Prevalence of Dermatophytoses amongst pupils from schools in some parts of Rivers State, Nigeria.

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Abstract: The prevalence and aetiology of dermatophytic infections amongst pupils in some parts of Rivers State, Nigeria, was monitored. This study was carried out between June 2010 and March 2012. A total of 2,538 pupils from twenty-seven primary schools within the age range of 4 – 16 years were randomly sampled. Clinical samples were aseptically collected and microbiologically analysed using standard methods. The results showed that of the 2,538 pupils, 340(13.4%) pupils had suspected dermatophytic lesions on different parts of their body, 282 (11.1%) were confirmed to be dermatophytic infections and 58 (2.3%) were non-dermatophytic infections. The aetiological agents isolated were - *Trichophyton rubum* [64(22.7%)] was the most prevalent infectious species. This was closely followed by *Trichophyton mentagrophytes* [58(20.6%)], *Microsporum gypseum* [54(19.1%)], *Microsporum ferregineum* [52(18.4%)], *Epidemiophyton floccosum* [24(8.5%)] and *Trichophyton tonsurans* [19(6.7%)] while *Microsporum canis* [11(3.9%)] was the least. The prevalence of the infection amongst the pupils of age range 4-7years was 106 (13.1%) increased to 139(14.2%) for 8-11years, and decreased to -95 (12.6%) for the age range 12-16yrs. The distribution of the infection among female pupils within the age range of 4-16years did not differ significantly but the males in the age range of 8 -11years were significantly ($p<0.05$) more infected than those in the other age ranges. Thus, the study revealed a high prevalence of dermatophytoses (11.1%) among pupils in Port Harcourt municipal and Obio/Akpor (urban area) and Okoma, Okporowo in Ahoada-East (rural area) Local Government areas.
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1. Introduction

Pathogenic fungi cause diseases that are generally called mycoses. Mycoses could be conveniently grouped as superficial, cutaneous, subcutaneous and systemic mycoses according to their location of infestation in the body of their host. (Jawetz *et al*, 1980; Abbey, 1995; Prescott *et al*, 2005). The superficial mycoses usually occur on the nail, skin, horns, hair, feathers and mucous membranes of their host. The fungi responsible for these are called dermartophytes. The pathological lesion is commonly referred to as ringworm as a result of its ring shape. The common belief is that such lesions were caused by worms (Weitzman and Summerbell, 1995). Ringworm infection medically known as dermatophytoses caused by dermartophytes- a highly specialized group of fungi. It is not a reportable disease as its reports are scanty (Egere and Gugnani, 1980) but is a cause for concern because of its contagious nature. Dermatophytes are closely related filamentous group of fungi that affect the superficial keratinized tissues of man and animals (Kern and Blevins, 1997). Dermatophytosis may not be fatal but causes high morbidity and the psychological embarrassment accompanying it could be traumatic among the sufferers (Abbey, 1995). It occurs primarily in prepubatal children over the age of 6 months (Elewski and Hay, 1996). It is highly contagious and represents a significant public health problem especially among primary school children (Fatini and Al-Samarai 2000; Omar, 2000; Higgins *et al*, 2000). The transmission of dermartophytes is generally fostered by poor hygienic condition, overcrowding through body contacts, contaminated hats, comb, hair-brushes or saloon equipments, pillow cases and other inanimate objects (Vidott *et al*, 1982; Abbey, 1995; Weitzman and Summerbell, 1995). Warm humid climate promotes the growth and spread of these infections (Soyinka, 1978).

Thus, this study aimed at determining the prevalence and aetiology of dermatophytic infections amongst pupils in some parts of Rivers State, Nigeria.

2. Materials and Methods

2.1. Study Area and Population

Twenty-seven State primary schools in Port Harcourt municipal (Urban) Okoma and Okporowo (Rural) were randomly selected. Questionnaires were administered to the pupils through their class teachers. A total of two thousand, five hundred and thirty-eight pupils within the age range of 4yrs-16yrs made up of one thousand, three hundred and thirty-six males and one thousand, two hundred and two females; two hundred and twelve females and two hundred and

fifty-two males giving a total of four hundred and sixty-four from the rural area; nine hundred and ninety females and one thousand and eighty-four males giving a total of two thousand and seventy-four from the urban area were examined (Table 1).

