EPIDEMIOLOGICAL STUDIES AND MOLECULAR CHARACTERISATION OF DERMATOPHYTES AMONG *ALMAJIRAI* IN MAKARFI LOCAL GOVERNMENT AREA OF KADUNA STATE

BY

AISHA ABDULLAHI AHMED

DEPARTMENT OF BIOLOGY,
FACULTY OF LIFE SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

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Aisha Abdullahi AHMED, BSc (A.B.U.) 2006

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DEPARTMENT OF BIOLOGY,

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DECLARATION

I declare that the work in this dissertation entitled "**Epidemiological Studies And Molecular Characterisation of Dermatophytes among *Almajirai* in Makarfi Local Government Area of Kaduna State**" has been performed by me in the Department of Biology, A. B. U. Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project report was previously presented for another degree or diploma at this or any other Institution.

Aisha Abdullahi AHMED _____ _____

Signature Date

CERTIFICATION

This thesis dissertation entitled "Epidemiological Studies and Molecular Characterisation of Dermatophytes among *Almajirai* in Makarfi Local Government Area of Kaduna State" by Aisha Abdullahi AHMED meets the regulations governing the award of Master of Science in Biology of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

Dr. T. T. Gbem, Chairman, Supervisory Committee Dept. of Biology, A. B. U. Zaria	_____ Signature	_____ Date
Prof. A. J. Nok Member, Supervisory Committee Dept. of Biochemistry, A. B. U. Zaria	_____ Signature	_____ Date
Prof. M. L. Balarabe Head, Department of Biology, Dept of Biology, A. B. U. Zaria	_____ Signature	_____ Date
Prof. Sadiq Z. Abubakar Dean, School of Postgraduate Studies Ahmadu Bello University, Zaria	_____ Signature	_____ Date

DEDICATION

This work is dedicated to my beloved parents Alhaji Abdullahi Ahmed and Hajiya Hajara Ahmed (late) for all they have done in my life and to whom I remain indebted.

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ABSTRACT

Dermatophytes are fungi that have the capacity to invade keratinised tissues of humans and animals to produce an infection. The study was conducted to determine the epidemiology and molecular characterisation to identify of the dermatophytes associated with *almajirai* in Makarfi Local Government Area (L. G. A.) of Kaduna State. Samples were collected from the *almajirai* that had lesions on their scalps. The sites of infection were cleaned with 70% alcohol and followed by the collection of scalp scrapings using sterile scalpel blades. Sabouraud's dextrose agar (DibenDiagnostics, U.K) containing cycloheximide and chloramphenicol was used. Identification of isolates was through observation of colonial morphology and microscopic appearance of lactophenol cotton blue stained fungal specimen obtained from culture. Molecular identification was carried out using primers which contained the ITS1-2, 18S rRNA and 28S rRNA regions. A total of 408 *almajirai* were selected and examined, out of which 153 (37.5%) were found to be infected with different species of dermatophytes. From the 153 samples collected 119 (78.1%) were culture positive while no growth was observed in 34 samples (21.9%). Dermatophytes identified were members of *Trichophyton* (*T.*) and *Microsporum* (*M.*) genera. The species isolated include *Trichophyton rubrum*45 (29.4%), *Trichophyton mentagrophytes* 16 (10.5%), *Trichophyton violaceum* 3 (2.0%), *Trichophyton soudanense* 2 (1.3%), *Trichophyton tonsurans* 17 (11.1%*) Trichophyton concentricum* 4 (2.6%), *Trichophyton quickeanum* 01 (0.7%), *Trichophyton megninii* 01(0.7%), *Trichophyton verrucosum* 01 (0.7%), *Microsporum canis* 05 (3.3%), *Microsporum fulvum* 07 (4.6%), *Microsporum gallinae* 10 (6.5%), *Microsporum audouinii* 01 (0.7%), *Microsporum equinum* 05 (3.3%), and *Microsporum nanum* 01 (0.7%). Highest rate of infection occurred in the *almajirai* of 5 - 7years (54.67%) and the least infection was found among 17-19 age group ($P< 0.05$). Those that reside with their parents or guardians had low infection rate (29.17%) compared to those that reside in the *tsangaya*. Based on the number of rooms in a house, rate of infection was higher among *almajirai* that live in houses that have one or two rooms (31.25%). Participants that share beddings had higher infection rate (31.17%) than those that do not share beddings (31.12%). The *almajirai* that shaved at the Barber's shop had the less infection (27.45%) than those that barb at home (31.37%). Contact with pets and livestock accounted for 29.35% infection while those that were not in contact with pets and livestock had an infection rate of 28.70%. From PCR result, regions of amplification consisting of expected sizes of between 200 and 500 base pairs were obtained for *Microsporum canis, Microsporum audouinii, Trichophyton rubrum, Trichophyton tonsurans and Trichophyton mentagrophytes* using a dermatophyte specific primer (ITS1-2). Using 18S ribosomal RNA primer, approximately 500 base pairs band on ITS1-2 was observed in *M. canis, T. rubrum, and T. tonsurans* while band patterns of 560 base pairs band on ITS1-2 were observed in *M. audoinii* and *T. mentagrophytes. M. canis, M. audouinii, T. violaceum, T. rubrum, T. mentagrophytes and T. verrucosum* were visible around 200 base pair long band. A 300 base pair long band was identified with the 28S ribosomal RNA primer PCR on *M. canis, T. violaceum, T. mentagrophytes T. tonsurans, T. verrucosum and M. gypseum.* A 300 base pair-long was observed in *M. audouinii, T. rubrum and M. fulvum* on the 18S ribosomal RNA primer. It is therefore necessary that the *Mallams,* parents/guardians as well as the *almajirai* are educated on maintaining adequate personal, community and environmental hygiene through general sanitation. Contact with pets and livestock should be minimised and also there is need for provision of good infrastructure and upgrading of the informal settlements by the government so as to improve the living conditions of the *almajirai*.

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CHAPTER ONE

1.0 INTRODUCTION

The word dermatophyte literally means "skin plant". The suffix 'phyte' implies that these organisms are plants and therefore in the present context, it is a misnomer because the fungi are phylogenetically not related to plants (Chander, 2002). Dermatophytoses are commonly referred to as ringworm or tinea infections. The name ringworm was coined to describe the circular lesion produced by the dermatophytes on skin or scalp (Wetizman and Summerbell, 1995). The term 'tinea' is derived from Latin word meaning "worm" or "moth" (Wetizman and Summerbell, 1995) and the second part of the name identifies the part of the body infected (Fisher and Cook, 1998). Hair, fur, skin cells and nail are the most important parts of human body that are enriched with keratin (Abdul and Al-Janabi, 2014). Therefore, dermatophytic infections are generally cutaneous and restricted to the non-living cornified layers because of the inability of the fungi to penetrate the deeper tissues (Wetizman and Summerbell, 1995). The serum fungal inhibitory factors in the extravascular space prevent the penetration of the fungi in the living tissues (Sehgal, 2004), also their inability to tolerance human body temperature (37ºC) and the antifungal activity of blood proteins (Rodwell *et al.*, 2008). Diseases caused by fungi known as mycoses can be clinically classified as superficial, deep, or systemic mycoses (Kwon-Chung *et al.,* 1992). Dermatophytes are the most important microorganisms causing superficial mycosis (Lacaz *et al.,* 1998; Monod *et al.,* 2002). Dermatophytosis lesion takes a ring shape with inflammatory edges and clear center of normal skin. The lesions are often roughly circular with raised border, but may coalesce to form confluent areas of dry, scaling skin, inducing itching and scratching which in severe cases may ulcerate (Abdul and Al-Janabi, 2014). Fungal elements are always found in active state in the edge of lesion and that is why it is preferable to take scraping

sample for microscopical diagnosis from the lesion edges and not from the clear zone (Hainer, 2003). The ring shape of dermatophytosis can also be used to differentiate it from other skin diseases, such as psoriasis or lichen planus in which the inflammatory responses tend to be uniform over the lesion (Hainer, 2003). The etiologic agents of the dermatophytoses are classified in three anamorphic (asexual or imperfect) genera, *Trichophyton*, *Microsporum* and *Epidermophyton*. World Health Organization (WHO) survey on the incidence of dermatophytic infections shows that about 20% the people worldwide come down with cutaneous infections (Marques *et al*., 2000). Based on their ecological characteristics, dermatophytes are divided into geophilic (from soil sources), zoophilic (from animal sources) and anthrophilic (from human sources) species (Chander, 2009). Anthropophilic species are responsible for the majority of human infections (Maraki *et al*., 2007) spreading from one individual to another either by exposure to infected macerated skin cells, direct inoculation through breaks in the skin more often in persons with depressed cell mediated immunity or direct contact with infected person. Geophilic where some species of dermatophytes live in keratinous materials of soil as saprophytes and transmitted to human on contact with contaminated soil (Rahbar *et al.*, 2010), and the third source of dermatophytosis are from animals especially domestic and some wild animals which called zoophilic source (Abdul and Al-Janabi, 2014) and also indirect transmission can occur from fomites such as upholstery, hair brushes, hats, clothing, under wears etc (Srinivasan *et al*., 2012). The different types of dermatophytosis are classified according to body sites or anatomical locations involved (Hay and Moore, 2004). Several anatomic sites may be infected by a single dermatophyte species and different species may produce clinically identical lesions (Weitzman and Summerbell, 1995). The conditions include ringworm of the scalp (*Tinea capitis*); ringworm of the body (*Tinea corporis*); ringworm of the beard (*Tinea*

barbae); ringworm of the groin (*Tinea cruris*); ringworm of the foot (*Tinea pedis*); ringworm of the hand (*Tinea manuum*); ringworm of the nail (*Tinea unguium*); and ringworm of the face (*Tinea faciei*) (Degreef, 2008). The Mycoses caused by fungal infections of the skin and nails are widespread, the most numerous group amongst all Mycoses (Havlickova *et. al*., 2008) and are found worldwide (Bindu, 2002) becoming a significant health problem affecting children, adolescents and adults (Beena and Singh, 2003). There has been an increase in the incidence of fungal infection (Madhavi, *et al*., 2011). The increase could be as a result of frequent usage of antibiotics and immunosuppressive drugs (Mishra *et al*., 1998). Studies have shown great correlation of dermatophytoses with immune-depression as evidenced by increased incidence of dermatophytoses in patients with HIV/AIDS and cancers as well as people on long term cytotoxic drugs and organ/stem cell transplantation patients (Weitzman and Summerbell, 1995). Reactions to a dermatophyte infection usually range from mild to severe as a result of the immune status of the host, host's reactions to the metabolic products of the fungus, virulence of the infecting strain or species, anatomic location of the infection, as well as local environmental factors (Prasad and Chayani, 2013). The estimated life-time risk of acquiring dermatophytoses is 10 to 20% (Sumathi *et al*., 2013). Though neither life threatening nor debilitating, dermatophytoses may be recurrent and require long term skin treatment with attendant skin depigmentation (Panackal *et al*., 2009) producing a dermal inflammatory response with intense itching and also of cosmetic importance (Mishra *et al.*, 1998). The relative occurrence of the etiological agents and predominating anatomical infection patterns vary with geographical location and environmental and cultural factors (Havlickova, *et al.*, 2008) as well as socioeconomic status and contact with animals (Mikali, *et al*., 2012), flooring, clothing, linens, furniture and barbers' shops instruments (Farzana, 2007). Dermatophytes thrive at surface

