

Full Length Research Paper

Prevalence of dermatomycoses in tertiary health institutions in Lagos State, Nigeria

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Accepted 12 February, 2013

Studies on dermatophytes isolated from patients in tertiary health institutions in Lagos State, Nigeria were carried out between August, 2009 and January, 2011. Collection, isolation, characterization and identification of the isolated fungi were achieved using both conventional laboratory methods (*in vitro* culture and microscopy) and molecular techniques (Deoxyribonucleic acid (DNA) extraction, Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and DNA sequencing). Twenty- one different isolates were obtained, in which into ten species and two strains belong to the dermatophytic group, two species of systemic mycoses agents and seven opportunistic mycoses agents. The adult emerge with the highest percentage of occurrence of dermatomycoses (82.32%) among the different age groups encountered in the study. There was significantly higher prevalence of dermatomycoses in female (53.81%) than in male (46.18%). Also, body infection was the most frequent (49.4%) while face infection (3.6%) had the least frequency in the studied areas.

Key words: Dermatomycoses, fungi, dermatophytes, mycoses, gender.

INTRODUCTION

Dermatophytes are the most common cause of fungal infections in humans worldwide, affecting approximately 20% of the population (Marques et al., 2000). Dermatophytes are pathogenic fungi specialized in the infection of skin, hair and nails, that utilize keratinous substrates as the carbon, nitrogen and sulphur sources. Dermatophytes have long been classified as anthropophilic, zoophilic and geophilic species on the basis of their primary habitat associations (Martinez-Rossi et al., 2008).

Anthropophilic dermatophytes are associated with humans and rarely infect other animals. Zoophilic dermatophytes usually infect animals or are associated with animals, but occasionally infect humans, while geophilic dermatophytes are primarily associated with keratinous materials such as hair, feathers, hooves and horns. When dermatophytes infect humans, they colonize

the keratinized outermost layer of the skin, and usually do not invade the living tissue (Martinez-Rossi et al., 2008).

Many epidemiological studies have investigated the prevalence of etiologic agents of superficial mycoses in different parts of the world (Ayadi et al., 1993; Ellabib and Khalifa, 2001; Anosike et al., 2005). A number of studies on the prevalence and etiological aspects of dermatomycoses have been carried out in different parts of Nigeria (Soyinka, 1978; Ajao and Akintunde, 1985; Iye, 1996; Anosike et al., 2005; Mbata and Nwajagu, 2007). Although, most of these reports are restricted among children of school age (Adetosoye, 1977; Ogbonna et al., 1985; Gughani and Njoku-Obi, 1986; Nweze, 2001; Anosike et al., 2005; Onayemi et al., 2005; Enemuor and Amedu, 2009; Chukwu et al., 2011). However, some researchers (Gughani et al., 1975; Soyinka, 1978; Alabi, 1980; Gughani, 1982; Yahya, 2007) did not limit their sampling to the age group of school children only.

Dermatomycoses is highly contagious and represents a significant public health problem in Nigeria, particularly Lagos State, an urban city with higher incidence of

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dermatomycoses due to hot humid climates and crowded living conditions (Adetosoye, 1977; Omar, 2000; Anosike et al., 2005). The present study is aimed at estimating the prevalence of dermatomycoses among different age groups and gender in Lagos State, since there is no recent data in literature to ascertain the prevalence of dermatomycoses in Lagos State Nigeria.

MATERIALS AND METHODS

Sampling and sample collection

Samples were collected weekly from two tertiary health institutions in Lagos State, Lagos University Teaching Hospital (LUTH) Idi-Araba, and Central Public Health Laboratory (CPHL) Yaba, for seventy-eight consecutive weeks (Agust, 2009 to January, 2011). Specimens were collected from infected areas which correspond to the active zone of the lesion from the different infected area on patient body. Prior to specimens' collection, the infected area was cleaned with methylated spirit in order to remove contaminants such as bacteria and some opportunistic fungi that may be present at the surface of the lesion area (Elewski, 1998). Specimens were collected in sterile envelop. Envelopes with collected specimen were then labelled with some necessary information concerning the patient. Information such as name, gender, age and infection site were collected and recorded.

Direct microscopy

A little part of the scraping was placed on a glass slide, and one to two drops of 10 to 20% potassium hydroxide (KOH) with dimethyl sulfoxide (DMSO) which is the most commonly used, was added and then viewed under the microscope for fungal hyphae (Lilly et al., 2006).