Table 1: Socio-demographic characteristics of pupils examined for Dermatophytoses in this Study

Characteristics	No. Tested (%)	No. Males (%)	No. Females (%)	No. Rural (%)	No. Urban (%)
Age (years)					
4-7	807(31.8)	424(52.5)	383(47.5)	167(20.7)	640(79.3)
8-11	979(38.6)	526(53.7)	453(46.3)	154(15.7)	825(84.3)
12-16	752(29.6)	386(51.3)	366(48.7)	143(19.0)	609(81.0)
Sex					
Males	1336(52.6)	1336(100.0)	0 (0.0)	252(18.9)	1084(81.1)
Females	1202(47.4)	0 (0.0)	1202(100.0)	212(17.6)	990(82.4)
Study area					
Rural	464(18.3)	252(54.3)	212(45.7)	464(100.0)	0 (0.0)
Urban	2074(81.7)	1084(52.3)	990(57.7)	0 (0.0)	2074(100.0)
Total	**2538(100.0)**	**1336(52.6)**	**1202(47.4)**	**464 (18.3)**	**2074(81.7)**

2.2. Sample collection

In each suspected case of dermatophytoses on the pupils examined, hairs on the affected parts were trimmed for easy sample collection. Physical examinations were thoroughly done for the evidence of scales, crusting, and follicular inflammations. Samples were collected from the affected areas by scraping of the affected part of the body by using the blunt-side of a sterile surgical blade for each pupil after thorough cleaning with cotton wool soaked in Methylated spirit. The scrapings were collected in a sterile filter paper properly folded and stored in brown envelopes and labeled with individual pupil's identities for proper identification and taken to the laboratory for analyses (Fatini *et al*., 2000).

2.3. Microscopic Examination

Each sample was aseptically collected and examined microscopically by mounting on a clean slide with 20% Potassium hydroxide solution and stained with Lactophenol cotton blue (Fatini and Al-Samaria, 2000; Hainer, 2003) for the presence of fungal elements such as hyphae, arthrospore and/or conidia (Rippon,1988, Mbakwem-Aniebo, 2010).

2.4. Culturing and Identification

Irrespective of the result from the direct microscopic examination, all samples were separately cultured on Potato Dextrose Agar (PDA) containing Cycloheximide and Chloramphenicol. The inoculated culture plates were incubated at room temperature (30□c) for 4 weeks before discarding. The inoculated plates were put in a white transparent silo-phenyl bag to avoid contaminants and dehydration and were physically examined at two- day intervals for evidence of growth.

The colonies were examined macroscopically and microscopically. A portion of each growth sample was aseptically collected and mounted on a clean grease-free glass slide and stained with Lactophenol cotton blue (Fatini and Al-samaria, 2000; Hainier, 2003, Mbakwem-Aniebo, 2010) and viewed under the microscope for fungal arthorspores, hyphae and conidia.

After the microscopic examination of the fungal isolates, Sub-cultures were made from each isolate on freshly prepared media of PDA media supplemented with Cycloheximide and Chloramphenicol and were incubated at room temperature (30□c) for up to 4 weeks, to allow the slow growing dermatophytic fungi to appear visibly and significantly (Abbey, 1995; Omar, 2000; Zuberand Baddam, 2001; Hainer, 2003; Kolhatkar and Ochei, 2008, Mbakwem-Aniebo, 2010). Slide cultures were also made. The molecular characterization and identification of the isolates through DNA extraction electrophorsesed on 1.5% Agarose gel and TBE as the running buffer (Plate1, Table 2). The Molecular characteratization was done on the isolated dermatophytes.

2.5. Data Analysis

The Univariate and Multivariate statistical analysis as provided by the SPSS Version 22.0, MS Excel 2007, ANOVA and graph pad Prism Software version 5.01 at $P< 0.05$ significant value were used to analyse the data.