temperatures of 25 – 28^{o}C and infection of human skin is supported by warm and humid conditions. For these reasons, superficial fungal infections are relatively common in tropical countries and are exacerbated by the wearing of occlusive clothing (Blanka *et al*., 2008). Although dermatophytic infections can be diagnosed by clinical presentation, the fungus can be demonstrated by easy laboratory procedures like microscopic examination of samples (KOH mount), biochemical characteristics and culture (Arbatzis, 2007). Species responsible for the infection can be identified based on demonstration of hyphae and both macroconidia and microconidia by colony characteristic and pigment production (Kim, 1997). Important characteristics are the rate of growth, shape and texture of the culture on solid media, color, dif-fusion of pigments into the agar and sporulation (Ninet *et al*., 2003). However, this system of identification is time-consuming and the same strains may show morphologically diverse colonies making it difficult to identify the dermatophyte species (Ninet *et al*., 2003). Moreover, the phenotypic features can be easily influenced by outside factors such as temperature variation, medium and chemotherapy (Liu *et al.*, 2000). In recent years, dermatophytes responsible for the infection can be identified with great precision by molecular methods such as Random amplified polymorphic DNA analysis- Polymerase Chain Reaction (RAPD-PCR), Nested-Polymerase Chain Reaction (Nested-PCR), Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP), and Real-time Polymerase Chain Reaction (Real-time PCR) (Rezaei-Matehkolaei *et al*., 2012). These novel molecular methods have advantages such as rapid identification of dermatophytes at genus and species level either directly in clinical samples or in fungal colonies (Kanbe *et al*., 2003; Mochizuki *et al*., 2003). The Internal Transcribed Spacer (ITS) regions of ribosomal DNA gene (rDNA) in the dermatophyte species used as a

reliable marker for species identification provide simple and precise method for dermatophyte species characterisation (Rezaei-Matehkolaei, 2012).

The word *Almajiri* is the adultrated spelling of the Arabic word *Almuhajir* which means somebody who migrates for the purpose of learning or seeking for knowledge (Yusha'u, 2013). The ancient culture of migration is tied to a system in which yearly, people inhabiting a given neighborhood gather their male children of school age usually after harvest and hand them to a teacher (*Mallam*). The purpose is for the *Mallam* to teach these children the basics of Islam through the Qur'anic schools where they are tutored how to read the Qur'an and write the Arabic alphabet. A pupil of any of these Qur'anic schools is known as *Almajiri* (*Almajirai* plural) (Alkali, 2001). According to Bambale (2003), *almajirai* are categorized into 3 classes:

i. Adult (*Gardi*): (17-25 years)

ii. Adolescent (*Titibiri*): (11-16 years) and

iii. An infant (*Kolo*): (5-10 years)

In order to escape domestic distractions, the *Mallam* may relocate his pupils to a distant area such as a city or another village and camp them there. It is at this camp that the *Almajirai* learn self-reliance and discipline as well as the essence of life (Alkali, 2001). To support the *Mallam* and his *Almajirai*, the local population provides accommodation and food for pupils and their teacher (Alkali, 2001). The place where they settle and take lessons is known as *tsangaya* which literally means learning centre in Hausa. A typical *tsangaya* has *alaramma* as the head, who is supported by other scholars such as *mahiru, gwani* and *gangaran* who have all memorized the Holy Qur'an and can write it off hand. About 15-20 houses with occupants being either learners or teachers make up a *tsangaya* (Hassan, 2015).

1.1 Statement of Research Problem

Dermatophytoses is one of the most common cutaneous infections worldwide (Havlickova *et al.,* 2008), posing a great public health problem to humans and animals (Ameen, 2010; Ghojoghi *et al.,* 2015). The prevalence of dermatophytosis varies from place to place throughout the globe and reported to vary in different parts of same continent (Havlickova *et al.,* 2008). In tropical and subtropical countries, it occurs in increased frequencies (Havlickova *et. al.,* 2008) due to warm and humid climate, poor nutrition, poor hygiene, overcrowding and poor sanitary conditions all promote the spread of these infections (Nweze, 2001). During the last decades, mycotic infections have increased to more than 20% - 25% of the world's population (Havlickova, *et al.,* 2008; Sharma *et al.,* 2015). Nigeria being a developing nation located in the tropic with wet humid climate fall into the category of regions with high prevalence of dermatophytosis, especially in school children of rural, suburban and urban extract (Gugnani and Njoku-Obi, 1995; Rudy, 1999). Socio-economic predisposing factors include; children's interaction patterns, poor living conditions marked by poor sanitation, housing (congestion), limited water supply as well as low economic power. Children living in informal settlements are more at risk to such skin diseases spread through contact as a result of living conditions and overcrowding both in school and at home (Chepchirchir *et al.,* 2009). The *almajirai* represent an accepted informal educational system that is widespread in the nation with several centres in the Northern Nigeria. The system is an integral part of the cultural and religious education that is meant to instill religious discipline in growing children. The increase in population as well as the desire/quest for the childhood training has led to overcrowding and establishment of multiple centres for the education. This has led to some centres housing many children often crowded with insufficient housing accommodation where they spend the night as well as share other materials such as

beds/beddings. This overcrowding has also overstretched the few available facilities in these centres and in most cases due to lack of funding these facilities are absent. It is these situations that provide suitable environment for the spread of dermatophytes and establishment of infection in these centres. There are efforts by the Government to integrate these centres with formal educational system and this is yet to be implemented in many centres. The poor infrastructural facilities found in the centres often has led to poor hygiene, dirty environment, poor health concern, a suitable environment for the spread of various pathogenic organisms including dermatophytes. Also, they have little or no access to medical facilities. Dermatophyte infections are often untreated or unreported. The inadequate health attention accorded this infection allows it to continue in the population unnoticed and spread to others. They are often overlooked as they do not produce severe debilitating illness and their treatment is not pursued vigorously. Molecular studies on dermatophytes are still scarce and not much work has been done on it in Nigeria. Some of these centres are attached to The Integrated Islamiya, Qur'anic and *Tsangaya* Schools.

1.2 Justification

Dermatophytosis is highly contagious and represents a significant public health problem in Nigeria and Africa at large, particularly among children. The study will establish the prevalence and predominant dermatophyte species that are associated with the *almajirai* system with a view to making recommendation on its control. It also intends to identify the risk factors associated with the disease spread within the study population. Since plans are on the way by the Government to get the system integrated with the formal educational system, the study on the risk factors will provide information on areas that need to be addressed to prevent its spread.

Though the disease poses no apparent health challenges, this study will bring to fore its prevalence and serve as framework for further studies and development of its control programmes. Distribution pattern and causative agents of infection continuously change therefore it is essential to update our knowledge in regard to the epidemiology of dermatophytes. The dermatophytosis caused by various dermatophyte species cannot be easily differentiated on the basis of clinical manifestations methods and for many years, conventional laboratory methods based on the detection of phenotypic characteristics, such as microscopy and *in-vitro* culture, have played an essential role in dermatophyte identification. However, these procedures generally suffer from the drawbacks of being either slow or non-specific (Liu *et al.,* 2000). More so, due to the high degree of phenotypic similarity between dermatophyte species, identification problems are imminent. Conventional approaches for identification down to the species level in the diagnostic laboratory are based on morphological and physiological criteria, need several days or weeks to be concluded and are frequently unspecific. Therefore, alternative molecular tools with sufficient specificity, reproducibility and sensitivity are necessary **(**Mohammadi *et al*., 2015). The use of ITS regions of ribosomal DNA gene (rDNA) in the dermatophyte species are reliable marker for species identification (Rezaei-Matehkolaei *et al*., 2012). For an effective control management strategy of dermatophytosis in Nigeria, adequate information on the epidemiology of dermatophytosis is indispensible. This study therefore will investigate the epidemiology of dermatophytic infection among *almajirai* in Makarfi Local Government Area relative to nature of school facilities, interaction with domestic animals and their play pattern with the aim of assessing a correlation and also carry out molecular studies on dermatophytes in the country and possible strains that circulate within the system.

1.3 Aim of the Study

To assess the epidemiology and characterisation of dermatophytes among *almajirai* in Makarfi Local Government Area of Kaduna State using molecular method.

1.4 Objectives of the Study

1. To determine the prevalence of dermatophytes among *almajirai* in relation to age.

2. To determine the associated risk factors for dermatophytosis among *almajirai* in Makarfi Local Government Area.

3. To isolate and conduct a molecular characteristic of dermatophytes from infected *almajirai.*

1.5 Hypotheses

1. There is no difference in the prevalence of dermatophytes among the various age groups of *almajirai*.

2. There are no associated risk factors for dermatophytosis among *almajirai* in Makarfi Local Government Area.

3. There is no difference in the isolated dermatophytes from infected *almajirai* using molecular tools.

CHAPTER TWO

LITERATURE REVIEW

2.0 General Properties of Fungi

Fungi are eukaryotic organisms with membrane bound nucleus, well differentiated apparatus and a cell wall. They are much larger than bacteria and usually characterized by vegetative cells of 2-10μm in diameter (Prescott *et al*., 2013). Fungi are heterotrophic in nature and spread as colonies of isolated cells (yeasts) or mycelium (multicellular filament or hypha) which branches to form networks (Sendron and Araro, 1999). Each fungal cell contains one or several haploid or diploid nuclei and is delimited by a β 1-3 and β 1-6 glucan wall and chitin (Ajello *et al*., 1966). Reproduction in Fungi involves both sexual and asexual methods and often results in the production of spores which are wind-disseminated depending on the species and conditions (Ajello *et al*., 1966). Spores are generated on hyphae or in micro or macroscopic sporangia which show limited tissue differentiation. Most fungi are non-motile throughout their lifecycle (Sendron and Araro 1999). The asexual and sexual forms in the same species are morphologically different. Most fungi are dimorphic and exist in two forms - the unicellular and yeast like forms and the filamentous forms. Almost all fungi that exhibit dimorphism are pathogenic to man (Sendron and Araro, 1999). They replicate sexually by fusion of gametes and asexually by spore formation, and exist in macroscopic or microscopic forms (Prescott *et al.*, 1999).

