Mycoses: Molecular Characterization and Phylogenetic Analysis of Some Human Pathogenic Fungi Associated with School Pupils' Scalp in Lagos.

[1,2]Irechukwu, P. G., [2]Ejikeme, C. E., [1]Samuel, T. O., [3]Adesida S. A. and [2]Shoneye, O. T.

[1]Department of Botany, Faculty of Life Science, University of Lagos, Lagos, Nigeria
[2]Department of Biology, Faculty of Science and Computing Education, Federal College of Education (Technical) Akoka, Yaba Lagos.
[3]Department of Microbiology, Faculty of Life Science, University of Lagos, Lagos, Nigeria

irechukwuprecious55@gmail.com

Abstract

This study investigated the molecular characterization of the non-dermatophytes and phylogenetic analysis of the fungi causing dermatomycoses among primary school children in Bariga, Lagos, Nigeria. The study ran from April to October 2019. The study population was drawn using a multi-stage sampling technique. Three primary schools were first purposively selected, while the pupils were randomly selected from all grades of the selected primary schools. A cluster random sampling method was used to screen a total sample of 45 pupils for the study. Samples were obtained by scraping the infected scalp area with a sterile toothed comb and collected into a sterile paper, folded in an envelope to avoid exposure to moisture and to prevent the growth of contaminants. Sabouraud Dextrose Agar (SDA) culturing and morphological identification of samples were carried out at the Mycology Botany Laboratory, UNILAG, while the molecular characterization analysis was done at the Federal Institute of Industrial Research, Oshodi, Lagos (FIIRO). Results of the morphological identification of the cultured samples showed colonies of white, cream, and yellow club-shaped microconidia with septate hyphae under the microscope among twelve samples, while the molecular characterization of the isolates revealed that the purity of the extracted DNA was within the range of 1.8 - 2.0, as expected for pure DNA. Agarose gels of PCR amplicons showed products ranging from 500 base pairs to 600 base pairs, as expected for a successful amplification of the internally transcribed spacer regions of fungal species. Based on these results, it is recommended that school-based hygiene education, routine screening, and improved sanitation infrastructure be prioritized among primary and secondary schools.

Keywords: Dermatophytes, Fungi, Pathogen, Molecular characterization, Mycoses

Introduction

Fungi are eukaryotic organisms distinguished by membrane-bound nuclei, organelles, and cell walls composed primarily of chitin. They exhibit remarkable morphological diversity, ranging from unicellular yeasts to multicellular filamentous molds (Nielsen et al., 2007; Griffin, 2002).

Most fungi are heterotrophic saprobes that decompose organic matter, while others parasitize plants, animals, or humans (Alexopoulos et al., 1996; Kathleen, 2005). Although approximately 80,000–120,000 fungal species have been formally described, global diversity is estimated at ~1.5 million species, suggesting that the majority remain undiscovered (Hawksworth, 2001; Kirk et al., 2001). Fungi are ubiquitous, inhabiting soil, vegetation, aquatic environments, and animal hosts. Their growth and distribution are influenced by environmental factors such as temperature, pH, moisture, aeration, and nutrient availability (Gaddeyya et al., 2012). Many pathogenic fungi are dimorphic, existing as filamentous molds in the environment and yeast-like forms in host tissues. Reproduction occurs sexually by gamete fusion or asexually by spore formation; fungi reproducing only asexually are termed Deuteromycota (Fungi Imperfecti), a group that includes many clinically important pathogens (Cheesbrough, 2000).

Mycoses range from superficial infections of keratinized tissues to disseminated systemic diseases involving vital organs (Santos & Davies, 2006). Pathogenic fungi are classified by site of infection, virulence, and route of acquisition. Superficial mycoses affect the stratum corneum and cause minimal inflammation, cutaneous mycoses involve the integument and appendages, subcutaneous mycoses follow traumatic inoculation, and systemic mycoses affect internal organs such as lungs and the central nervous system (El-Megeed et al., 2015; Sarosi & Davies, 1993). Primary pathogens infect immunocompetent hosts, while opportunistic pathogens exploit compromised immunity (Gupta et al., 2004). Infections may be exogenous (airborne, cutaneous, percutaneous) or endogenous (colonization or reactivation of latent infection) (Gupta et al., 2014).