3. Result

Out of the 2,538 pupils examined (1,336 males and 1,202 females) (Tables 1), 340 (13.4%) presented with suspected superficial dermatophytic lesions according to age, sex and location (Table 2). The study also revealed that the infection was highest in the 8-11yrs group and lowest in the 12-16yrs group (Table 2). The distribution among female pupils within the age range of 4-16yrs did not differ significantly but the males in the age range of 8-11yrs were significantly ($p<0.05$) more infected than those in the other age ranges (Table 2).

Table 2: Prevalence of pupils with suspected superficial lesions

Characteristics	No. Tested (%)	No. with Superficial lesions (%)	No. Males (%)	No. Females (%)	No. Rural (%)	No. Urban (%)
Age (years)						
4-7	807(31.8)	106(13.1)	57(53.8)	51(46.2)	57(53.8)	49(46.2)
8-11	979(38.6)	139(14.2)	81(58.3)	58(41.7)	63(45.3)	76(54.7)
12-16	752(29.6)	95(12.6)	56(58.9)	39(41.1)	57(60.0)	38(40.0)
Sex						
Males	1336(52.6)	194(14.5)	194(100.0)	0 (0.0)	97(50.0)	97(50.0)
Females	1202(47.4)	146(12.1)	0 (0.0)	146(100.0)	80(54.8)	66(45.2)
Study area						
Rural	464(18.3)	177(38.1)	97(54.8)	80(45.2)	177(100.0)	0 (0.0)
Urban	2074(81.7)	163(7.9)	97(59.5)	66(40.5)	0 (0.0)	163(100.0)
Total	**2538(100.0)**	**340(13.4%)**	**194(57.1)**	**146(42.9)**	**177 (52.1)**	**163(47.9)**

Of the 2538 pupils tested, 282 (11.1%) had dermatophytic lesions, 58(2.3%) had non-dermatophytic lesions and 2198 (86.6%) were without any physical infection (Table 3).

Table 3: Prevalence of pupils with suspected superficial lesions

Characteristics	No. Tested (%)	No. with dermatophytic lesions (%)	No. with non-dermatophytic lesions (%)	No. without any physical infections (%)
Age (years)				
4-7	807(31.8)	94(11.6)	12(1.5)	701(86.9)
8-11	979(38.6)	111(11.3)	28(2.9)	668(82.8)
12-16	752(29.6)	77(10.2)	18(2.4)	39(87.4)
Sex				
Males	1336(52.6)	170(12.7)	24(1.8)	1530(85.5)
Females	1202(47.4)	112(9.3)	34 (2.8)	1056(87.9)
Study area				
Rural	464(18.3)	144(31.0)	36(7.8)	284(61.2)
Urban	2074(81.7)	138(6.7)	22(1.2)	1914(92.3)
Total	**2538(100.0)**	**282(11.1%)**	**58(2.3)**	**2198(86.6)**

The study also revealed the prevalence and distribution of the different dermatophytoses amongst the pupils in the study areas with *Tinea capitis* as the most prevalent and much more in the rural area than the urban area (Table 4).

Table 4: Prevalence of dermatophytosis

Clinical Type	No. (%)	No. Males (%)	No. Females (%)	No. Rural (%)	No. Urban (%)
Tinea capitis (ringworm of the scalp	234(83.0)	132(56.4)	102(43.6)	126(53.8)	108(46.2)
Tinea coporis (ringworm of the skin)	17(6.0)	13(76.5)	4(23.5)	10(58.8)	7(41.2)
Tinea mannum (ringworm of one or both hands)	13(4.6)	7(53.8)	6(46.2)	7(53.8)	6(46.2)
Tinea faciei or*Tinea incognito* (ringworm of the face)	18(6.4)	13(72.2)	5(27.8)	8(44.4)	10(55.6)
Total	**282(100.0)**	**163(57.8)**	**119(42.2)**	**151 (53.5)**	**131(46.5)**

Three genera of dermatophytes -*Trichophyton, Microsporum* and *Epidermophyton* were isolated. A total of Seven (7) different dermatophytes were identified and confirmed from the 282 samples confirmed to be superifical dermatophytic lesions. The isolated fungi were identified using microscopic and molecular characterization and identification as- *Trichophyton mentagrophytes*, *Microsporum ferrugineum*, *Microsporum gypseum*, *Trichophyton rubrum, Trichophyton tonsurans*, *Epidermophyton floccosum* and *Microsporum canis* (Plate 1, Table 5).