Culture method

The remaining part of the skin scrapings were cultured on Sabouraud dextrose agar (SDA) containing three antibiotics; chloramphenicol, gentamicin and cycloheximide, for the isolation of the fungi. The cultures were incubated at 30°C (Robert and Pihet, 2008). Culture plates were examined at least twice a week since some morphological traits can appear transiently, even though some can take up to four weeks before showing any morphological characteristics.

Identification of isolates

Once growth occurred, in order to accurately identify these isolated human pathogenic fungi, each specific type of colony morphology by gross features (topography, texture, and pigmentation) is noted. A little portion of the growth colony was teased with an inoculation needle and mounted in a drop of Lactophenol cotton blue on a clean microscope slide. Covered with a cover slip, this was squashed with the butt of the inoculation needle and the excess fluid then blotted off. This was to observe the precise arrangement of the conidiophore and the way in which their spores were produced. The identities of these fungi were certified using their cultural, morphological as well as comparing them with confirmed representatives of different species in relevant texts such as Alexopolous et al. (2007) and Ellis et al. (2007).

DNA extraction

The DNA extraction and analysis was carried out at the Molecular Laboratory, Department of Botany, University of Lagos, Akoka, Lagos State, Nigeria. Total genomic DNA was extracted with the Zymo kit by Zymo Research Corp (Hatfield-Pretoria 0028, South Africa).

Procedure for DNA extraction

Ten (10 g) of freshly prepared pure fungal culture plate of each isolate was scrapped and suspended in 50 µl of water to the tubes of a ZR BashingBead™ Lysis Rack. Four hundred microlitres (400 µl) of Lysis solution was added to each tube. Tubes cap were tight to prevent leakage. The ZR BashingBead™ Lysis Rack was centrifuge at 5,500 rpm for 5 min. Two hundred and fifty microlitres (250 µl) of the supernatant was transferred to each well of a deep-well block. Seven hundred and fifty microlitres (750 µl) of Fungal DNA binding buffer was added to the supernatant in the deep-well block from culture method (step 3), completely covered with cover foil, and then mixed thoroughly by vortexing block for 2 min. The deep-well block was centrifuge at 5,000 rpm for 5 min. After removing cover foil, 500 µl of each of the supernatants from DNA extraction (step 5) was transferred to the wells of a Silicon-A™ plate on a collection plate. The assembly was centrifuge at 5,000 rpm for 5 min. The flow was discarded from the collection plate and DNA extraction (step 5) repeated. Two hundred microlitres (200 µl) of DNA Pre-wash buffer was added to the wells of the Silicon-A™ plate on the emptied collection plate and the assembly centrifuged at 5,000 rpm for 5 min. Five hundred microlitres 500 µl fungal DNA wash buffer was added to the wells of the Silicon-A™ plate on the collection plate and the assembly centrifuged at 5,000 rpm for 5 min. The Silicon-A™ plate was transferred to an elution plate and 100 µl DNA elution buffer was added directly to the matrices in the plate. The assembly was centrifuged at 5,000 rpm for 5 min, after which it was eluted and the ultra-pure DNA was now ready. Then, the DNA verification was carried out on 1% agarose gel electrophoresis.

DNA verification on 1% agarose gel electrophoresis

One percent (1%) of agarose gel was prepared by mixing 1.5 g agarose with 150 ml 1× Tris-borate-Ethylenediaminetetraacetic acid (TBE) buffer and was placed in the microwave oven at 50°C for 3 min. On cooling, 6 µl of ethidium bromide was added to 100 ml agarose gel. The mixture was allowed to solidify after 10 min. 6 µl of loading dye was added to the extracted DNA samples. This mixture and 1 kb ladder of the marker (Lambda DNA HindIII) were then loaded on the electrophoresis gel machine which was allowed to run at 110 mA for 45 min. Genomic DNA was then viewed under the ultraviolet trans-illuminator for DNA quality and yield assessments and the photograph was taken (Figure 1a and b).

Polymerase chain reaction (PCR) and DNA sequence

For the amplification of regions of rDNA, PCR analysis and DNA sequencing, the voucher extracted genomic DNA samples in properly labelled PCR tubes were sent to Macrogen Incorporation, Washington, USA for subsequent DNA sequence analysis. Procedure-terms and conditions was applied (Macrogen Inc., Washington, U.S.A.).