2.1 Historical Background

Historically, medical mycology specifically relating to human disease began with the discovery of the fungal etiology of favus and centered around three European physicians in the mid-19th century: Robert Remak, Johann L. Scho¨nlein, and David Gruby (Seeliger, 1985). Remak in 1835 first observed peculiar microscopic structures appearing as rods and buds in crusts from favic lesions. Although he did not publish his observations, he permitted those observations to be cited in a doctoral dissertation by Xavier Hube in 1837. Remak (1837) detected fungal elements in the scutula in a case of favus and Schoenlein (1839) described the nature of the fungus and recognized its aetiological role in favus. Remak (1845) named the fungus Achorion (the Greek name of scab or scurf) and coined the name of Schoenlein to the fungus as *Achorion schoenleinii.,* Gruby (1843) described small fungal spores surrounding hairs of a patient suffering from ringworm and named the fungus *Microsporum audouinii* in the honor of his boss Dr. Audouin. Malmsten (1845) described fungal spores inside hairs from cases of ringworm and named the fungus *Trichophyton tonsurans* (trichos= hair, phytos= plant). By the year 1945 all the three genera of dermatophytes were identified and had one species each. Harz in 1870 described a fungus which he named *Trichothecium or Acrothecium floccosum* and was later named *Epidermophyton floccosum.* In 1881 Megnin described the cause of favus in poultry naming the fungus *Achorion gallinae,* while Zopf (1890) described and named *Trichophyton quinckeanum* as the cause of favus in rodents. Blanchard (1895) described and named two new Trichophyton species, namely *Trichophyton megninii* and *Trichophyton concentricum.* Bodin (1902) described *Microsporum canis* and in 1907 *Microsporum gypseum,* while Castellani (1909) described and named *Trichophyton rubrum.*

Raimond Sabouraud, one of the best known and most influential of the early medical mycologists, began his scientific studies of the dermatophytes around 1890, culminating in the

publication of his classic volume, *Les Teignes* (Sabouraud, 1910). Sabouraud's contributions included his studies on the taxonomy, morphology, and methods of culturing the dermatophytes and the therapy of the dermatophytoses. He classified the dermatophytes into four genera, *Achorion*, *Epidermophyton*, *Microsporum*, and *Trichophyton*, primarily on the basis of the clinical aspects of the disease, combined with cultural and microscopic observations. The medium that he developed is in use today for culturing fungi (although the ingredients are modified) and is named in his honor, Sabouraud Glucose (Dextrose) Agar (Odds, 1991). Chester Emmon (1934) redefined the dermtophytes according to the botanical rules of nomenclature and taxonomy. He grouped all the 42 species of dermatophytes known to cause human infections into the genera Microsporum, Trichophyton and Epidermaphyton (Emmons *et al*., 1974; Rippon, 1985; Chander, 1995; Larone, 1995; Anaisse, *et al*., 2003). Generally, *Microsporum* spp. infects hair and skin not nail; *Trichophyton* spp. Infects skin, hair and nails and *Epidermatophytes* spp. infects skin, nails, but not hair (Howard, *et al*., 1987).

At present, the use of physiological characteristics, mating types and critical antigenic analysis, in addition to the classical methods of descriptive morphology had placed the taxonomy of dermatophytes and other pathogenic fungi on a firm of scientific basis (Abanmi *et al*., 2008; Ameen, 2010; Lakshmipathy and Kannabrian, 2010).

The discovery of sexual reproduction in the dermatophytes opened the door to classical genetic studies with these fungi, e.g., determining the cause of pleomorphism (Weitzman, 1965) and clarifying the taxonomy and understanding of the incompatibility systems operating in these fungi (Weitzman, 1964).

2.2 Etiology of Dermatophytosis

There are approximately 40 different species of dermatophytes characterised by their capability to digest keratin and are divided among three genera: *Trichophyton*, *Microsporum* and *Epidermophyton*.

2.2.1 Trichophyton

Species of Trichophyton are among the six superficial fungi reported to cause infections of the skin (Aly, 1994). The genus *Trichophyton* was identified by Malmsten in 1845 with the discovery of species *T. tonsurans* (Gotz, 1964). It has 24 species (Chander, 1995). Colonies on agar media are powdery, velvety or waxy. The predominant spore type is microconidia with sparse macroconidia (Chander, 1995). Reverse side pigmentation is characteristics of the species and is used for the identification of the species within genus (Wagner and Sohnle, 1995; Davies, (1995). The macroconidia are thin-walled with smooth surface and variable shape, pyriform about 2 - 3µm or irregular in form. Macroconidia are thin walled and cigar-shaped (Mucoma, 2000). Members of the genus *Trichophyton* are the common agents of dermatophytosis (Gotz, 1964). They are especially significant in onychomycosis, but also invade the skin and hair, causing infection associated with substantial morbidity (Ajello, 1974).

2.2.2 Microsporum

The genus *Microsporum* was identified by Gruby in 1843 with the discovery of *Microsporum audouinii*. It was microscopically confirmed in USA in 1956 (Gotz, 1964). The genus has 16 species and their colony morphology on agar surface is either velvety or powdery with white to brown pigmentation (Chander, 1995). It produces both macroconidia and microconidia with macroconidia being predominant. Macroconidia are multiseptate, with thin or thick echinulate

cell wall, spindle shaped and may be numerous or scarce (Emmons, 1934; Mucoma, 2000; Ali *et al*., 2009).

2.2.3 Epidermophyton

The *Epidermophyton* genus was first established by Sabouraud in 1910 with the discovery of *Epidermophyton rubrum* (*Epidermophyton interdigitale*—Kanfmam Wolf). The genus *Epidermatophyton* has only two recognized and phylogenetically separated species (*Epidermophyton floccosum* and *Epidermophyton stockdaleae*). Both species lack microconidia while macroconidia are abundant and are produced in clusters (Chander, 1995). The macroconidia are thin walled with smooth surface and occur in clustered branches (Emmons, 1934; Mackenzie and Philpot, 1981; Hussain *et al*., 2012). The colonies are slow-growing, powdery with unique brownish yellow color (Emmons, 1934).

2.3 Ecological Classification

Dermatophytes are also classified into three habitual species based on their host specificity as Anthropophilic (human), Zoophilic (animals), and Geophilic (soil) species in an environment (Maraki, *et al.*, 2007; Lakshmipathy and Kannabiran, 2010; Chowdhry, *et al.*, 2013).

2.3.1 Anthropophilic

The primary hosts of anthropophilic species are human being but they may also cause infection in animals. Transmission of infection is from human to human and is therefore more prevalent among urban populations especially in developed countries (Havlickova *et al.*, 2008; Nweze, 2010). The have predilection for human keratinized epidermis (Rippon, 1982; Silva and Oliveira,

2008) while in animals they may cause Tinea infection (Achterman *et al.*, 2011). Infections by anthropophilic fungi are more common among population with poor socioeconomic status where it is spread directly or indirectly. It is common in schools, barracks, prisons where it is spread through flooring, clothing, linens, furniture, barber shop instruments (Rippon, 1982; Farzana, 2007; Mikaili *et al.,* 2012). Examples include: *T. rubrum, T. tonsurans, M. audouinii*, *M. langeroni, M. rivalieri, M. ferrugineum* and *M. langeroni* (Simpanya, 2000).

2.3.2 Zoophilic:

These are dermatophytes that attack the animal keratin substratum. Zoophiles are also reported to infect human beings when human come in contact with infected animals (English, 1972; Cahtterjee *et al*., 1980; Starova *et al.,* 2010). Infection is common among dairy workers and children due to increased contact with farm animals as well as pets (Spiewak and Szostak, 2000; Chowdhry *et al.,* 2013; Jahromi, 2013). Pathogenic spores are hardly active as saprophytes but endure in a resting position on infected resources of an animal foundation (Ajello, 1961). *Microspom canis, T. verrucosum, T. mentagrophytes, A. vanbreuseghemii, A. benhamiae* are widespread representatives of zoophilic species that cause mycosis in animals as well as human infection (Marples, 1956). Other species include *T. simi* (monkey), *M. canis* (cats and dogs), *M. nanum* (pigs), *T. mentagrophyte* (bovine and sheep).

2.3.4 Geophilic

These are generally saprophytes and derive nourishment from keratinous substrates (Ajello, 1961). They are pathogenic to human and animals (Matsumoto and Ajello, 1987; Mucoma, 2000). The presence of these typical dermatophytes in nature is affected by natural

environmental conditions such as soil pH, temperature, humidity, environmental light, climate, chemical composition and amount of organic material in the soil (Bohme and Ziegler, 1969). This group of fungi mainly consists of *M. gypseum, T. ajelloi*, *T. terrestre*, and *M. fulvum.*

2.4 Clinical Manifestation of Dermatophytes

Dermatophytoses in humans presents a wide range of clinical features that are influenced by many factors depending on the site of infection, the species, size of inoculum and immune status of the host among other factors (Hiruma andYamaguchi, 2003; Enemuor and Amedu, 2009). These clinical manifestations are named with reference to the area of the body involved and are as follows:

2.4.1 Tinea capitis (ringworm of scalp):

This is a dermatophytic infection of the hair and the scalp where spores are formed within or outside the hair shaft (Fuller *et al.*, 2003). The causative agent of tinea capitis varies with geography, socioeconomic status and time (Elewski, 2000). It is endemic among school children worldwide especially in tropical Africa where they cause significant public health problems (Hogewoning *et al*., 2011). The clinical appearance varies from scaling (diffuse scaling with discrete patches of hair loss), hair loss, black dots or pustules, nodules to massive purulent secretion (Kerion) (Woldeamanuel *et al*., 2006).

2.4.2 Tinea barbae

Tinea barbae is also known as "Barber's itch. It is the ringworm of the beard (James and Werger, 2006). The disease affects the skin and hair in the beard and mustache region and results mostly

from shaving and abuse of steroids (Rapini *et al*., 2007; Marcus *et al*., 2008). Common symptoms include loose and broken hairs, kerion-like plaques, rash, itching and pimples near hair follicles in the neck, and genital areas (Szepietowski, *et al*., 2008; Vorvick, *et al*., 2010). Examples of zoophilic dermatophytes responsible for infection in human include *T. mentagrophytes, T. verrucosum, T. megninii, T. rubrum* and *T. violaceum*.