Cutaneous mycoses include dermatophytosis (caused by dermatophytes) and dermatomycoses (caused by non-dermatophytes). Dermatophytes—Trichophyton, Microsporum, and Epidermophyton are specialized fungi that degrade keratin in skin, hair, and nails (Weitzman & Summerbell, 1995). They invade the stratum corneum but rarely penetrate deeper tissues in immunocompetent hosts (Chastain et al., 2001). Dermatophytes are ecologically classified as anthropophilic (human-associated), zoophilic (animal-associated), or geophilic (soil-associated) (Hainer, 2003). Transmission occurs via direct contact, contaminated fomites, or zoonotic routes.

Children are particularly susceptible due to close contact and poor hygiene (Enweani et al., 1996). Dermatophytosis has been linked to social stigma and reduced academic performance in school

children (Chepchirchir et al., 2009). Globally, superficial mycoses affect 20–25% of the population (Havlickova et al., 2008), underscoring their public-health importance.

Tinea capitis, a dermatophytic infection of the scalp and hair shafts, is especially common in school-aged children and presents with scaling, alopecia, folliculitis, and sometimes kerion formation (DiSalvo & Reiss, 2018). While dermatophytes are the principal agents, non-dermatophyte molds and yeasts are increasingly implicated in scalp infections (Ameen, 2010). Traditional identification methods based on morphology and culture are useful but limited in specificity, often failing to resolve cryptic species (Howell et al., 1999).

In Nigeria, dermatophytosis remains endemic. Surveys at Lagos University Teaching Hospital (LUTH) reported tinea capitis in 72.2% of fungal infections among children aged 0–9 years, with prevalence declining in older age groups (Ayanlowo & Akinkugbe, 2013). Infections are more frequent among primary school children aged 5–10 years and more prevalent in larger, poorer families (Ajao & Akintunde, 1985). Multiple studies confirm widespread prevalence across Nigerian communities (Nweze & Okafor, 2005; Chukwu et al., 2011). Immunological studies show that dermatophyte infections elicit variable cytokine responses in keratinocytes, with Trichophyton mentagrophytes inducing strong inflammatory reactions and T. tonsurans eliciting weaker responses (Shiraki et al., 2006; Tani et al., 2007).

Conventional diagnosis relies on microscopy, culture, and phenotypic identification, but these methods are slow, nonspecific, and prone to false negatives (Laupland, & Valiquette, 2013). Molecular methods, particularly PCR amplification and sequencing of the internal transcribed spacer (ITS) region, provide rapid, sensitive, and specific identification (Esteve-Zarzoso et al., 1999; Higgins, 2012). ITS sequencing is the official fungal barcode, while additional loci such as TEF1α and β-tubulin improve phylogenetic resolution (Gräser et al., 1999). Techniques such as RAPD, AP-PCR, and RFLP further support strain typing and epidemiological studies (Li et al., 1997; Faggi et al., 2001).

Materials and Methods

The study was conducted in Bariga, a district within Shomolu Local Government Area of Lagos State, southwestern Nigeria. Bariga lies between latitudes 6°31′20″–6°33′30″ N and longitudes 3°22′0″–3°24′0″ E. It is bounded by the Lagos Lagoon to the east, Mushin Local Government to