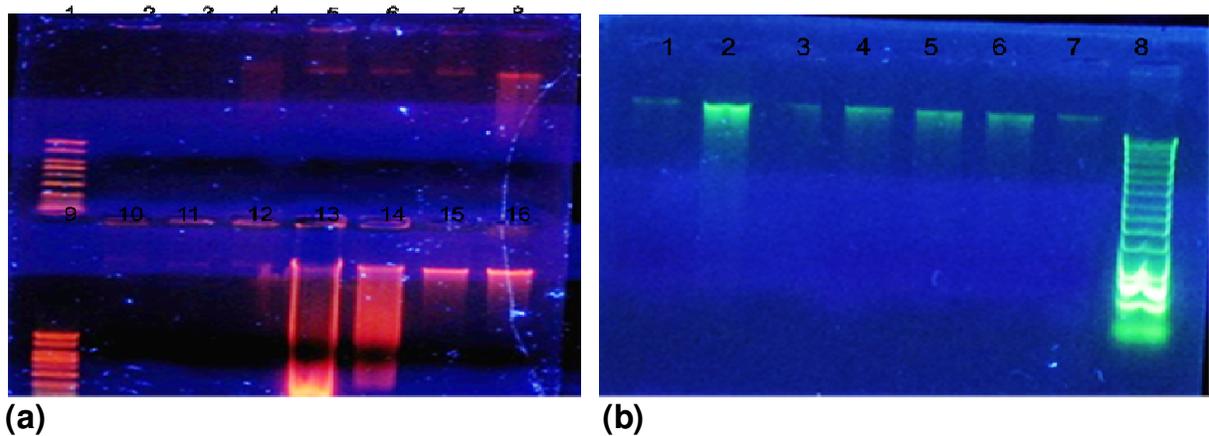


Figure 1. Electropherogram of the PCR products of some extracted fungal DNA band viewed under the ultraviolet light.

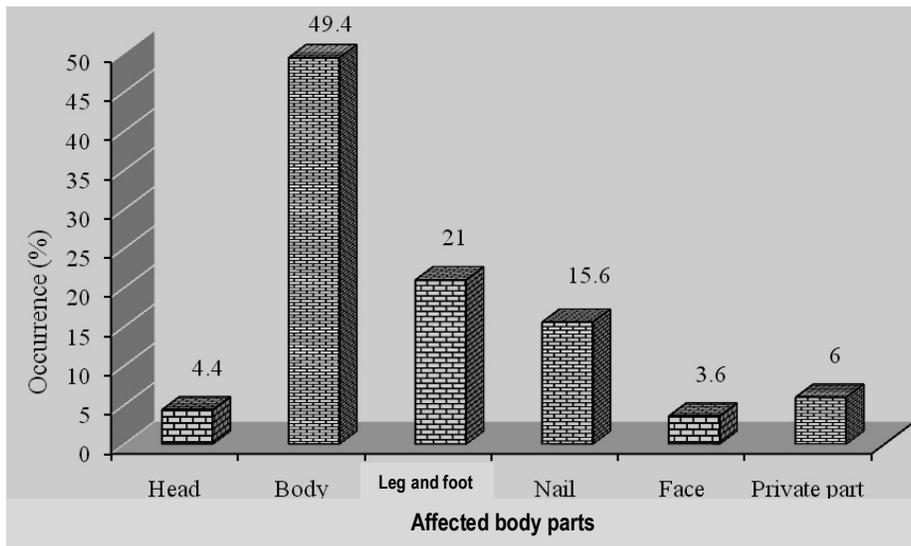


Figure 2. Percentage occurrence of dermatomycoses from all infected body parts.

Basic local alignment search tool (BLAST) sequence

The basic local alignment search tool (BLAST) of both the ITS 1 and ITS 4 sequences of each of the fungi was carried out to find the region of local similarity between sequences. The BLAST search was also carried out to compare a query sequence of the genomic DNA fungal sequences with the library database of the GenBank (<http://www.ncbi.nlm.nih.gov>) and identify library sequences that resemble the query sequence above a certain threshold. This was achieved through the online analysis tool provided by the GenBank.

Statistical analysis

The data obtained from the study were represented in graphs using Microsoft Excel Spreadsheet.