2.4.3 Tinea faciei

Tinea faciei occur on the non-bearded area of the face (Lin, *et al*., 2004; Rapini, *et al*., 2007). The lesions are mostly itchy and with burning sensation on exposure to sunlight (Nweze, 2010). Infections are more frequent in females than males (Ghilardi *et al*., 2005). Most common aetiologic agents are *T. tonsurans, T. verrucosum, T. mentagrophytes, M. canis* and *T. rubrum* (Lin *et al*., 2004; Starova *et al*., 2010).

2.4.4 Tinea manuum (hands):

This is a fungal infection of the palms caused mainly by *Trichophyton rubrum*. Infection is common in patients with tinea pedis. In patients with Tinea manuum, the palmar surface appears diffusely dry and exhibits hyperkeratosis (Goldstein *et. al*., 2000).

2.4.5 Tinea unguium

Tinea unguium also called onychomycosis is caused by dermatophytes in nails (Harvey and Stoppler, 2011). It attaches to the tip of the toe nail and steadily spreads to the nail matrix (Hiroshi, 2007). It is usually observed in men and also in pregnant women as lines and ridges on nails (Harvey and Stoppler, 2011). Infection is characterised by thickened; broken and discolored

nails and may result in the separation of the nail plate from the nail bed (Nenoff *et al*., 2007). It is commonly caused by *Trichophyton rubrum, T. mentasgrophytes T. interdigitale, E. floccosum, T. violceum, M. gypseum, T. tonsurans* and *T. Soudanense* (Macura, 1993; Nenoff *et al*., 2007).

2.4.6 Tinea cruris (ringworm of the groin):

Tinea cruris is infection of the groin. It is also known as crotch itch, crotch rot, eczema marginatum, gym itch, jock itch, jock rot, and ringworm of the groin (Rapini *et al*., 2007). It is also an infection of the proximal medial thighs and buttocks (Sharma *et al*., 2012). The fungal spores are usually transferred to the groin part by scratching from location in underclothing or pants. Affected areas usually present as red, tan, rippling brown, peeling, or cracking skin (Sharma *et al*., 2012). Common etiologic agents include *T. rubrum, E. floccosum, M. magnum, T. mentagrophytes* and *T. raubitschekii* (Nweze, 2010; Sharma *et al*., 2012).

2.4.7 Tinea pedis (athlete's foot):

This is an infection of the foot that is characterised by fissures, scales and maceration in the toe web or scaling of the soles and lateral surface of the feet (Gupta *et al*., 2003; Sharma *et al*., 2012). Tinea pedis which sometimes is called atlete’s foot is a universal infection commonly observed in many adults which boost up with age from adolescence (Havlickova *et al.*, 2008). The etiological agents of Tinea pedis are *T. rubrum, T. mentagrophytes,* and *T. tonsurans* and *E. floccosum* (Weitzman and Summerbell, 1995).

2.4.8 Tinea corporis (ringworm on the trunk)

This affects the body usually the trunk, shoulders or limbs causing red patches. It is more common in children than in adults and occurs most frequently in hot climates (Macura, 1993). Tinea corporis presents as typical round lesions with central healing, hair loss and scaling on the edges. Affected area can be large and widespread due to lack of treatment or in case of immunosuppression. Dermatophytes of the genera Trichophyton and Microsporum are the most common causative agents. On the trunk and the legs antropophilic infections like *Trichophyton tonsurans, Trichophyton violaceum, Trichophyton sudanense and Microsporum audouinii*, which are most prominent in Africa, are most common causative agents (Gupta *et al*., 2003; Woldeamanuel, *et al*., 2006).

2.5 Identification of Dermatophytes

There are two methods of identifying dermatophytes. The first method depends on the phenotype differences (conventional method) while the second method depends on the molecular differences like Polymerase Chain Reaction method.

2.5.1 Conventional method

The conventional laboratory method is based on detection of phenotypic characteristic such as microscopy, culture and physiologic properties and plays an essential role in dermatophytes identification (Rippon, 1988). Species can be identified by the colony structure and color, microconidia, macroconidia and other microscopic structures (Moriello, 2004). Direct microscopic examination is usually performed using KOH and is based on the detection of hyphae and shape of conidia. The advantage of direct microscopic examination is that it is rapid

and inexpensive but does not provide genus or species identification (not species-specific) (Koneman and Roberts, 1985; Weitzman and Summerbell, 1995; Mohanty, *et al*., 1999).

2.5.1.1 Cultural Characteristics

Dermatophytes can be cultured on various fungal media, including Sabouraud agar (with cycloheximide and antibiotics) and dermatophyte test medium (DTM). These cultures are usually incubated at room temperature (26°C-30ºC), but higher temperatures can be used when certain organisms such as *T. verrucosum* are suspected. Colonies often become visible within 1-2 weeks but, some species grow more slowly and may require longer time to appear. *In-vitro* culture identification of species takes longer time to give result and require a range of culture media such as Dermatophyte test medium, Sabouraud's dextrose agar, Urea test medium, Corn meal agar which are used to simulate condition or pigment production but in many reported literature Sabouraud dextrose agar (SDA) medium is conventionally used for comparative purposes. It is used to obtain colonies which can be compared to others (Ajello, 1974; Ameh and Okolo, 2004). Colony morphology usually differs with the medium and descriptions are based on Sabouraud agar. Dermatophyte test medium (DTM) contains a pH indicator (phenol red) that turns the medium red when a dermatophyte is growing. However, the mycelial growth must also be examined microscopically, as this color change alone is not diagnostic and could be produced by other fungal or bacteria (Chermette *et al.*, 2008). In presumptive identification of a dermatophyte culture, five important colony characteristics should be taken into consideration- when it is one to three weeks old, rate of growth , general topography (flat, heaped, regularly or irregularly folded), texture (yeast-like, glabrous, powdery, granular, velvety or cottony), surface pigmentation and reverse pigmentation (Ajello *et al*., 1966). It is based on these criteria that

dermatophyte species are classified on differences in conidial morphology into three genera namely *Epidermophyton, Microsporum,* and *Trichophyton* (Emmons, 1934; Caputo *et al*., 2001; Ghannoum *et al*., 2003).

2.5.1.2 Macroscopic morphology

Growth occurs on Sabouraud dextrose agar containing chloramphenicol, cyclohexamide (Actidione) and nicotinic acid and incubated at room temperature (26°C-37°C) for 3-4 weeks. *Trichophyton verrucosum* on the other hand grows better at 37°C. Colonies may be circular, asteroid, polygonal or lobulate. Colour of the colony may be white to gray, rosy, vinaceous, reddish, apricot, orange, yellow, violet and the back of the colony varies in colour from yellow to dark red to purplish black. There are three forms of colonies according to Ajello, *et al.,* (1968) and they are:-

The membranous form (glabrous, waxy, faviform): the aerial mycelium is entirely absent and the vegetative mycelium is in compact masses e.g. *M. ferrugineum, T. concentricum, T. schoenleinii, T. violaceum and T. verrucosum*.

The filamentous form (cottony, fluffy, hairy, velvety, woolly): the aerial mycelium is more or less high and dense e.g. *E. floccosum, M. audouinii, M. canis, M. distortum, M. nanum and T. rubrum.*

The granular-powdery form: characterised by excessive conidia and absence of aerial filamentous elements e.g. *M. equinum, T. mentagrophytes* and *T. megnini* (Ajello *et al*., 1968).

2.5.1.3 Microscopic morphology

Microscopic morphology of the microconidia and /or macroconidia is a more reliable identifications character but one needs good slide preparation.The main microscopic structures of dermatophytes are microconidia and macroconidia, chlamydospores, septated hyphae, racquet hyphae spirals. The main diagnostic structures in dermatophytes are the macroconida and they are usually single-celled, sessile or on a stack, spindle, pencil or club-shaped (Hasegawa, 2000). Some dermatophytes consist of only sterile hyphae and rarely produce spores. In *Epidermophyton*, the macroconidia are broadly clavate with typically smooth, thin to moderately thick walls and one to nine septa. They are usually abundant and borne singly or in clusters. Microconidia are absent. The genus is represented by two species and only *E. floccosum* is pathogenic. *E. floccosum* has smooth thin walled macroconidia, lacks microconidia and colonies have green to brown to khaki colour (Greenwood *et al.*, 2005). In *Microsporum*, the macroconidia are characterised by the presence of rough walls which may be asperulate, echinulate or verrucose. They are spindle-shaped or fusiform, obovate or cylindrofusiform (Feuerman *et a*l., 1975). Microconidia are sessile or stalked and cleavate and usually arranged singly along the hyphae. The genus comprises at least 12 species, *M. audouinii, M. canis, M. equinum, M. ferrugineum, M. fulvum, M. gallinae, M. gypseum, M. nanum, M. persicolor, M. preecox, M. racemosum and M. vanbreuseghemii. Microsporum* species have rough walled macroconidia and microconidia may or may not be present. In Trichophyton, the macroconidia when present are smooth, usually thin walls and 1 to 12 septa are born singly or in clusters and may be elongated and pencil shaped, clavate, fusiform or cylindrical. Example in *Trichophyton mentagrophytes* macroconidia are cigar-shaped with thin smoothed walls, *Trichophyton verrucosum* macroconidia are rare, long, thin and smooth wall with many chlamydospore,

Trichophyton equinum macroconidia are rare, clavate, thin and smooth wall. Trichophyton microconidia are usually more abundant, may be globose, pyriform, or clavate, sessile or stalked, and are borne singly along the sides of the hyphae or in grape-like clusters (Greenwood *et al.,* 2005). The genus comprises about 15 species, e.g. *T. concentricum, T. equinum, T. gourvilii, T. kane, T. megninii, T. mentagrophytes, T. raubitschekii, T. rubrum, T. schoenleinii, T. simii, T. soudanense, T. tonsurans, T. verrucosum, T. violaceum and T. yaoundei* (Feuerman *et al.***,** 1975). Gently touch a small piece of clear acetate tape to the surface of the fungal colony, and then apply the tape to a glass slide over a drop of blue stain (methylene blue, lactophenol cotton blue, or the blue Diff-Quik solution (basophilic thiazine dye) . Examine the slide under 10X and 40X objective lens to identify the characteristic dermatophyte features (Rebell *et al.,* 1974). In the early stages of growth, only fungal hyphae with no macroconidia may be seen, especially in cases of *Trichophyton* species infections. Incubating these cultures longer allow spore development for more reliable identification (Refai *et al*., 2013).