the west, Lagos Mainland to the south, and Kosofe Local Government to the north. The study population comprised pre-adolescent children aged 6–15 years enrolled in three public primary schools in Bariga. Between April and August 2019, 100 pupils were screened using cluster random sampling (Cheesbrough, 2000). Of these, 45 pupils presented with clinical signs of scalp infection (scaling, lesions, alopecia) and were included for further analysis. Scalp scrapings were obtained by gently combing affected areas with sterile toothed combs, one per child, to prevent cross-contamination (Enweani et al., 1996). Scrapings were collected into sterile paper, sealed in envelopes, and transported to the Research Laboratory, University of Lagos, for culture and identification. Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) were prepared following the manufacturer's instructions with modifications (Howell et al., 1999). Sixty-five grams of SDA powder was dissolved in 1 L distilled water, autoclaved at 121 °C for 15 minutes, and supplemented with 500 mg chloramphenicol to inhibit bacterial growth. The medium was poured into sterile petri dishes and allowed to solidify. Hair scrapings were inoculated onto SDA plates using sterile loops. Plates were incubated at 27–30 °C for up to 3 weeks. Pure cultures were obtained by sub-culturing onto fresh SDA. Plates without growth after 4 weeks were considered negative. For morphological identification, isolates were identified based on colonial morphology, pigmentation, and microscopic features (Weitzman & Summerbell, 1995). Slides were prepared with lactophenol cotton blue stain and examined under 10× and 40× objectives. Photomicrographs were captured using a Motic MC Camera attached to a light microscope. DNA extraction, spectrophotometric analysis, agarose gel electrophoresis, and PCR were performed at the Molecular Biology Laboratory, Federal Institute of Industrial Research Oshodi (FIIRO), Lagos. Sequencing was conducted at Inqaba Biotec, South Africa. DNA was extracted from pure cultures using the Zymo Research Fungal/Bacterial DNA Extraction Kit with modifications (Li et al., 1997). Approximately 50–100 mg of mycelium/spores was homogenized in phosphate-buffered saline, lysed mechanically, and purified through spin-column filtration. DNA was eluted in pre-heated buffer and stored at −20 °C. Spectrophotometric Analysis DNA concentration and purity were determined using absorbance ratios at 260/280 nm (Esteve-Zarzoso et al., 1999). Agarose Gel Electrophoresis Genomic DNA integrity was assessed on 1% agarose gels stained with ethidium bromide and visualized using a Cleaver Scientific OMNIdoc System. Polymerase Chain Reaction (PCR). The ITS region of ribosomal DNA was amplified using ITS1 and ITS4

primers (White et al., 1990). PCR conditions included denaturation at 94 °C, annealing at 52 °C, and extension at 68 °C. Products were resolved on 2% agarose gels and compared against a 100 bp DNA ladder. Amplicons were sequenced, edited, and aligned using ClustalW in MEGA 7 (Tamura et al., 2013); Elewski, B. E. (1996). Phylogenetic trees were constructed using the neighbour-joining method with 1,000 bootstrap replicates. Sequences were deposited in GenBank, and accession numbers were obtained.

Results

A total number of forty-five pupils (samples) were accessed; thirty samples showed radial growth on the cultured medium used (SDA) after eight days of inoculation. Twelve different fungal isolates were identified based on the morphological features observed from the photomicrograph taken (Plate 1 - 2). Samples of the pictures are as follows

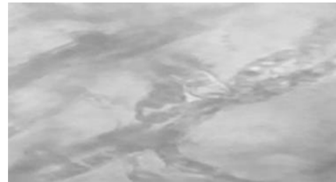

Plate 1A: Culture Plate of AP **1B**: Photomicrograph (Mag x 400

Molecular Characterization of Isolates

The agarose gels of extracted genomic DNA from the fungal isolates showed intact bands indicating that the DNA is suitable for further downstream analysis (Figures 1). The purity of the extracted DNA was within the range of 1.8 - 2.0 as expected for pure DNA. Agarose gels of PCR amplicons showed products ranging from 500 base pairs to 600 base pairs (Figure 2 and 3) as expected for a successful amplification of the internally transcribed spacer regions of fungal species.