RESULTS

The weekly collection for seventy-eight consecutive weeks produced two hundred and forty-nine isolate in all. These were collected from different parts of the body, with the following percentages: 3.6% of the population from the face; 15.6% from the nail; 4.4% from the head and 21.0% from the leg and foot part and 6.0 and 49.4% from the private part and body (chest, back, laps and buttocks), respectively (Figure 2). Of the 249 scrapes, 115 (46.19%) were collected from male while the remaining 134 (53.81%) were from females (Figure 3). There are also variations with age, for the age group zero months to age twelve, 14.45% were generated (that is,

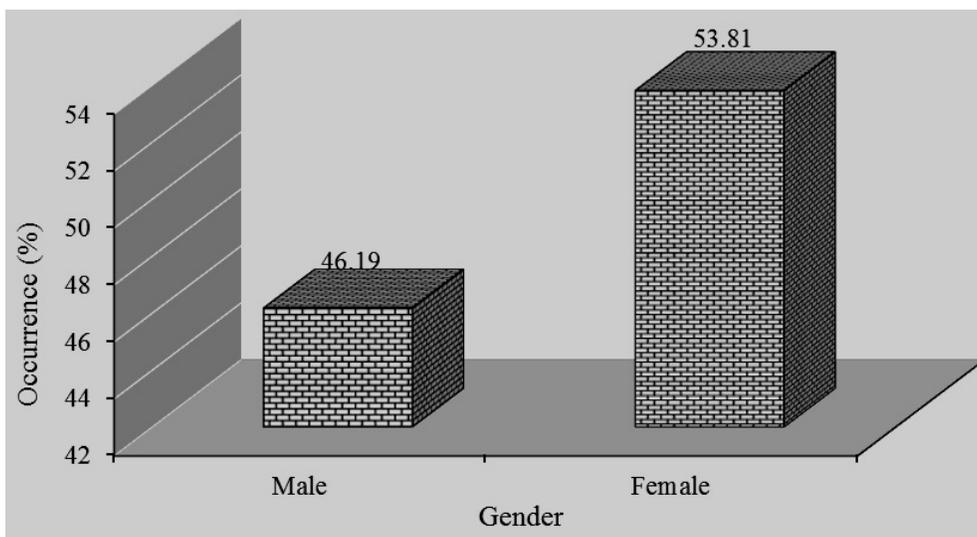


Figure 3. Percentage occurrence of dermatomycoses in male and female.

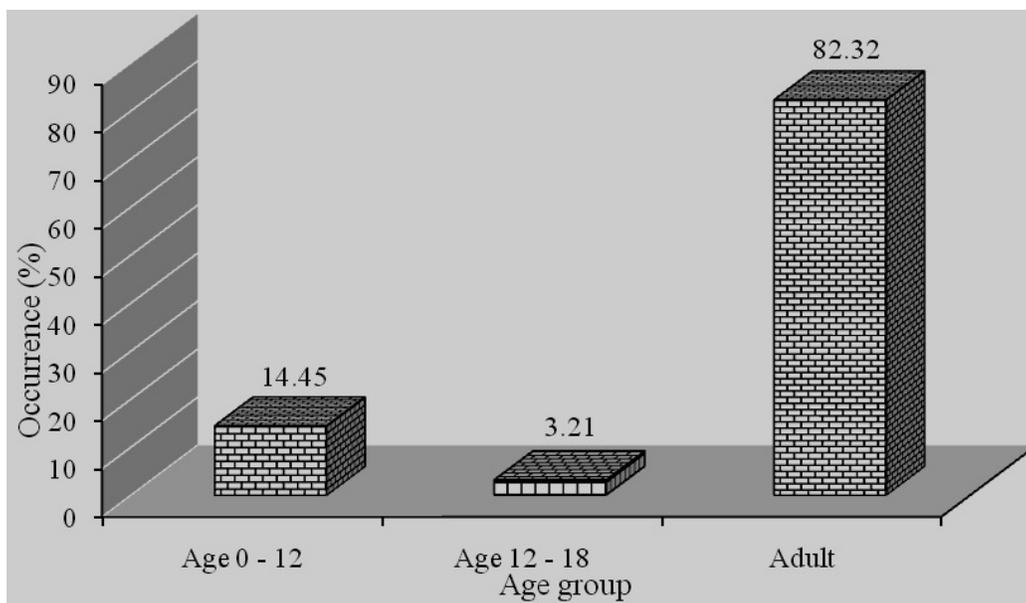


Figure 4. Percentage occurrence of dermatomycoses among different age groups.

