorized as geophilic, zoophilic and anthropophilic depending on the habitat they are most likely to be found in (46). Globally, dermatophytes have been reported around the world though with variation in distribution, incidence, epidemiology, etiology and target hosts from one location to another over the years. Studies reported the prevalence of dermatophytosis varies between 13% to 49% depending on the geographical distribution of the countries (50). For instance, more than 11.8% of the Nigerian population reportedly suffers from a serious fungal infection and approximately 960,000 of those who are affected suffer considerable mortality (27). Several factors have been highlighted for the aforementioned variation; these include geographic location, prevailing climate (temperature, humidity, wind), overcrowding, health care, immigration, environmental hygiene culture and socioeconomic dispositions have great implication for the proliferation of dermatophytosis (29,30).

Dermatophytes are usually found in tropical and subtropical regions which is the climate in most regions of the continent of Africa (34). The tropical location of a developing country like Nigeria and the favourable climatic conditions among other factors have sponsored high prevalence of dermatophytosis, especially in school children of rural, suburban and urban extract (Gugnani and Njoku-Obi, 1995). (37) opined that children are particularly susceptible to dermatophytic infections because of their poor personal hygiene habits and poor environment sanitation. Similarly, children who possess pets, wet skin conditions, skin injuries and those who use public showers, walk barefoot, and share hair brushes or unwashed clothing with other people are highly susceptible (32). Studies have also reported that the infection is more frequently seen in primary school children between 5 and 10 years of age, as well as poor families who are an average of five or more (29 - 30). (3) reported that the prevalence of superficial fungal infection (SFI) in Nigeria as reported ranges from 3.4% to 55%. In developing countries including Africa, *Microsporum audouinii* and *Trichophyton soudanense* are most frequently isolated aetiological agents. The most common and most widely distributed aetiological agent is *Trichophyton rubrum*, which causes different types of infection in different parts of the world (27). Many studies have been done on the isolation of dermatophytes among school pupils in Nigeria; however, most of them are limited to certain geographical area. Therefore, this study was conducted with the aim to isolate dermatophytes among school children in Akungba Akoko, Ondo State in order to provide information for public health measures.

MATERIALS AND METHODS

Collection of Clinical Samples

The samples were collected in the morning between 7am and 11am on each day of collection across seasons in both dry and raining seasons of the year. The nails, skin scrapings and hair samples were collected aseptically randomly from 145 school children suspected to have the symptoms of dermatophytosis of skin, nails and hair.

Preparation of Culture Media

The media used (Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose broth (SDB), Dermasel Agar, Christensen's Urea Agar) Oxoid and Merck products were all prepared according to the manufacturer's specification. Thereafter, each agar was poured under aseptic condition into sterile Petri dishes and allowed to solidify.

Direct Microscopic Examination

The skin scrappings, hair and nail samples were placed in two drops of 20% potassium hydroxide (KOH) on a clean glass slide and a cover slip was applied. The slide was warmed gently over the flame of a bunsen burner in order to soften and clear the epithelium. Each treated slide was examined microscopically under low (x10) and high (x40) objective for the presence of hyphae and chains of arthrospores.

Isolation of Dermatophytes from the Samples

The samples were inoculated on Sabouraud Dextrose Agar (SDA) containing antibiotics. Each sample was inoculated and incubated at 28°C for four weeks; this was examined daily for fungal growth at 2-3- day intervals. Identification was done based on the growth characteristics and morphology of the fungal isolates. Microconidia, macroconidia, chlamydospores and hyphae appearing as spiral pectinate or reflexive were sought for and features seen were compared with atlas (14). Then the pure isolates were further maintained in agar slants according to (28).

Characterization of Fungal Isolates Using Molecular Method

Fungi DNA Extraction Protocol:

Fungal mycelia (100mg) were weighed into sterile mortar and 1ml of DNA Extraction Buffer (DEB) containing proteinase K (0.05 mg/ml) was added and macerated with sterile pestle. The macerated mycelia were transferred aseptically into 1.5ml eppendorf tube. The fungal DNA was extracted according to (31) and stored for further molecular analysis.

Polymerase Chain Reaction (PCR) Analysis:

The ITS gene for characterization of fungi, ITS universal primer set which flank the ITS1 (5.8S) and ITS2 region was used; ITS 1: 5' TCC GTA GGT GAA CCT GCG G 3' and ITS 4: 5' TCC TCC GCT TAT TGA TAT GC 3'. PCR sequencing preparation cocktail was prepared and amplified according to (31). The integrity of the amplified 1.5Mb gene fragment was checked on a 1% Agarose gel run to confirm amplification.