2.5.2 Molecular Biological Methods

The use of molecular methods for genotyping of dermatophyte species have proven to be more specific, precise, rapid and less likely to be affected by external influences such as temperature variations and chemotherapy. It is therefore very useful in identifying strain of fungi which under normal circumstances may not possible with conventional methods. Recent developments and applications of nucleic acid amplification technology have provided the opportunity to enhance the quality and speed of dermatophyte diagnosis (Liu *et al.,* 2002). Polymerase Chain Reaction (PCR) (Blanz *et al.*, 2000; Machouart-Dubach *et al.*, 2001) and sequencing of Internal Transcribed Spacer (ITS) regions (Harmsen *et al*., 1999) have been developed for the diagnosis

of dermatophytes. Polymerase chain reaction is a technology used to amplify a single or few copies of DNA across several orders of magnitude, generating thousand to millions of copies of a particular DNA sequence (Mohini and Deshpande, 2010).

2.6 Transmission of Dermatophytes

Dermaphytoses are transmitted from person to person directly by means of contact or indirectly via formites contaminated with infected skin, scales or hairs. Infection occurs by contact with arthrospores (asexual spores formed in the hyphae of the parasitic stage) or conidia (sexual spores formed in the "free living" environmental stage). It can also be acquired by humans from infected animals and by direct exposure to infected soils (Greenwood *et al*., 2005). Transfer of infecting organisms from soil, animals or humans is accomplished by means of arthrospores which are vegetative cells with thickened cell walls formed by dermatophytes hyphae *in vitro* or *in vivo* (Mandell *et al.*, 2000).

2.7 Prevalence of Dermatophytosis

Infections are more common in children than adults. The real prevalence of dermatophytosis is not known because patients do not seek medical advice unless their quality of life is affected as these are not life threatening diseases (Joseph *et. al.,* 2000). This distribution pattern of dermatophytes infection in different part of the world has been attributed to climatic factors, life-style, and prevalence of immunodeficiency diseases in the community and also the reluctance of patients to seek treatment because of embarrassment or minor nature of disease unless the condition becomes sufficiently serious to affect the quality of life (Al-sheikh 2009). An epidemiological survey carried out in North America reported a prevalence of 12.8%

(Woodstock, 2005); while in Peru the prevalence of dermatophytosis among teenagers is around 12.61% (Flores *et al.,* 2009). In the developing countries the prevalence of dermatophytosis ranges from 10.1% -51 %, in Tunisia it is 30.3%, in Brazil 38.4% and in Iran 21.1% (Souza *et al*., 2008; Neji *et al*., 2008; Hashemi *et al.,* 2009). In Nigeria the prevalence of dermatophytosis among school going children is 51% (Uneke *et al.,* 2006), while in Zimbabwe the prevalence of ideologically proven fungus is 39% (Wright and Robertson, 2001). In Kenya dermatophytosis accounts for almost 19 % of the skin diseases affecting school going children (Thappa, 2002). According to recent data obtained from primary school going children in Kibera slums, Kenya, the prevalence rates was at 11.25% with Tinea capitis being the most common type of infection (Chepchirchir *et al*., 2009). In 1997 the prevalence was 10.1% according to data obtained from school going children in Kisumu (Schmeller *et al.,* 1997). In the United Kingdom, a survey found dermatophytosis to be the most common zoonosis with prevalence of 24%. In Switzerland, one study reported that 14% of those working with cattle had been infected.

2.8 Geographical Distribution of Dermatophytes Species

Although all dermatophytes are botanically closely related, each species has certain characteristics in its geographic distribution (Kwon-Chung and Bennett, 1992).The most prevalent species of dermatophytes may vary strikingly from one geographic locality to another. While many species are cosmopolitan, others have very limited geographic ranges. The reason for these differences is unknown. However, it is clear that changing patterns of prevalence occur and are caused by several factors including migration of labour, troop movement, emigration and other travel, changing world patterns of animals husbandry, evolution of new genotypes and the transfer or adaptation of species indigenous to wild animal populations to parasitism in man and

recent therapeutic advances, played important roles in speeding these fungi (Mackenzie *et al*., 1986). The rapid transit from continent to continent, and the increasing mobility of people; agents of disease are no longer geographically restricted. Disease contracted half way across the world may become manifest in a country in which the pathogen is not normally found (Philpot, 1978). Dermatophytosis of scalp (tinea capitis) due to *M. audounii*, *T. tonsurans*, *T. violaceum*, and *T. schoenleinii* was highly prevalent in Western Europe during the early 1900s, and then tinea capitis due to the four species has been disappearing since World War II from that region, excluding the Mediterranean area. Sporadic out breaks of tinea capitis still occur but they are usually due to *M. canis* (Kwon-Chung and Bennett, 1992). Geographic dermatophytes are more significant as agents of ringworm in hotter, drier climates, while zoophilic fungi usually are more important in colder climates where they may represent over 80% of human infections and this directly related to techniques in farming and animals husbandry in colder are as (Verma, 1978). The high humidity, warmth, and low standard of living, poor hygienic conditions of living as well as customs and traditions are etiological factors causing the high incidence of dermatophytes in a tropical country (Verma, 1978; Kamalom *et al*., 1981). The incidence and type of mycoses vary according to age, geographical distribution of the organism, and the epidemicity of the prevalent species (Ali, 1990).

2.9 Epidemiology

Dermatophytosis is among the most common of all communicable diseases but because many cases are not brought to medical attention reliable incidences do not exist (Mignon and Losson, 1997). The infections are found throughout the world although some are endemic and they tend to spread rapidly throughout non endemic regions (Mandell *et al.*, 2000; Cohen and Powdery,

2004). The epidemiology of dermatophyte infection has been altered with changing patterns of migration, growth in tourism, and changes in socioeconomic conditions (Bhatia and Sharma, 2014). Dermatophytes endemic to Asia (Macura, 1993) and Africa (Morar *et al.*, 2006) such as *T. soudanense*, *T. violaceum*, and *M. audouinii*, have increased in frequency in Europe and North America as a result of migration (Ginter-Hanselmayer *et al*., 2007). Changes to the epidemiology of causative agents are also a reflection of changing patterns of dermatophytosis.

2.9.1 Reports in Nigeria

In the study carried out by Ayorinde (2013), on A Microbiological Study of Dermatophyte Infection among Primary School Children in Mowe, Ogun State, Nigeria, where they examined seventy-eight children, 45 (57.7%) were positive while 33 (42.3%) were negative. The following species were identified - *Microsporum audouinii* 3 (6.7%), *Trichophyton tonsurans* 8 (17.8%), *Trichophyton terrestre* 4 (8.9%), *Trichophyton mentagrophyte* 3 (6.7%), *Microsporum gypseum* 2(4.4%), *Microsporum canis* 3 (6.7%) and *Trichophyton verrucosum* 4 (8.9%). They found out that the infection was more prevalent in males (94.8%) than in females (5.1%) with *T tonsurans* (42.1%) having the highest percentage of infection, while *M. gypseum* (4.9%) had the least. The highest infection occurred among the 5-7 age bracket (53.3%), ($p>0.005$) compared with other age brackets. Age group 12-14 (15.6%) had the lowest number of infection. Ogbu *et al. in 2015 carried a research on* Prevalence of superficial fungal infections among primary school pupils in Awka South using six public primary schools where 870 pupils were examinedout which 353 (40.57%) had various skin fungal infections; *Tinea capitis* 229 (64.87%), *Tinea corporis* 96 (27.20%), *Tinea faciei* 25 (7.08%), and *Tinea pedis* 3 (0.85%). Sixty-five (18.41%) had multiple skin fungal infections; 21 (31.34%) pupils from Obiora Primary School, Mbaukwu, recorded the

highest, while Community Primary School, Amawbia 3 (7.32%) recorded the least infection (*P*> 0.05). Two hundred and twenty-nine pupils (64.87%) had the highest prevalence of *Tinea capitis*, while 3 (0.85%) pupils had the least infection recorded for *Tinea pedis*. Pupils from Central Primary School, Nibo, 82 (56.55%), had the highest prevalence, while 39 (26.90%) pupils from the Central Primary School, Umuawulu had the least infection. The age group of five to nine years was more infected than the 10–14-year age group. More male (199 (56.37%)) than females pupils were infected with skin fungal infections; this difference was not statistically significant. Chukwu (2011) undertook a study to evaluate the prevalence of dermatophytoses among one hundred and ten children between the ages of 3 to 14 years in rural primary school children of Barkin-Ladi Local Government Area. 108 (98.2%) were positive for fungal infections and 2 (1.8%) were negative was found to be highly significant at 5 and 10% level of probability at (P = 0.01 and P = 0.05). Of these 108 fungal isolates, 91(84.3%) were dermatophytes while 17 (15.7%) were non-dermatophytes. Among the pupils investigated, 56.5% fungal isolates were from males while 43.5% from females. From the site of fungal infections on the body of the pupils screened, 90 (83.4%) were from the head (hair) which is significant at 5 and 10% level of probability at (P = 0.01 and P = 0.05) compared to, 13 (12%) from the body trunks and 5 (4.6%) from the face. The dermatophytes isolates were; *Trichophyton mentagrophytes*, 26 (24%), *Trichophyton violaceum*, 11(10.2%) *Microsporum audouinii* 10(9.3%), *Trichophyton verrucosum* 8(7.4%), *Microsporum ferrugineum* 8(7.4%), *Microsporum canis* 7(6%), *Trichophyton tonsurans* 6(5.5%), *Trichophyton concentricum* 4(3.7%), *Trichophyton schoenleinii* 3(2.8%) and *Trichophyton megninii* 2(1.9%) and *Trichophyton rubrum* 6(5.5%). The non-dermatophytes were; *Aspergillus fumigatus* 4 (3.7%), *Mucor* species 3 (2.8%), *Penicillium* species 2(1.9%), *Aspergillus niger* 2(1.9%), *Aspergillus flavus* 2(1.9%), *Candida*

albicans 2(1.9%), *Trichoderma* species 1(0.9%) and *Alternaria* species 1(0.9%). From the work of Dike-Ndudim *et al.* (2013) on Fungal agents associated with dermatophytosis among pupils in Isu local government area (L.G.A), Imo State, Nigeria, they collected samples of hair and skin scrapings from 234 pupils cultured on Sabouraud agar and microscopy was carried out for fungal agents using 10% KOH and lactophenol cotton blue. Seventy-four (74) (31:6%) were positive for dermatophytosis, while 160 (68:.4%) were negative. *Microsporum audounii* (24.3%), *M. canis* (18.9%), *M. gypseum* (13.5%), *Trichophyton soudanense*(9.5%), *T. mentagrophytes* (6.8%), *T. rubrum* (4.1%) and *T. tansurans* (22.7%) were recovered. *Micosporum spp.* predominated (56.7%), than*Trichophyton spp.* (43.3%) and infection is age and sex dependent decreasing with increased age. Male children had a higher prevalence (54.1%) than females (45.9%) but was not statistically significant. Enemuor and Amedu (2009) carried out a study in Anyigba, a university town in Kogi State, Nigeria. 2184 primary school children were sampled randomly from four schools and screened for superficial mycoses. 144 (6.6%) had lesions suggestive of superficial mycoses. In a total of 155 samples collected, 108 (69.67%) yielded significant growth by culture and the distribution of superficial mycoses is dependent on age and sex. Eight species of fungi belonging to two genera were isolated, including: *Microsporum gypseum* (13.5%), *M canis* (12.4%), *M. ferrugineum* (3.4%), *Trichophyton rubrum* (30.3%), *T. tonsurans* (12.4%), *T. soudanense* (5.6%), *T. verrucosum* (11.2%) and *T. schoenleinii* (11.2%). Poor infrastructure (residential house and classrooms), contact with soil during outdoor activities (especially in children), intimate association with pet animals and poor personal hygiene may contribute to the spread of these infections among children. Maori (2011) in his work on "The Prevalence of Dermatophytes among Almajiri (Disciples) In Bauchi State" which was carried out between June and July, 2011with a total of 120 samples collected and cultured for bacterial isolates. Out of the