36 scrapes); 3.2% (that is, eight scrapes) for the age 13 to 18 while the adult age group produced 82.32% (that is, 245 scrapes) (Figure 4).

Out of the 249 isolates, only seven isolates did not yield any fungal growth irrespective of the body part they were isolated from. Based on the morphological comparison with the confirmed representatives of standard medical mycology text books, the remaining 242 produced 21 morphological different species of fungi, whose identities were later confirmed by the BLAST sequence query

below (Table 1) to give the following 12 species/ strain of dermatophytes;

Epidermophyton floccosum, *Microsporum audouinii*, *M. ferrugineum*, *M. nanum*, *Trichophyton concentricum* strain A, *T. concentricum* strain B, *T. mentagrophytes* var. *quinckeanum*, *T. rubrum*, *T. soudanense*, *T. tonsurans* strain A, *T. tonsurans* strain B and *T. violaceum* (Figure 5), and two species of systemic mycoses agents otherwise known as the “deep

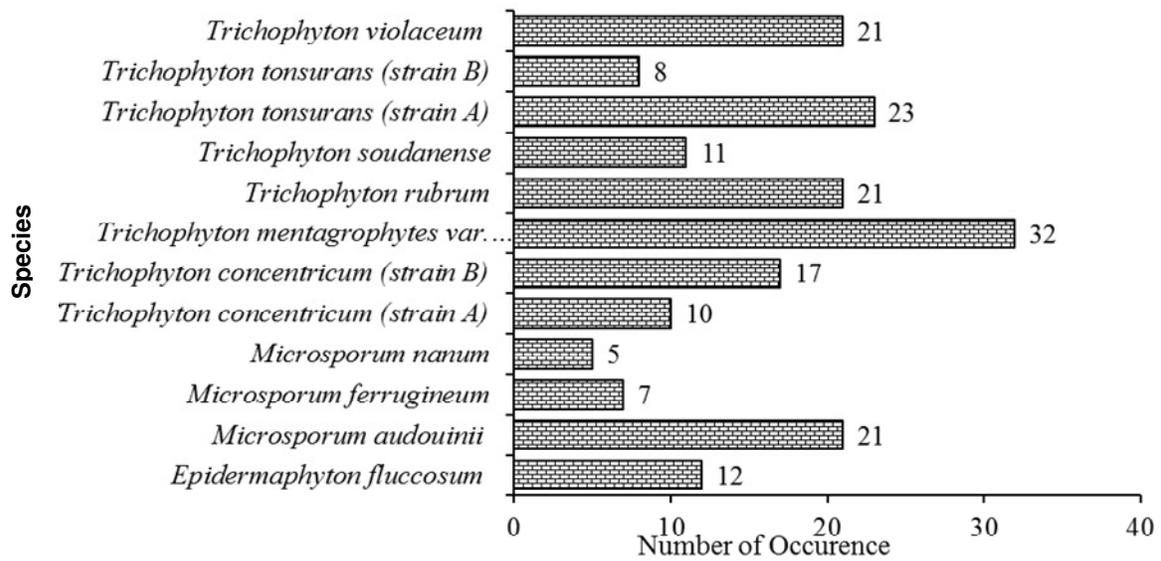


Figure 5. Frequency of occurrence of all isolated dermatophytes.