The frequency of occurrence of fungi isolated are shown in Table 6. *Trichophyton rubum* [64(22.7%)] was the most prevalent infectious species. This was closely followed by *Trichophyton mentagrophytes* [58(20.6%)], *Microsporum gypseum* [54(19.1%)], *Microsporum ferregineum* [52(18.4%)], *Epidemiophyton floccosum* [24(8.5%)] and *Trichophyton tonsurans* [19(6.7%)] while *Microsporum canis* [11(3.9%)] was the least (Table 6).

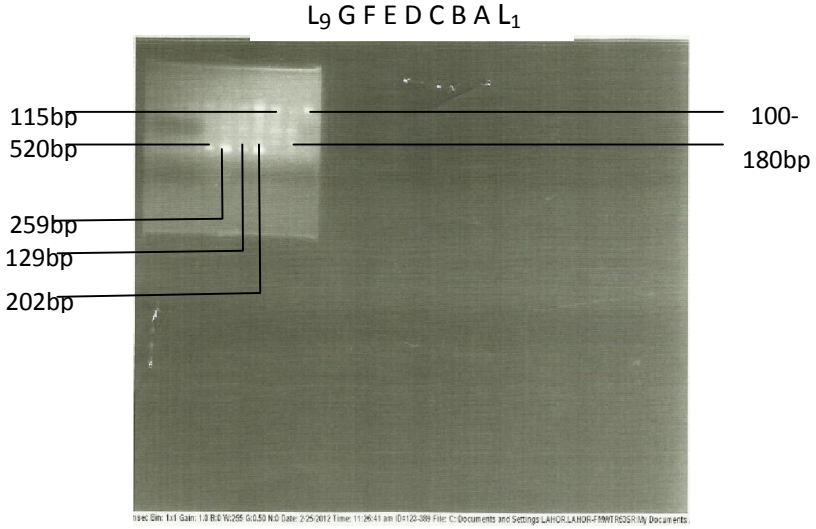

Plate 1: DNA Ladder size were 129,180,520,202, 129, 259 and 115 base pairs forTrichophyton mentagarophytes(D), Microsporum ferregineum(A), Microsporum gypseum(B), Trichrophyton rubrum(C), Trichophyton tonsurans(D), Epidermophyton flocossum(E) and Microsporum canis(F) respectively.

Table 5: Result of the Molecular Characterization and Identification of the Isolates obtained

S/NO	Dermatophytes	Primer	Primer Sequence	Band Size	References
1	*Trichophyton mentagrophytes*	*Chitin syntase genefragment*	Uni-5'-GCAAGACATGGGGTAAAGAAGCC-3' Rev- 5'GCCTATCTGGGTGGTATATTCGTG-3'	129 base pair	Baeza *et al*., 2004; Dobrowolska *et al*.,2008; Cafarchia *et al*., 2009; Malinovschi *et al*;2009
2	*Microsporum ferrugineum*	*Actin gene fragment*	Uni-5'-CCAGGGAGGTTGGAAACGACCG-3' Rev-5'-GCCATTAAAGGCTGAAGCCA-3'	180 base pair	Baeza *et al*., 2004; Dobrowolska *et al*.,2008; Cafarchia *et al*., 2009; Malinovschi *et al*;2009
3	*Microsporum gypseum*	*Actin gene fragment*	Uni-5'-GGCTCCTGGGCGAATGGGACA-3' Rev-5'-TTCAGCGGGTATCCCTACCTGATCCG-3'	115 base pair	Baeza *et al*., 2004; Dobrowolska *et al*.,2008; Cafarchia *et al*., 2009; Malinovschi *et al*;2009
4	*Trichophyton rubrum*	*ITS-2 gene fragment*	Uni-5'-TCTTTGAACGCATTGCGCC-3' Rev- 5'- CGGTCCTGAGGGCGCTGAA-3'	202 base pair	Baeza *et al*., 2004; Dobrowolska *et al*.,2008; Cafarchia *et al*., 2009; Malinovschi *et al*;2009
5	*Trichophyton tonsuans*	*Chitin syntase gene fragment*	Uni-5'-GCAAGACATGGGGTAAAGAAGCC -3' Rev-5'- GCCTATCTGGGTGGTATATTCGTG-3'	129 base pair	Baeza *et al*., 2004; Dobrowolska *et al*.,2008; Cafarchia *et al*., 2009; Malinovschi *et al*;2009
6	*Epidermophyton floccosum*	*ITS-1 gene fragment*	Uni-5'-TCTTTGAACGCATTGCGCC -3' Rev-5'-CCGACGGAAACTAGGGCCAGAG-3'	259 base pair	Baeza *et al*., 2004; Dobrowolska *et al*.,2008; Cafarchia *et al*., 2009; Malinovschi *et al*;2009
7	*Microsporum canis*	*Actin gene fragment*	Uni-5'-ACGTCTCCATCCAGGCTGTGCTCTC -3' Rev-5'-GCGAGGTGTTAGAAGGAAAAACGGTCC-3'	520 base pair	Baeza *et al*., 2004; Dobrowolska *et al*.,2008; Cafarchia *et al*., 2009; Malinovschi *et al*; 2009