120 samples collected 61 (51%) were positive to dermatophysis and 59 (49%) were negative. Skin scrapings were collected and examined microscopically, cultured onto Sabouraud Dextrose Agar and incubated at room temperature. *M. auduinii* had the highest incidence with 38 (62.3%), *M. canis* 22 (36%) and *M. distortum* 1 (1.6%) were identified. In the work of Ndako *et al.,* 2012 which investigated the prevalence of dermatophytosis and associated non-dermatophytes among Islamiyya school children of ages 5-13 years old in Kano metropolis, 100 samples were collected and 91 (91%) yielded positive to fungal growth from which 66 (72.5%) were identified from males and 25 (27.5%) from females school children respectively. Fifty-three dermatophytes (58.2%) were recorded out of which 39 (73.6%) were isolated from males and 14 (26.4%) on females. Non-dermatophytes were also more in males (27 isolates) than females which had 11. The etiological agents of dermatophycoses recorded were *M. ferrugineum* (15.4%), *M. canis* (15.4%), *M. audounii* (9.9%), *T. concentricum* (5.5%), *T. verrucosum* (3.3%), *T. rubrum* (3.3%), *T. mentagrophyte* (2.2%), *T. tonsorans* (1.1%) and *T. schoenleini* (1.1%). *A. flavus* (9.9%), *A. niger* (8.8%), *Penicillium* sp. (7.7%), *Candida albicans* (5.5%), *Mucor* sp. (4.4%), *Trichoderma* sp. (3.3%) and *A. fumigatus* (2.2%) constituted the non-dermatophytes associated with these cutaneous infections. They observed that there was higher frequency of dermatophytosis in children with greater propensity for play, interaction with domestic animals and who lacked the luxury of school seats during classroom learning. Onwuliri *et al*., (2014) investigated Dermatological Disordersamongst Primary School Children in Riyom Community, North-Central Nigeria using 150 samples from pupils of seven (7) primary schools within the community comprising 80 (53.3%) males and 70(46.7%) females were randomly selected. They observed that infection was more common among the males than the females and frequent among the children between ages of 3 and 8 years. Species of dermatophytes isolated from the

various forms of the infection included Trichophyton 57(38%), Aspergillus 40(26.7%), while the Microsporum were found 34(22.6) pupils. Sanuth and Efuntoye in 2010 studied the distribution of dermatophytes infection among primary School Children in Ago-Iwoye, Ogun State, Southern Nigeriawith a population of 1404. Two hundred and seventy-four children representing 19.52% of the children were found to be infected by the disease with *Tinea capitis* accounting for 82.4% of the infection. It was more common among the males than the females and found to be frequent among the children between ages of 7 and 10 years. Shehu *et al.,* (2014) conducted a study to investigate the prevalence of superficial mycotic infections among the primary school children in Maru emirate, Zamfara State, Nigeria. Three hundred and fifteen pupils were implicated with clinical skin, hair and nail infections Out of 3251 pupils examined. *Trichophyton mentagrophytes* dominated (33.3%), followed by *Trichophyton gallinea* (15.2%), while *Microsporum distortum* was the least encountered dermatophytic fungus (2.0%). Females had high (22.1%) significant (p<O.05) prevalence than the males (9.7%). Innocent *et al.,* 2008 carried out a study on Dermatophyte Infections in Children: A Prospective Study from Port Harcourt, Nigeria. They tried to evaluate the spectrum of dermatophyte infections among children attending the Dermatological Clinic of the University of Port Harcourt Teaching Hospital (UPTH), Nigeria and they examined 432 children (ages between 2 months and16 years) with with skin diseases and they found out that 49 had dermatophyte infection. Males 31(63.3%) were more affected than females 18(36.7%). There was a predominance of tinea capitis 23(46.9 %). Trichophyton 28(57.1 %) and Epidermophyton 11(22.4%) were common. Most children with dermatophytosis 41(83.7%) were below 10 years. Wariso, *et al. in* 2015 carried out a 5-year desk review of the dermatophytes isolated in the medical microbiology laboratory of UPTH. Relevant Patients' data were retrieved and analyzed and a total of 70 dermatophytes were isolated over the five-year

period with *Trichophyton* species accounting for 55(79%), *Epidermophyton* species 8(11%) and *Microsporum* species 7(10%). Highest number of dermatophyte species was isolated from patients of age between 31 and 40 years with a male to female ratio of 1:1.7. Emele *et al*., 2015 investigated the role of barbers in ringworm transmission in Nigeria and Seventy eight barbers were randomly selected for the survey. They were given questionnaires to determine their knowledge and attitude toward ringworms infection. Disinfecting agents used by the barbers to decontaminate their clippers were tested for sporicidal activity against fungi. Seventy five (96%) of the barbers were aware that their clippers could transmit different diseases including ringworm and all of them (100%) habitually decontaminated their clippers either by exposure to chemical agents or by flaming with chemical agents with methylated spirit being the most frequently applied agent 57(73%). Twenty seven (35%) of the barbers decontaminated their clippers by flaming with petrol while 13(16%) flamed with methylated spirit. *In vitro* evaluation of the chemical agents for fungicidal activity showed that sodium hypochlorite was the most effective with a minimum disinfectant contact time of 5minutes, while flaming of clippers with methylated spirit or petrol did not inactivate fungal spores on clippers.

2.10 Social Economic Impact of Dermatophytosis

Skin infections can affect one's self esteem especially in children where it may interfere with individual performance despite adequate potential to excel. In some type of employment such as health care, bar and resultant, infected staff may require sick leave to access medical attention and mycological cure would take months resulting to loss of revenue. This aspect of the diseases has not been considered to date nor has any consideration been made of the cost (Chepchirchir *et al.*, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Makarfi Local Government Area located on latitude 75^{o}2'E and longitude 11^{o}22'N and occupies an area of about 2,520 sq mile. Makarfi Local Government area has a population of 146,259 (National Population Commission, 2006) with agriculture being the main occupation of the people. It shares boundaries with Rogo Local Government Area of Kano State to the north, Ikara Local Government Area to the south and east, Kudan Local Government Area to the west and Soba Local Government Area to the south-east in Kaduna State. The community cultivates sugarcane, maize, sorghum, rice, beans, groundnuts, tomatoes, onions, pepper and chili. The area is most famous for its leading position of sugarcane in Kaduna State. Average annual rainfall is about 1,000 mm to 2,000 mm which is marked by a dry season from October to May. In this area, the rainfall reached its peak by the month of August. The temperature description of the area is between the ranges of 24° - 27°C. However, there are changes with response to changes in season; it could be as low as 15°C in the harmattan and as high as 30°C in the rainy season respectively.

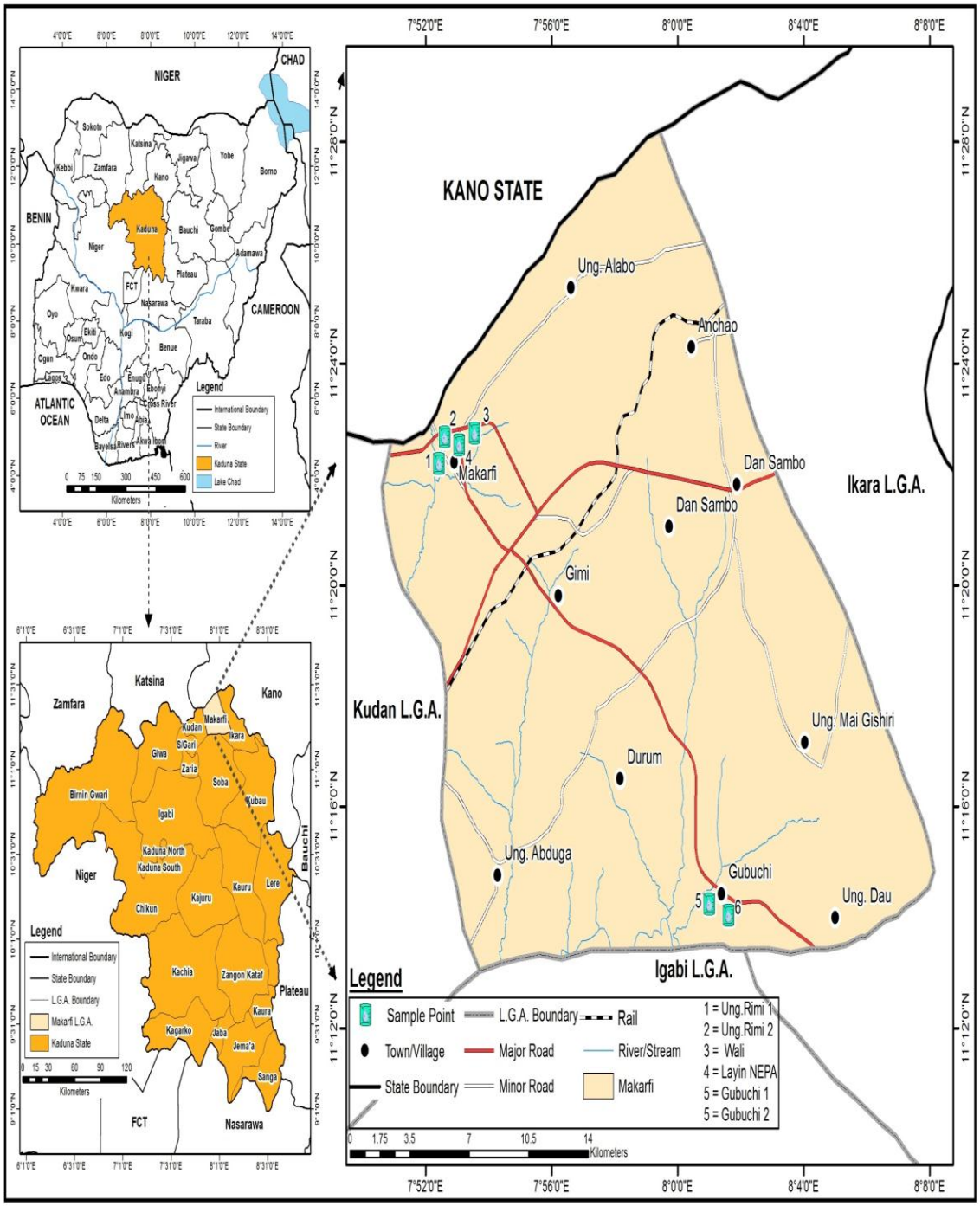


Figure I: Map of Makarfi L. G. A. showing sampling areas

3.2 Study Design

This was a cross-sectional descriptive study. Convenience sampling method was used to select seven sstudying sites located in Makarfi, Tudun Wada, Danguzuri, Gazara and Gubuchi wards. Sampling was by proportionate to size of *almajirai* population in each *Tsangaya* at the time of sample collection.