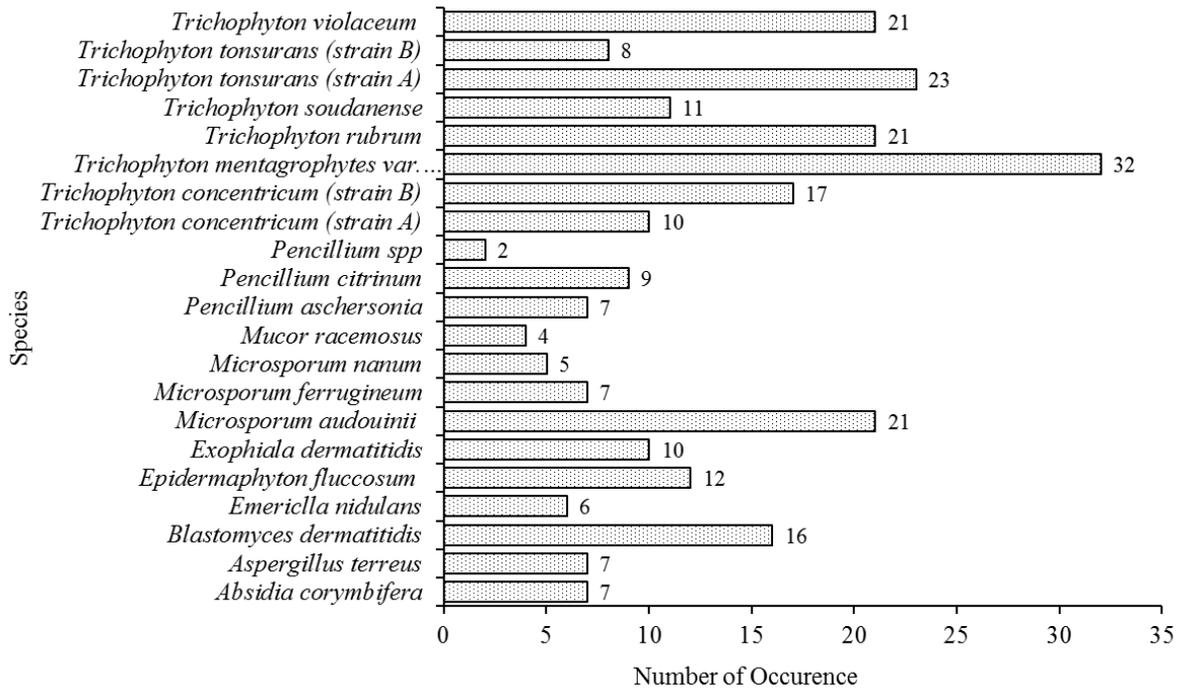


Figure 6. Frequency of occurrence of all isolated fungi.

mycoses” and these are *Blastomyces dermatitidis* and *Exophiala dermatitidis*. The remaining seven species of the isolated fungi belong to the opportunistic mycoses group, and these include:

Absidia corymbifera, *Aspergillus terreus*, *Emericlla nidul-*

ans, *Mucor racemosus*, *Pencillium aschersonia*, *P. citrinum*, and *Penicillium* species (Figure 6).

On the basis of the primary habitat association, the isolated above mentioned dermatophyte are of three groups. These are the anthropophilic, zoophilic, and geophilic geophilic (Table 2).

Table 1. BLAST sequence query and percentage of significant matches.

Sequence data	Percentage of significant match
110719-03_C07_TOA-ITS1 AACATGCGTGAGATTGTAAGTCTAGCTTTCCCCCTGTCC ATCACTTCCCCTCTATTTGCTTGACACCATTAGTATCACT GGCTCTCA	This sequence data was 98% significantly matched with <i>T. concentrum</i>
110719-09_O05_TOB-ITS4 AACATGCGTGAGATTGTAAGTCTAGCTTTCCCCCTGTCC ATCACTTCCCCTCTATTTGTCAACATGGAGTAACGTGAT CTTAATAAAC	This sequence data was 96% significantly matched with <i>T. tonsurans</i>
110719-03_A07_TOC-ITS1 AACATGCGTGAGATTGTAAGTCTAGCTTTCCCCCTGTCC ATCACTTCCCCTCTATTTGGATAATGTTGCCCACTTAAG CGTCCGCTTCA	This sequence data was 99% significantly matched with <i>T. mentagrophytes var. quinckeanum</i>
110719-09_I05_TOD-ITS4 GGACGAGGGCCCAACACACAAGCCGGGCTTGAGGGCA GCAATGACGCTCGGACAGGCATGCCCCCGGAATACC AGGGGGCG	This sequence data was 99% significantly matched with <i>Aspergillus terreus</i>
110719-03_K05_TOE-ITS1 TGGCCACTTGAACACAACCTTGTGGACCACGCTTACAT GCATGTGGGTTCAATTTTTCGAACCGACGCCGCCTTCTA ACTC	This sequence data was 99% significantly matched with <i>Blastomyces dermatitidis</i>
110719-03_I05_TOF-ITS4 AGTCTGAGACCTATAACGAAATTAGTTAAAACCTTCAAC AACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAG CGAAATGCG	This sequence data was 95% significantly matched with <i>T. tonsurans</i>
110719-03_E07_TOG-ITS4 CACTGAACTTCATGCCTGAGAGTGATGCAGTCTGAGCC TGAATACAAATCAGTCAAACCTTTCAACAATGGATCTCT GGTTCCGGCA	This sequence data was 97% significantly matched with <i>Exophiala dermatitidis</i>
110719-09_A07_TOH-ITS CGGCTGGCGCCCGGCCGCCCTAATCGAGCGGGTGAC AAAGCCCATACGCTCGAGGACCGGACACGGTGCCGC CGCTGCCTTTC	This sequence data was 98% significantly matched with <i>M. audouinii</i>
110719-03_G07_TOI-ITS1 ACCACTGAACTTCATGCCTGAGAGTGATGCAGTCTGAG CCTGAATACAAATCAGTCAAACCTTTCAACAATGGATCT CTTGGTTC	This sequence data was 97% significantly matched with <i>P. citrinum</i>
110719-09_C07_TOJ-ITS4 AACATGCGTGAGATTGTAAGTCTAGCTTTCCCCCTGTCC ATCACTTCCCCTCTATTTGGATTCACTGAATTCTGCAATT CACATTAC	This sequence data was 98% significantly matched with <i>T. rubrum</i>
110719-03_I07_TOK-ITS1 AACATGCGTGAGATTGTAAGTCTAGCTTTCCCCCTGTCC ATCACTTCCCCTCTATTTGCTTGTAAATCAGTTAAAACCT TCAACAATG	This sequence data was 99% significantly matched with <i>T. soudanense</i>