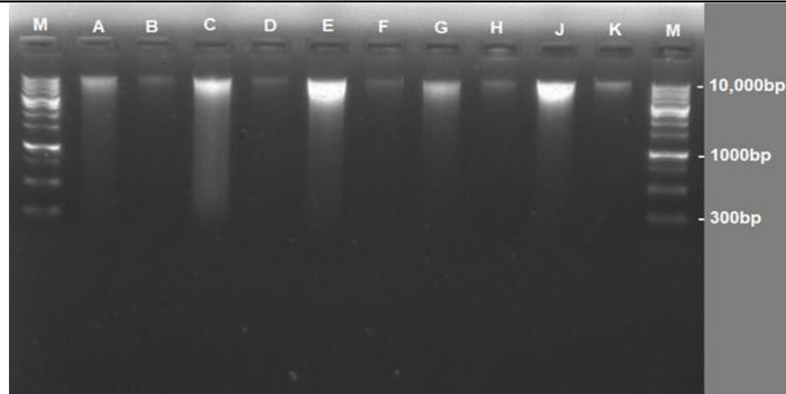

Figure 1: Gel Electrophoresis of DNA Extracted from Fungal Samples

Lane M= 1kb DNA Ladder, Lane A to K = DNA band of Fungal isolates

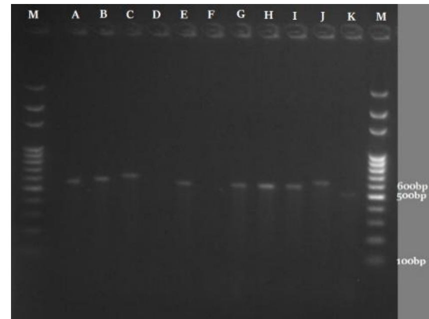

Figure 2: PCR Amplicons Agarose Gel

M = 100bp DNA Ladder, Lane 2 to Lane 12 = Amplicons from Samples AP – MP

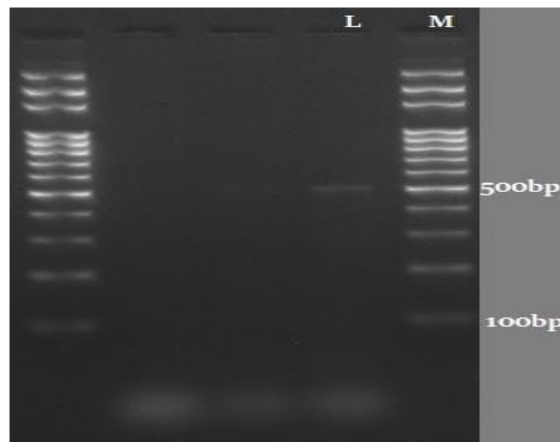

Figure 3: PCR Amplicons Agarose Gel of Isolate LP
M = DNA Ladder; L = Amplicon from Sample LP

Twelve isolates were characterized using molecular biology techniques; out of the twelve, six isolates (AP, BP, FP, GP, HP and IP) belonged to the *Aspergillus* genera. Other isolates belonged to the *Trichoderma* (CP), *Neopestalotiopsis* (DP), *Chaetomium* (EP), *Taloromyces* (JP) and *Candida* (KP and LP) genera. Isolates AP (Figure 4), FP, GP, HP (Figure 5) and IP showed 100 %, 99.81 %, 100 %, 99.34 % and 100 % homology with some strains of *Aspergillus sydowii,* respectively. Isolates BP (Figure 6), CP, DP and EP (Figure 7) showed 100 %, 99.81 %, 100 % and 100 % homology with *Aspergillus niger* 109INT-1.4.4, *Trichoderma longibrachiatum* NCQ3-2, *Neopestalotiopsis* sp FX24 and *Chaetomium globosum* CES5, respectively. Isolate JP showed 100 % homology with *Talaromyces islandicus* NWUSeq40. Isolate KP and LP showed 99.75 % and 100 % with *Candida parapsilosis* CMC_182and *Canidida orthopsilosis* Milk3, respectively. Table 1 shows locations of the strains with closet homology to isolates AP – LP. The accession numbers of the 12 isolates issued by the NCBI-GenBank, the sizes of submitted sequences, and their new NCBI-GenBank codes are shown in Table 2 below.