3.3 Study Population

The study population comprised of *almajirai* aged between 5 and 19 years who are attending different *Tsngaya* in Makarfi Local Government Area of Kaduna State that are under the Integrated Islamiyya, Qur'anic and *Tsangaya* Schools registered with Kaduna State Government. The *Tsangaya* are located in five wards of the Local Government namely- Gubuchi, Makarfi, Tudun Wada, Danguzuri and Gazara.

3.4 Sample Size Determination

The sample size was determined using a reported 10.1% prevalence of superficial mycoses among pupils in rural areas of Zamfara State by Shehu *et al.* (2014) and the formula described by Naing *et al.*, 2006.

$$n = \frac{Z^2 p\,(1-p)}{d^2}$$

Where n = Sample size

Z = confidence interval 95% = 1.96

P = prevalence rate = 10.1% = 0.10

d = precision allowable error = 5% = 0.05.

Therefore,

$$n = \frac{1.96^2 \times 0.10 \times (1-0.10)}{0.05^2}$$

n = 138.

Based on the sample size calculated, the minimum number of *almajirai* to be selected for this study is 138 but a total of 408 *almajirai* were selected for the study being the total number of *almajirai* available in all the *tsangaya* as at the time of sample collection and the total number examined.

3.5 Sampling technique and Specimen Collection

All specimens were collected according to the method described by Olusola *et al.* (2014) and Akinboro *et al.* (2011). Briefly each *almajiri* was examined in a well-lit room and the scalp was examined for scaly grey patches, lusterless hair strands and purulent lesions. Those participants that presented lesions, rashes, and other skin infections suggestive of dermatophylic infections were selected for the study. The site of infection was cleaned with 70% alcohol and followed by the collection of scalp scrapings from actively growing margins of the lesions using sterile scalpel blades. Hair specimens were collected by removing dull broken hair from the margin of the lesion using sterile surgical blade. The specimens were collected in a sufficient amount from the edge of the infected area which corresponds to the active zone of the lesion. The scrapings were handled separately and no individual scraping was allowed to mix up with the other. The samples collected were sealed in paper envelopes and labeled appropriately. The samples were transported to the Department of Microbiology, Ahmadu Bello University Zaria for culture and identification. Standard normal sterile procure was observed in the collection of samples from

the study participants. Samples were collected weekly for a period of five (5) months (from January to May).

3.6 Inclusion Criteria

Almajirai who were present in the registered *tsangaya* at the period of study, whose parents, guardians or *mallam* consented as well as those who consented to participate in the study.

3.7 Exclusion Criteria

Almajirai whose *tsangaya* is not a member Integrated Islamiyya, Qur'anic and *Tsangaya* Schools, whose parents, guardians or *mallam* did not consent were excluded from this study.

3.8 Administration of Structured Questionnaire

A structured questionnaire was used to obtain information on the age, playing habit, association with domestic animals, number of children and adults in the house, information on the sharing of beddings as well as other questions relevant to the study (Appendix I). Questionnaires were administered by the researcher.

3.9 Laboratory Investigation

The methods described by Devise (1995) and Cheesbrough (2000) for macroscopy and cultural methods were adopted for this work.

3.10 Preparation of Media

Sabouraud's dextrose agar (DibenDiagnostics, U.K) containing dextrose 40.0g/L, bacteriological agar 13.0g/L, peptone mixture 10.0g/L, cycloheximide 0.40g/L, chloramphenicol 0.50g/L) was

used for the work. A total of 65.9g of the media was suspended in 1000 ml of distilled water in 1500ml conical flask, then plugged with cotton wool and wrapped with aluminium foil. It was dissolved by heating to boil with frequent agitation and then sterilised at 121^{o}C for 15 minutes. The media was allowed to cool to 45°C before pouring into sterilised Petri-dishes and allowed to solidify after sterilisation before inoculation of the samples. To a sterile petri dish, 20ml of sterile sabouraud dextrose agar was poured. Cycloheximide prevents growth of majority saprophytic fungi while chloramphenicol is an antibacterial agent (Raymond and Piphet, 2008).

3.11 Isolation of Dermatophytes using Cultural Techniques

The scraped samples were picked with a heat sterilised forceps and aseptically placed at the centre of the Agar plates. The plates were sealed with masking tape to avoid dehydration and minimize contamination. Each plate was incubated at room temperature (28^{0}C) for six weeks and examined daily for growth, mycelia characteristics and changes in the medium of growth. A culture plate was kept for a maximum of six weeks before being adjudged as negative for growth. The plates were observed for colony and the colony types were noted and recorded. Growths were sub-cultured and then transferred to slant culture for identification. Identification was done based on the growth characteristic and morphology of the fungi using fungi atlas.

3.11.1 Identification of the Isolated Dermatophytes

Identification of fungal isolates was carried out by both macroscopic and microscopic examination which included growth rate, general topography, surface and reverse pigmentation. Microscopic identification of positive fungal cultures was carried out using the method described by Devise (1995), Murray *et al*. (2005) and Refai *et al.* (2013).

3.11.2 Macroscopical Examination of the Cultures

Examination involved rate of growth, colour on the surface, texture of the colony or consistency (Cottony, velvety, folded, fluffy, suede-like and wiry), colonial morphology and reverse side of colony (pigmentation of the media), margins, elevation and detachability from the agar surface.

3.11.3 Microscopical Examination of the Cultures

A drop of lactophenol cotton blue was placed on a clean glass slide and a pin-size of the culture was placed on the slide. It was gently teased with flamed inoculation needles. It was satisfactorily spread and cover slip was placed on it, pressed gently and avoiding air bubbles. Excess stain around the cover slip was removed with the edge of a piece of blotting paper. The stain was allowed to penetrate for 5 minutes. The preparation was examined with x 10 and x 40 objectives of Research microscope (). The identification was based on features such as organization of hyphae (pencil shaped, spiral, pyriform, septations etc.), microconidia and macroconidia (tear shaped, drop like, spherical, in bunches, abundance or rare etc.) and features seen were compared with atlas (Devise, 1995 and Refai *et al.*, 2013).

3.12 Molecular Identification of Dermatophytes

3.12.1 Genomic DNA Extraction from Dermatophyte Cultures

Preparations were carried out according to manufacturer's instructions with slight modifications. Approximately 100mg each dermatophyte was placed in a microcentrifuge tube containing 100µL of nuclease free water. Ninety-five µL (95 µL) of 2X digestion buffer and 5 µL of proteinase K were then added to the solution and allowed to stand for 5 minutes at room temperatue. Seven hundred µL (700 µL) of Genomic Lysis Buffer was added to the tube, mixed

thoroughly by vortexing for 5 seconds mixed and incubated at 55°C for 30 minutes in a heating block. A total of 800 µL the lysate was transferred to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuged at 10,000 x g for 2 minutes at 5°C (refrigerated centrifuge). The flowthrough was discarded and column replaced back. Two hundred µL (200 µL) of DNA Pre-Wash Buffer was added to the spin column in a new Collection Tube and centrifuge for 2 minutes at 10,000 x g at 5°C. The flowthrough was discarded as before and column was placed in a new Collection Tube and centrifuge empty for 2 munutes at 5°C. Five hundred µL (500 µL) of g-DNA Wash Buffer I was added to the spin column and centrifuged at 10,000 x g for 2 minutes at 5°C. The flowthrough was discarded. Five hundred µL (500 µL) of g-DNA Wash Buffer II was added to the spin column and centrifuged at 10,000 x g for 2 minutes at 5°C. The flowthrough was discarded, column replaced and centrifuged empty at 10,000 x g for 2 minutes at 5°C. The spin column was transferred to a new sterile 1.5ml microcentrifuge tube. Seventy-five µL (75 µL) of DNA Elution Buffer was added to the spin column, incubated for 5 minutes at room temperature (28°) and centrifuged at 14,000 x g for 2 minutes to elute the DNA. The spin column was removed, decanted and the eluted DNA obtained was used for PCR.

3.12.2 Polymerase Chain Reaction (PCR) Procedure

The pcr tubes were briefly centrifuged at 5 000 g for 30 second.

The PCR amplification was performed with a reaction volume of 25µl consisting of 12.5 µL of PCR Master mix, 1µL of Forward primer, 1µL Reverse primer (Table 3.) and 3.5µL Nuclease free water giving a total of 18 µL to which 7µL of the DNA template was added making a total of 25µL reaction mix. The pcr tubes were centrifuged at 5 000 g for 30 seconds.

3.12.3 Cyclic Parameters for PCR Amplification

The Polymerase Chain Reaction (PCR) tubes were arranged in the thermocycler. The amplification program consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 repeated cycles of denaturation at 94°C for 30 seconds then annealing at 55°C for 30 seconds and extension 72°C for 30 second. Final extension at 72°C was for 7 minutes and Final hold at 4°C.