Table 6: Frequency of occurrence of isolated fungi

Fungi Isolates	No. (%)
Trichophyton mentagrophytes	58(20.6)
Microsporum ferrugineum	52(18.4)
Microsporum gypseum	54(19.1)
Trichophyton rubrum	64(22.7)
Trichophyton tonsurans	19(6.7)
Epidermophyton floccosum	24(8.5)
Microsporum canis	11(3.9)
Total	**282(100.0)**

4. Discussion

Ringworm is a common dermatophytic infection that forms an important public health problem among humans especially children worldwide, including Nigeria (Ive, 1966; Egere and Gugnani, 1980; Ajao and Akintude 1985; Ogbonna *et al*., 1985; Mbata *et al*, 2007). The disease remains endemic in Nigeria, largely because of the absence of control and effective preventive measures. The present study revealed that out of the 2,538 pupils examined, 340 (13.4%) presented with suspected superficial dermatophytoses; 282(11.1%) were confirmed to be dermatophytic infections while 58(2.3%) were non-dermatophytic infections, 2198 (86.6%) pupils were without any superficial infection. This result obtained revealed a prevalence of 11.1% dermatophytic infections from the pupils within the study areas affected by different species of dermatophytes. This could be as a result of the numerous challenges like unemployment facing the majority of the population here by resulting in hardship, poverty, living in uncomfortable and unhygienic environment, overcrowded houses with increased risk of infections and diseases. Although dermatophytes have a widespread distribution some

are limited geographically and their prevalence reflects the living conditions and habits of the population (Abbey, 1995).

Majority of the populace go about their usual business harboring these infections and disease unknown to them as asymptomatic carriers. Some, even when they are aware are not too worried since it is not a life-threatening illness. This attitude contributed to the spread of infections and diseases in as there is no geographical location that is spared of this infection and disease (Abbey, 1995; Congly, 1999). It is generally believed that people in the urban areas have higher levels of personal and environmental hygiene which makes them to be less infected than the people in the rural areas. This has also been confirmed in this study (Jacky *et al.,* 1982; Abbey, 1995; Brooks *et al.,* 2004). This finding agrees with other public reports on this disease in Nigeria, though are scanty (Egere and Gugnani, 198; Enendu and Ibe 2005; Murkthar, 2005).

The observation in the growth of the dermatophytes within the days of inoculation did not differ significantly, in that there was homogeneity in their growth. There was no competition with other organisms because the presence of antibiotics in the media prevented the organisms that could have competed with the dermatophytes (Anosike *et al*., 2005; Omar, 2000). A total of seven (7) pathogenic species of dermatophytes were identified. Of all with, *Trichophyton rubrum* as the predominant causative agent of dermatophytoses with a prevalence of 64 (22.7%) followed by *Microsporum canis* 11(3.9%).