Table 1: Locations of Strains with Closet Homology from NCBI-BLAST

S/N	Isolate Code	Organism with Closet Homology	Location Isolated
1	AP	*Aspergillus sydowii* AsN19C03	China
2	BP	*Aspergillus niger* 109INT-1.4.4	Vietnam
3	CP	*Trichoderma longibrachiatum* NCQ3-2	China
4	DP	*Neopestalotiopsis* sp FX24	China
5	EP	*Chaetomium globosum* CES5	India
6	FP	*Aspergillus sydowii* DTO245H7	South Africa
7	GP	*Aspergillus sydowii* DTO245H7	South Africa
8	HP	*Aspergillus sydowii*	China
9	IP	*Aspergillus sydowii* DTO245H7	South Africa
10	JP	*Talaromyces islandicus* NWUSeq40	South Africa
11	KP	*Canidida parapsilosis* CMC_182	Italy
12	LP	*Canidida orthopsilosis* Milk3	Brazil

Table 2: NCBI-GenBank Submission Information of Isolates AP -LP

S/N	Isolate Code	Identity and NCBI-GenBank Code	Nucleotide sequences (bp)	Accession Number
1	AP	*Aspergillus sydowii* strain SIF1	508	MN700115
2	BP	*Aspergillus niger* strain SIF2	538	MN700116
3	CP	*Trichoderma longibrachiatum* strain SIF3	533	MN700117
4	DP	*Neopestalotiopsis sp.* strain SIF4	495	MN700118
5	EP	*Chaetomium globosum* strain SIF5	431	MN700119
6	FP	*Aspergillus sydowii* strain SIF6	513	MN700120
7	GP	*Aspergillus sydowii* strain SIF7	516	MN700121
8	HP	*Aspergillus sydowii* strain SIF8	457	MN700122
9	IP	*Aspergillus sydowii* strain SIF9	505	MN700123
10	JP	*Talaromyces islandicus* strain SIF10	438	MN700124
11	KP	*Candida parapsilosis* strain SIF11	394	MN700125
12	LP	*Candida orthopsilosis* strain SIF12	429	MN700126