Purification of Amplified Product and Sequencing:

The amplified fragments were ethanol purified in order to remove the PCR reagents after gel integrity. The purified fragment was checked on a 1.5% Agarose gel run on a voltage of 110 volts for about 1 hr, to confirm the presence of the purified product and quantified using a nanodrop of model 2000 (Thermoscientific) (31). The pellets were re-suspended in 20 µl of sterile distilled water and kept at -20°C prior to sequencing. The amplified purified fragments were then sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystem using manufacturers' manual while the sequencing kit used was from Big Dye terminator v3.1 cycle sequencing kit.

RESULTS

Sixty-five (65) of the study population (145 pupils) was infected with dermatophytosis. Eight fungi species belonging to *Microsporum* and *Trichophyton* were identified which include *M. audouinii*, *T. tonsurans*, *M. gypseum*, *T. soudanense*, *T. mentagrophytes*, *M. canis*, *T. verrucosum* and *T. schoeleni* (Table 1). The prevalence and distribution of dermatophytosis recorded varied among studied school as shown in (Table 2). The percentage distribution of dermatophytosis in relation to sex shows that infection was higher in males than females. School-one had the highest number of infected pupils (Table 2). *M. gypseum* and *M. schoeleni* was not isolated from

age group 11-15 years, but appeared mainly in the 6-10 years group. *M. gypseum* was the least isolated species.

Table 1. Morphology characteristic of the isolates

Isolates	Shape	Appearance	Elevation	Surface	Hyphae	Septa	Spore	Spread features	Probable organism
1	Spindle-shaped	Pale buff	Flat	Rough	Branched	Septate	Large conidia	Hyphal growth, pectinate hyphae sometimes present	<i>M. audouinii</i>
2	Club-shaped	Buff to cinnamon	Flat	Rough	Branched	Septate	No spores	Numerous macroconidia, sparse microconidia	<i>M. gypseum</i>
3	Spindle-shaped	Pale buff to white	Flat	Rough	Branched	Septate	No spores	Large macroconidia, most common in the centre of the colonies	<i>M. canis</i>
4	Cylindrical	White to cream	Flat	Smooth	Branched	Septate	Large conidia	Abundant microconidia, some macroconidia and spiral hyphae	<i>T. mentagrophytes</i>
5	Complex	Deep orange-yellow	Flat	Smooth	Branched	Septate	Short conidia	Few microconidia, reflexive branching at edge of colonies, arthrospores and chlamydospores may present	<i>T. soudanense</i>
6	Cylindrical	Cream-white to pale brown	Flat	Smooth	Branched	Septate	Chlamydospores	Abundant microconidia, chlamydospores	<i>T. tonsurans</i>
7	Complex	White to cream	Flat	Smooth	Branched	Septate	Chlamydospores		<i>T. verrucosum</i>
8	Complex	White to light grey	Heaped	Smooth	Un branched	Aseptate	No spores	Characteristic antler hyphae with swollen tips	<i>T. schoeleinii</i>

Result shows that male pupils had a higher prevalence and distribution of the individual aetiological agents than female pupils. This is with the exception of *M. canis* which appeared more in females (60%) than in males. All the other species of dermatophytes were isolated more in males than females as presented in (Table 3). Result shows that majority of the identified dermatophytes occur on the scalp (93.85%) compared with those that occurred as skin lesions (6.15%) in the same population (Table 4). Generally, *M. audouinii* was most frequently encountered among the isolates followed by *T. tonsurans*. Notably, in respect to the infection site, only *Microsporum gypseum* and *Trichophyton tonsurans* were isolated from the

skin lesions, others species including *Trichophyton tonsurans* were present on the scalp as detailed in (Table 4)

Table 2: Percentage distribution of dermatophytosis among sampled schools in relation to sex.

Aetiological agents	No of isolates	Sex		Age		
		Male	Female	1-5	6-10	11-15
<i>M. audounii</i>	21(32.30)	15	16	7	10	4
<i>T. tonsurans</i>	11(16.92)	9	2	5	4	2
<i>M. gypseum</i>	3(4.61))	2	1	1	2	-
<i>T. soudanense</i>	6(9.23)	5	1	1	4	1
<i>T. mentagrophytes</i>	7(10.76)	5	2	2	3	2
<i>M. canis</i>	5(7.69)	2	3	2	2	1
<i>T. verrucosum</i>	4(6.15)	2	2	1	2	1
<i>T. schoeleinii</i>	8(12.30)	5	3	2	6	-
Total	65(100)	45(69.23)	20(30.77)	21	33	11

Table 3: Prevalence and distribution of aetiological agents based on sex and age

School	No examined	No infected %	Sex	
			Male	Female
School i	78	29(37.17)	17	12
School ii	32	18(56.25)	13	5
School iii	35	18(51.42)	11	7
Total	145	65(44.82)	41	24