Table 1. Contd.

Sequence data	Percentage of significant match
110719-03_K07_TOL-ITS1 CCACTCCCGAGCGTGATTGCTCGGTTTCTTTACTCTAC AATATGGCCGTATAATGCACCACTTGGTGAATTGGCTA GAATCTA	This sequence data was 98% significantly matched with <i>M. ferrugineum</i>
110719-03_M07_TOM-ITS1 CTCCGTCAGGCTTTCTTCTCCAGTTTTGACAGGGATT CTTGTCGTTATACCTATTTCCCTACAATTACCTCCTGCCT TCGATTGCTGG	This sequence data was 99% significantly matched with <i>P. aschersonia</i>
110719-09_G07_TON-ITS4 CAGTTCACCTGCGAAAATTTTTTTGTGCTGACTGCCG GCTTTTTAGGGAGCGGGGAATGAAACCAAGAATCTCC AGGAC	This sequence data was 99% significantly matched with <i>Penicillium</i> species
110719-09_K07_TOO-ITS4 GGCCCTAGTCGAGCGGGTGACAAAGCCCCATACGCTC GAGGACCGGACACGGTGCCGCCGCTGCCTTTCGGGCC CGTCC	This sequence data was 99% significantly matched with <i>Epidermaphyton flucosum</i>
110719-03_A09_TOP-ITS1 ACTTCATGCCTGAGAGTGATGCAGGCTGGCTCTGAATC TAAAGCAAACCTTTCTTCTGAATCTTTGCTTCCCGCC TCCA	This sequences data was 98% significantly matched with <i>Mucor racemosus</i>
110719-03_C09_TOQ-ITS1 AACATGCGTGAGATTGTAAGTCTAGCTTCCCCCTGTCC ATCACTTCCCCTCTATTTGTGGTAGATTGCGACGTGCC GCGAAAACA	This sequence data was 95% significantly matched with <i>T. concentricum</i>
110719-09_O07_TOR-ITS4 AACCATGTGGATGGGCAACGAACATGTTTACCGGCCTG ATAATTTTGCCTAAGCCTGGTATCCTATTAACCTCTACC TTCAGAT	This sequence data was 96% significantly matched with <i>Absidia corymbifera</i>
110719-09_K05_TOS-ITS4 ATGCGTCGGCGGGCGCCGGCCCTACGGAGCGG AAGACGAAGCCCCATACGCTCGAGGACCGGACGCGGT GCCGCCG	This sequence data was 98% significantly matched with <i>M. nanum</i>
110719-09_I05_TOT-ITS4 AACATGCGTGAGATTGTAAGTCTAGCTTCCCCCTGTCC ATCACTTCCCCTCTATTTGCCCGGGAGCCGGGGGAC GAGGGCCCA	This sequence data was 99% significantly matched with <i>T. violaceum</i>
110719-03_E05_TOU-ITS1 ACGGATCTTTGGTTCCGGCATCGATGAAGAACGCAGC GAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAA TCATCG	This sequence data was 99% significantly matched with <i>Emericella nidulans</i>

Table 2. The three groups of isolated dermatophytes based on their primary habitat.