Phylogenetic Tree of Isolates

The legend logo

The unknown organisms = ⬬

The out group = ⬬

The organism from the database = ⬭

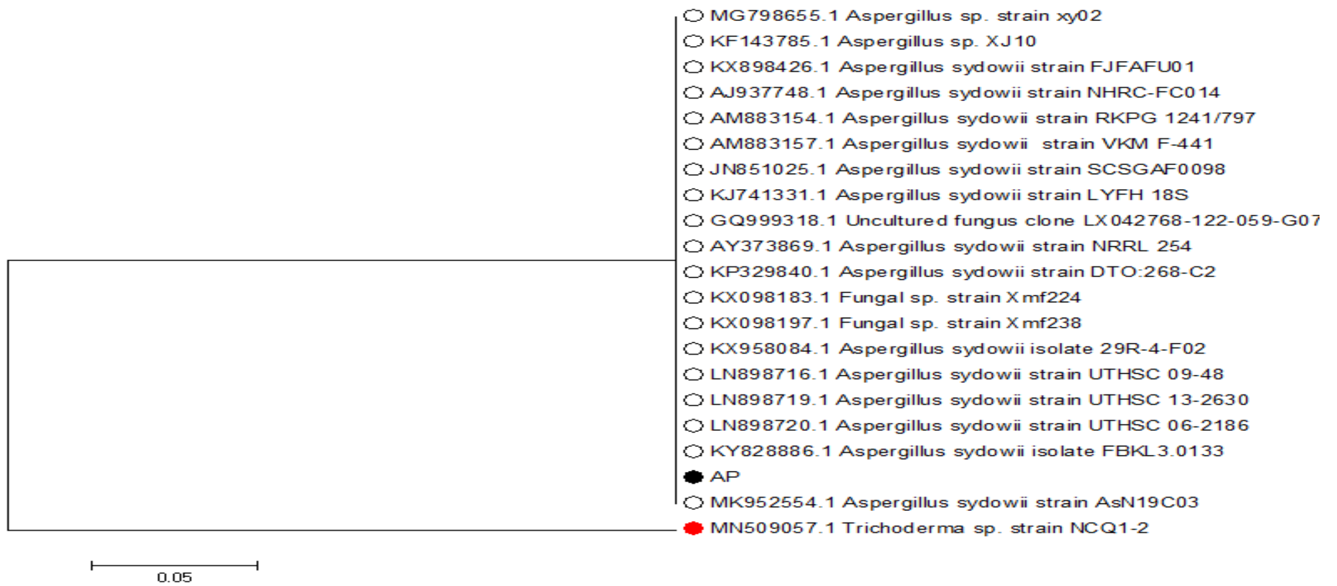

Figure 4: Phylogenetic Tree of Isolates AP

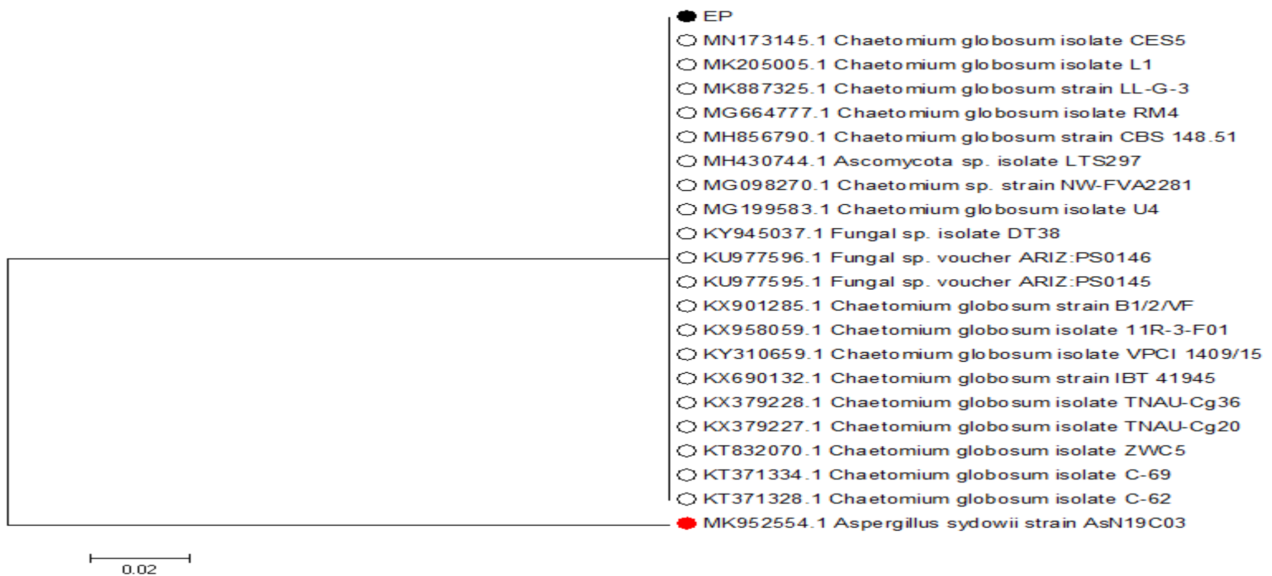

Figure 5: Phylogenetic Tree of Isolates EP

Discussion

This study demonstrated that non-dermatophyte fungi, particularly Aspergillus and Candida species, were the predominant causes of Tinea capitis among Nigerian school children, with a

prevalence rate of 99.7%, which is much higher than reports from Palestine (1%) and Iraq (2.7%) (Ali-Shtayeh et al., 2002; Fathi & Al-Samarai, 2000). The findings differ from earlier studies that identified Trichophyton species as the most common agents (Enweani et al., 2007; Ezeronye, 2005; Adefemi et al., 2011). The spread of infection was linked to poor hygiene, contaminated goods, and social practices such as sharing combs, contact sports, and farming activities. Molecular tools such as PCR and DNA sequencing provided more accurate identification and tracing of fungal origins (Li et al., 2004).