Table 4: The occurrence of aetiological agents according to site of infection

Aetiological agents	No of isolates %	Site of lesion		
		Scalp	Skin	Nail
<i>M. audounii</i>	21(32.30)	21	-	-
<i>T. tonsurans</i>	11(16.92)	10	1	-
<i>M. gypseum</i>	3(4.61)	-	3	-
<i>T. soudanense</i>	6(9.23)	6	-	-
<i>T. mentagrophytes</i>	7(10.76)	7	-	-
<i>M. canis</i>	5(7.69)	5	-	-
<i>T. verrucosum</i>	4(6.15)	4	-	-
<i>M. schoeleinii</i>	8(12.30)	8	-	-
Total	65(100)	61(93.84)	4(6.15)	-

DISCUSSION

Dermatophytes remain a public health problem among children globally and particularly in Nigeria (2). This present investigation constitutes the first reported survey of dermatophytes involving school children in Akungba Akoko part of Ondo State of Nigeria. This study revealed that the prevalence of dermatophytes among school children is 44.82%. The prevalence obtained is an indicator that dermatophytes is a public health problem in Akungba Akoko, Ondo State. The prevalence obtained is comparably lower to the 53% and 72.2% reported by (2) in

Delta and (11) from Kebbi state respectively. The prevalence in this study is almost equal to the 40.57% recorded in Awka south, Anambra State (21). Similar studies have reported lower prevalence of 35%, 31.6%, 23.4%, 9.6%, 6.6%, 5.0% respectively (20; 16; 12; 22; 19; 10). The variation in the prevalence of superficial fungal infection in different regions have been linked to the variation in climatic, socioeconomic status, time, lifestyle and environmental conditions of the areas being studied (12; 2). In respect to gender difference, this study revealed a higher prevalence in boys than in girls which was statistically insignificant. This study showed higher prevalence and distribution of dermatophytosis in boys (69.23%) than in girls (30.77%). This study is in consonance with similar studies which showed that prevalence of dermatophytosis was higher in males than in females (20; 16; 22; 23; 15). We opined that the higher prevalence in boys than in girls may be linked to the playing habits of boys compared to girls. The playing habit of boys is a risk factor for dermatophytosis which is not present in girls. (2) submitted that the fact that boys visit barbers often can also contribute to this higher prevalence, since barbers instruments have been noted to play a role in the spread of these infections. (10) also commented that personal hygiene cleanliness can also play a factor in dermatophytic infection, females appear to be neater than males as they pay more attention to their outlook, especially, as they approach teenage age.

Eight fungi species belonging to *Microsporum* and *Trichophyton* were identified which include *M. audounii*, *T. tonsurans*, *M. gypseum*, *T. soudanense*, *T. mentagrophytes*, *M. canis*, *T. verrucosum* and *T. schoeleni*. In a similar study, Anya and Sunday, (2019) isolated similar dermatophytes which belonged to two genera: *Trichophyton* and *Microsporum*. Among the isolated dermatophytes from the present study, *M. audounii* had the highest frequency of 32.20%. while *M. gypseum* had the least frequency of 4.61%. The most prevalent dermatophytes in our study population is absolutely the same with a study conducted in Ile-Ife, Osun state, a neighboring state to the state where our study was carried out, *M. audounii* was reported as the most prevalent organism among the pupils who were studied (10). However, other studies reported slightly different dermatophytes. For instance, (4) and (17) reported *T. rubrum*, as the most isolated organism among children with tinea capitis infection in Osun state and certain parts of Northern Nigeria. Also, contrary to this present report, (2) reported that *T. mentagrophytes* was isolated at the highest frequency of 43.4%. Another notable finding in this study is that *M. gypseum* was isolated from just three of the pupils making it the least dermatophytes from this study. Justifiably, *M. gypseum* has been said to be rarely isolated in Africa (5). Studies generally have showed variation in the species of dermatophytes isolated from a particular geographical region (8)

Dermatophytes affect persons of all age. Although from this study, school children of age group 1 - 5 and 6 - 10 years appeared more susceptible. This was in agreement with the previous reports that fungal infection was usually higher in pupils with age lower than 10 years than in older pupils (21; 9). Similarly, in a study by (4) conducted among primary school children in Osogbo, the authors reported a high prevalence of the infection among children aged 4 - 7 years. Presence of superficial fungal infection in younger age group observed in this study agrees with the proposition that the infection is related to the poor hygiene at the younger age and absence of saturated fatty acids that provide a natural protective mechanism against fungal infections (27).

In conclusion, the prevalence of dermatophytes among school children in Akungba akoko area of Ondo state demands an urgent public health measure to mitigate the consequential effect of the infections in school children. Meanwhile, a survey of larger population size is recommended to validate the findings from other study.

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