S/N	Group
	Anthropophilic
1	<i>Epidermophyton floccosum</i>
2	<i>M. audouinii</i>
3	<i>M. ferrugineum</i>
4	<i>T. rubrum</i>
5	<i>T. soudanense</i>
6	<i>T. violaceum</i>
7	<i>T. concentricum</i>
8	<i>T. tonsurans</i>
	Zoophilic
9	<i>T. mentagrophytes var. quinckeanum</i>
	Geophilic
10	<i>M. nanum</i>

DISCUSSION

The survey result in this present study revealed that dermatomycoses can occur irrespective of age, gender, occupation, custom and tradition of people. The ratio of school children below the age of twelve years to adults encountered in this present study was approximately 1: 6. This may be due to the fact that most parents do not take dermatomycoses in their kids/wards serious to the extent of seeking medical help. They tend to resort to the use of some medicinal plants or sometimes go for self-medication. Recently, Adekunle and Ikumapayi (2006) had reported the use of the aqueous extracts of *Funtumia elastica* (bark) and *Mallotus oppositifolius* (leaf) by the Nigerian populace to treat skin disease in children. Adekunle et al. (2011) also reported the use of *Acalypha wilkesiana* leaves by Nigerian natives to cure skin diseases (skin rashes in children) caused by *Candida albicans*, *T. mentagrophyte* and *M. audonii*. They provided some scientific justification for the utilization of extracts from these plants, by reporting that the antifungal activities of the extracts might be due to the presence of phytochemical compounds in the plants. They concluded that this is probably the rationale behind the use of these plants by the local populace.

The frequency of dermatomycoses is higher in female patients than in the male patients encountered. According to Kane et al. (1997), they reported that reduction in triacylglycerides in sebum (an oily substance secreted by the sebaceous glands in mammalian skin which helps to make the skin and hair waterproof and to protect them from drying out) may predispose postmenopausal women to the development of dermatomycosis more frequently than other adults. Yahya (2007) and Atraiide et al. (2011)

also observed a higher frequency of dermatomycoses in female patients in their researches. All these agreed with the findings in this study. Also, the results from the prevalence study of dermatomycoses in this study showed the skin lesion (49.4%) to have the highest percent of frequency compared to other dermatomycoses encountered in this study. This finding was supported by the findings of Alabi (1980) and Yahya (2007).

It was also observed in this study that some dermatophytes are peculiar to certain parts of the human body for their manifestation. For example, *T. rubrum* was more frequently isolated from the nails and foot than any other part of the human body, *T. soudanense* was isolated more from hairy region like the head and "private part" while *T. violaceum* was found to be associated with the head, feet and the nail regions. All these agree with the findings of Zurita and Hay (1967), Weitzman and Summerbell (1995), Gupta and Summerbell (2000) and Ellis et al. (2007). In their works, they reported that the anthroconidia or chlamydospores of dermatophytes species have a tendency to adhere *in vitro* to corneocytes derived from a particular body part. Also, *M. audouinii*, an anthropophilic fungus was found to be peculiar to the human skin, most especially on the skin of children below the age of twelve. Kane et al. 1997 and Ellis et al. 2007 reported that this is possibly due to an increase in fungistatic action of triglycerides in the sebum that is produced following puberty.

Ten species and two different strains of dermatophytes were isolated in this study. These include *M. audouinii*, *M. ferrugineum*, *M. nanum*, *T. concentricum* strain A and B, *T. mentagrophytes var. quinckeanum*, *T. rubrum*, *T. soudanense*, *T. tonsurans* strain A and B and *T. violaceum*. The identification of two different strains of *T. concentricum* and *T. tonsurans* was made possible by the application of the molecular techniques which provide the accurate identification based on the result from DNA sequences data generated, which revealed that no single queried isolated fungal sequence from this study was 100% homologous with the GenBank library database. This implies that the isolated dermatophytes from this study were probably different strains. This is probably the first work to report the identification of dermatophytes using both conventional and molecular laboratory methods and to report these numbers of dermatophytes (species/strains) in Lagos State, Nigeria.

ACKNOWLEDGEMENTS

The authors thank the permanent secretary, Health Service Commission, Lagos State for granting the permission to access the patients. Also, we appreciate the assistance of Mr Ohakem of the Centre Public Health Laboratory, Yaba, Lagos and Mr Nwanyanwu of Lagos University Teaching Hospital Lagos State, Nigeria.

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