It can therefore be recommended, based on the findings of this study, that school-based hygiene education, routine screening, and improved sanitation infrastructure be prioritized through coordinated government and community action to reduce the burden of Tinea capitis among Nigerian school children (Oladele & Denning, 2014; Enemour & Amedu, 2009).

Conclusion

It may be concluded based on the results of this study that non-dermatophyte fungi, particularly Aspergillus and Candida species, are major contributors to Tinea capitis among Nigerian school children, with an alarmingly high prevalence (99.7%). The findings highlight that poor hygiene, environmental exposure, and social practices significantly drive transmission. Molecular diagnostic tools proved more reliable than traditional methods, enabling accurate identification and epidemiological tracing.

Recommendation

It can therefore be recommended, based on the findings of this study, that school-based hygiene education, routine screening, and improved sanitation infrastructure be prioritized through coordinated action by government and community stakeholders to reduce the burden of Tinea capitis among Nigerian school children.

References

Adefemi, S. A., Odeigah, L., & Alabi, K. M. (2011). Prevalence of dermatophytosis among primary school children in Oke Oyi, Kwara State, Nigeria. *African Journal of Clinical and Experimental Microbiology, 12*(2), 68–74. https://doi.org/10.1099/0022-1317-49-6-493. PMID: 10847201.

Ajao, T. A., & Akintunde, C. (1985). Studies on dermatophytosis in primary school children in Ilorin, Nigeria. *Mycopathologia, 89*(1), 43–48. https://doi.org/10.1007/BF00436764

Alexopoulos, C. J., Mims, C. W., & Blackwell, M. (1996). *Introductory mycology* (4th ed.). Wiley.

Ameen, M. (2010). Epidemiology of superficial fungal infections. *Clinical Dermatology, 28*(2), 197–201. https://doi.org/10.1016/j.clindermatol.2009.12.005

Anya, T. C., & Sunday, B. C. (2019). Prevalence of dermatophytic fungal infections among primary school children in Aba North, Abia State, Nigeria. *International Journal of Research and Innovation in Applied Science,* 4(8), 231–236. https://doi.org/10.33564/IJRIAS.2019.v4.i8.001

Ayanlowo, O. O, Akinkugbe, A. O. (2013). Overview of dermatophyte infections at the dermatology outpatient clinic of a tertiary institution in Nigeria. Nig Q J Hosp Med. 23(1):1-6. PMID: 24579485. https://doi.org/10.4103/1119-3077.106733

Ion, A., Popa, L. G., Porumb-Andrese, E., Dorobanțu, A. M., Tătar, R., Giurcăneanu, C., & Orzan, O. A. (2024). A Current Diagnostic and Therapeutic Challenge: Tinea Capitis. *Journal of Clinical Medicine*, *13*(2), 376. https://doi.org/10.3390/jcm13020376

Chastain, M. A., Reed, R. J., & Pankey, G. A. (2001). Cutaneous fungal infections. *American Journal of Clinical Dermatology, 2*(2), 89–97. https://doi.org/10.2165/00128071-200102020-00001

Cheesbrough, M. (2000). *District laboratory practice in tropical countries*. Cambridge University Press.

Chepchirchir, A., Bii, C., & Ndinya-Achola, J. O. (2009). *Dermatophyte infections in primary school children in Kibera slums of Nairobi*. East African Medical Journal, 86(2), 59–68**.** https://doi.org/10.4314/eamj.v86i2.46934.