One of the greatest problems hindering the prevention and eradication of dermatophytic infections is the presence of healthy asymptomatic carriers. Majority of the pupils examined (2,538) showed no physical symptoms of infection, yet samples collected from some parts of the body of these asymptomatic pupils yielded significant growth of dermatophytes. This observation is in line with the reports of Ive (1966) who found that asymptomatic carriers of dermatophyte may be equal to symptomatic sufferers (Schmeller, 1998; Hainer, 2003). This should alert parents, teachers, government and the public to make adequate control and preventive measures to reduce the rate of spread in the schools. The prevalence (11.1%) and distribution of dermatophytic infections observed from this study agrees with the reports of Ajao *et al.,* (1985) amongst school children in lle-ife, Nigeria (14.02%) and those of Omar (2000) in Alexandria (7.4%). The differences may be due to variation in environmental and climatic conditions of the areas studied as well as the standard living conditions of the people in these environments (Jacyk *et al*., 1982; Ajao and Akintunde, 1985; Abbey, 1995; Elewski, 2000).

Gender and area related studies on the prevalence of dermatophytic infection in Nigeria had been broken down into different parts (Ogbonna *et al.,* 1985). A Pair-wise comparison in the distribution of dermatophytosis among pupils by gender and area revealed that the distribution of dermatophytosis differ significantly. The sex distribution of the pupils with dermatophytoses in this study, revealed that it was higher in the male pupils than in the females. This agrees with some reports on this disease which stated that the males were more infected than the females (Congly, 1999; Faitni and Al-Samaria, 2000; Nurimar *et al*., 2001; Obire *et al.,* 2010). This could be because of the constant physical body contacts with infected persons, soils and animals during regular play of the boys thereby increasing their risk of the infection. Also their regular visits to the barbing salon and exposure to unsterilized barbing equipments which facilitates the transmission of the spores of dermatophytes, and their short hair exposes their scalps to the spores, thus giving them more access to infections (Egere and Gugnani 1982; Proenca and Assumpcao, 1989; Omar, 2000; Obire *et al.,* 2010).

The females in the rural areas were more infected than those in the urban areas. This could be attributed to lack of adequate personal hygiene of the females in rural areas, their regular visits to the farms; not showing adequate concern to their personal hygiene thereby increasing the risk of the infection. Most female pupils in the urban areas on the other go to salons where they are opportune to use some chemicals (Hair Relaxer, shampoos and Hair cream) on their scalp that may prevent or reduce the risk of these infections directly and indirectly (Anosike *et al.*, 2005). This finding confirms that infection is related to personal hygiene and its prevalence can be reduced by adequate health education and good personal. Higher prevalence of this infection was found amongst pupils within the age range of 8-11yrs than the younger and older ones (Table 2 and 3) as dermatophytic infection is mainly a pre-pubertal disease (Congly, 1999; Nurimar *et al*., 2001). This fact can be explained by poor hygiene as well as the absence of saturated fatty acids that could have provided a natural protective mechanism at this age (Wagner and Sohnie, 1995, Fisher and Cook 1998).

Poor infrastructures and lack of good social amenities are contributing factors to the high prevalence of dermatophytosis amongst the pupils in this study. The schools sampled, lacked good accommodation for study irrespective of locations. The pupils sat on the floor. Children can also contact the infection from the soil (Ogbonna *et al.,* 1985; Abbey, 1995). The play habits of these children, their habit of accompanying their parents to the farm bring these children in close contact with the soil. Most of

the pupils rarely had regular baths and the fungal spores once deposited on their skins from the soil or animals have enough chance of germinating and colonizing their skin. Their constant play with pet animals such as cow, goat, sheep, cats and local dogs which are known sources of infection also predispose them to infection (Ogbonna *et al*., 1985; Fatini *et al*., 2000).

Three genera of dermatophytes -*Trichophyton, Microsporum* and *Epidermophyton* were isolated in this study. *Trichophyton rubum* was the most prevalent infectious species, followed by *Trichophyton mentagrophytes*, *Microsporum gypseum*, *Microsporum ferregineum*, *Epidemiophyton floccosum* and *Trichophyton tonsurans* while *Microsporum canis* was the least. The study revealed a high prevalence of dermatophytoses (11.1%) among pupils in Port Harcourt municipal and Obio/Akpor (urban area) and Okoma, Okporowo in Ahoada-East (rural area) Local Government areas.

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