Chukwu, O. S., Ogbu, C. I., & Nweze, E. I. (2011). Dermatophytosis among school children in Nigeria. *Mycoses, 54*(2), 176–180. https://doi.org/10.1111/j.1439-0507.2009.01803.x

DiSalvo, A. F., & Reiss, E. (2018). Cutaneous mycoses. In *Medical mycology* (pp. 145–162). Springer. https://doi.org/10.1007/978-3-319-71707-4_8

Elewski, B. E. (1996). Tinea capitis: Pathogenesis, diagnosis, and management. *Journal of the American Academy of Dermatology, 35*(4), 529–543. DOI: 10.1016/S0190-9622(00)90001

Ilkit, M., Bingöl, O., Durdu, M., Spruijtenburg, B., Kaplan, E., do Prado, C. M., Demircili, M. E., Ünal, N., & Meijer, E. F. J. (2026). Comparison of DermaGenius and In-House qPCR Methods for Detection of Recalcitrant Dermatophyte Infections: A Practical Approach for Dermatologists and Mycologists. Mycoses, 69(6). https://doi.org/10.1111/myc.70199

Enweani, I. B., Ozan, C. C., & Agbonlahor, D. E. (2007). Prevalence of *Trichophyton* species among school children in southwestern Nigeria. *Mycoses, 50*(6), 494–497. https://doi.org/10.1111/j.1439-0507.2007.01402.x

Gugnani, H.C., Paliwal-Joshi, A., Rahman, H., Padhye, A.A., Singh, T.S.K., Das, T.K., Khanal, B., Bajaj, R., Rao, S. and Chukhani, R. (2007), Occurrence of pathogenic fungi in soil of burrows of rats and of other sites in bamboo plantations in India and Nepal. Mycoses, 50: 507-511. https://doi.org/10.1111/j.1439-0507.2007.01402.x

Esteve Zarzoso, B., Belloch, C., Uruburu, F., & Querol, A. (1999). Identification of yeasts by PCR amplification of the ITS1–5.8S–ITS2 rDNA region and restriction analysis. *International Journal of Systematic Bacteriology, 49*(3), 329–337. https://doi.org/10.1099/00207713-49-3-329

Gugnani, H. (2009). Etiology of Dermatophytoses amongst School Children in Cross River State of Nigeria/Die ätiologie der Dermatophytosen an Schulkindern im Cross River State, Nigeria. Mycoses. https://doi.org/10.1111/J.1439-0507.1987.TB03835.X

Fathi, H., & Samarai, A. (2002). Prevalence of tinea capitis among schoolchildren in Iraq. Eastern Mediterranean Health Journal, 6(1), 128–137. https://doi.org/10.26719/2000.6.1.128

Havlickova, B., Czaika, V. A., & Friedrich, M. (2008). Epidemiological trends in skin mycoses worldwide. *Mycoses, 51*(Suppl. 4), 2–15. https://doi.org/10.1111/j.1439-0507.2008.01606.x

Hawksworth, D. L. (2001). The magnitude of fungal diversity: The 1.5 million species estimate revisited. *Mycological Research, 105*(12), 1422–1432. https://doi.org/10.1017/S0953756201004725

Higgins, D. G. (2012). Molecular approaches to fungal identification. Journal of Clinical Microbiology, 50(4), 1234–1240. https://doi.org/10.1128/JCM.05012-11

Laupland, K. B., & Valiquette, L. (2013). The changing culture of the microbiology laboratory. *Canadian Journal of Infectious Diseases and Medical Microbiology,* 24, 101630. https://doi.org/10.1155/2013/101630

Liu, G., He, C., & Zhang, H. (2014). Identification and characterization of dermatophyte species and strains with PCR amplification. *Experimental and Therapeutic Medicine, 8*(2), 545–550. https://doi.org/10.3892/etm.2014.1785

Nweze, E. I., & Okafor, J. I. (2005). Prevalence of dermatophytosis in children: A study in Nigeria. *Mycoses, 48*(6), 431–436. https://doi.org/10.1111/j.1439-0507.2005.01158.x

Oladele, R. O., & Denning, D. W. (2014). Burden of serious fungal infections in Nigeria. *West African Journal of Medicine, 33*(2), 107–114.