3.12.4 Agarose Gel Electrophoresis of PCR products

Two grams (2g) of agarose powder was weighed and poured into 150ml beaker. One hundred ml (100 ml) of 1X Tris Borate EDTA (TBE) was added and the mixture dissolved using microwave oven at full strength for 2 minutes. It was allowed to cool to about 45^{o}C and 5µL of ethidium bromide was added gently and swirledto mix. Gel casting tray was assembled with the combs placed in position and the mixture was carefully poured into the casting tray and allowed to solidify. The combs were carefully removed, the tray was transferred to the electrophoresis tank and tank filled to the gauge with 1X TBE running buffer. The first well was loaded with 5µL of 100 bp DNA ladder, the second well was loaded with 10 µl nuclease free water mixed with 5 µL 6X loading dye while the other wells were loaded with 10 µL PCR productssamples mixed with 5µl 6X loading dye. Voltage was set at 75V and the preparation was run for 45 minutes after which gel-tray was removed and transferred to gel- documentation unit. The gel was observed images were captured using the Versadoc imaging system (Bio- Rad, CA).

3.13 Data Management

Data was analysed using IBM SPSS statistics 20. Data analysis was done in accordance with study objectives presented using tables, and charts as appropriate. Associations were also determined using Chi-square test with level of Significance at P=0.05.

3.14 Ethical Approval and Informed Consent

Ethical clearance was obtained from the Kaduna State Ministry of Health for this research (Appendix II). Permissions were obtained from the spiritual teachers (*Mallams*) while verbal consent was obtained from the *almajirai* that took part in the study. Informed verbal and written consents were obtained from the parents/guardians for participation of their children/wards in the study.

Table 3.1: Nucleotide sequences of the primers used in this study, at the ITS1-2, 18S ribosomal RNA, and 28S ribosomal RNA sites (Kim *et al.*, 2011).

Primer name	Nucleotide sequences
Der ITS1-2 Fw	ATCATTAACGCGCAGGC
Der ITS1-2 Rv	5’ 3’
	TGGCCACTGCTTTTCGG
Der 18S Fw	AAGTTGGGTCAAACTCGGT
Der 18S Rv	5’ 3’
	TGATCCTTCCGCAGGTT
Der 28S Fw	ACAGGGATTGCCCCAGTA
Der 28S Rv	5’ 3’
	CTTGTTCGCTATCGGTCTC

CHAPTER FOUR

4.0 RESULTS

4.1 Number of Samples collected from each *tsangaya*

A total of 153 samples were collected from the *almajirai* that presented with lesions on their scalps out of 408 *almajirai* selected for this study. Seventeen samples were collected from *Tsangayar* Mallam Sirajo located in layin NEPA, Makarfi; 32 samples from *Tsangayar* Mallam Kabiru located in Layin Pampo, Tudun Wada; 21 samples *Tsangayar* Mallam Umar located in Unguwan Rimi, Tudun Wada; 26 samples were collected from *Tsangayar* Mallam Gwani located in Unguwan Rimi Gazara; 09 samples were collected from *Tsangayar* Mallam Abubakar Sadiq located in Kadage, Gubuchi; 24 samples were collected from *Tsangayar* Mallam Abdullahi Me Dan Gemu located in Kari, Gubuchi; and 24 samples were collected from *Tsangayar* Mallam Haruna located in Wali, Gazara (Table 4.1).

Table 4.1: Samples collected from participants in each *tsangaya*

S/No.	*Tsangaya*	No. of samples collected
1	Mallam Sirajo located in layin NEPA, Makarfi	17
2	Mallam Kabiru located in Layin Pampo, Tudun Wada	32
3	Mallam Umar located in Unguwan Rimi, Tudun Wada	21
4	Mallam Gwani located in Unguwan Rimi, Danguzuri	26
5	Mallam Abubakar Sadiq located in Kadage, Gubuchi	09
6	Mallam Abdullahi Mai Dan Gemu located in Kari, Gubuchi	24
7	Mallam Haruna located in Wali, Gazara	24
8	Total	153

-

4.2 Frequency of Dermatophyte Species Isolated

Out of the 408 *almajirai* examined during the study, 153 (37.5%) were found to be infected with different species of dermatophytes out of which 119 samples (78.1%) were culture positive and no growth was observed in 34 samples (21.9%). Out of the positive dermatophytes cultures, 59.0% belong to *Trichophyton* group while 19.1% belong to the group *Microsporium* but no member of the *Epidermophyton* group was isolated in samples collected. Fifteen species of dermatophytes were isolated with the following frequencies- *Trichophyton rubrum* was the commonest isolates 45(29.4%), other isolates were *Trichophyton mentagrophytes* 16(10.5%), *Trichophyton violaceum* 3(2.0%), *Trichophyton soudanense* 2(1.3%), *Trichophyton tonsurans* 17(11.1%*)* *Trichophyton concentricum* 4(2.6%), *Trichophyton quickeanum* 1(0.7%), *Trichophyton megninii* 1(0.7%), *Trichophyton verrucosum* 1(0.7%), *Microsporum canis* 5(3.3%), *Microsporum fulvum* 7(4.6%), *Microsporum gallinae* 10(6.5%), *Microsporum audouinii* 1(0.7%), *Microsporum equinum* 5(3.3%), and *Microsporum nanum* 1(0.7%) (Figure II).

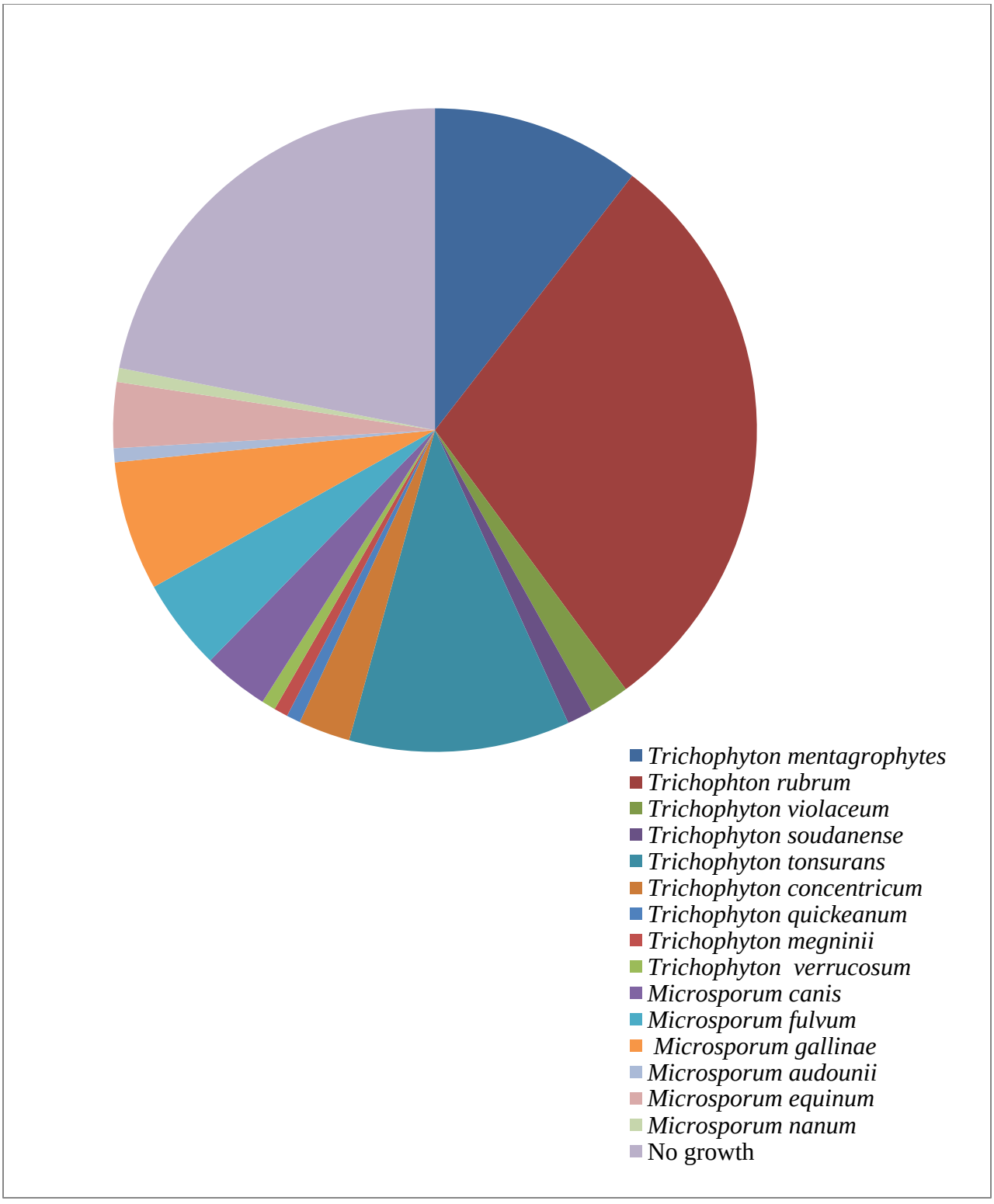


Figure II: Dermatophytes species isolated

Plate I: Showing scalp lesions on a participant

Plate II: Showing (arrow) scalp lesions on a participant

4.3 MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS

Dermatophyte species identification was done based on cultural characteristics, growth rate, texture, colony size and pigmentation produced on obverse and reverse sides of SDA plates as well as microscopic features.

4.3.1 *Trichophyton rubrum*

Trichophyton rubrum colonies showed slow growth on Sabouraud's Dextrose Agar (SDA) plates. They were flat to slightly raised centre, white, suede-like with a deep red colour on the reverse. It is well septate and has pencil shaped hyphae with numerous spherical microconidia and macroconida (Plate III).

4.3.2 *Trichophyton mentagrophytes*

The growth *T. mentagrophytes* on SDA was rapid and appearance was powdery and creamy on the obverse while the reverse pigmentation showed brown coloration. On microscopic examination well septate spiral hyphae with numerous spherical microconida were visible (Plate IV).

4. 3.3 *Trichophyton tonsurans*

Growth of *Trichophyton tonsurans* was slow and colonies were suede – like and powdery. Obverse morphology was white and brown while reverse was reddish brown. Microconidia were present with different shapes and sizes ranging from pyriform to balloon-shaped (Plate V).

4. 3.4 *Microsporum gallinae*

Growth rate is moderately rapid. Colonies are moderately wrinkled and with velvety to wooly or cottony texture. The surface colony color is white to gray turning pink as it matures and the reverse is deep red pigment. Macroconidia are club – shaped, commonly curved or narrow at the tip (Plate VI).

4. 3.5 *Microsporum canis*

Microsporum canis colonies appeared were flat and spreading. Obverse texture was cream-coloured with dense cottony surface showing some radial grooves. Reverse colonies showed golden yellow and brownish yellow pigment (Plate VII).

4. 3.6 *Trichophyton violecium*

Growth was very slow and texture of colonies was glabrous. Both surface and reverse colony appeared violet. Hyphae had an irregular diameter while microconidia and macroconidia were absent (Plate VIII).

4. 3.7 *Microsporum fulvum*

Colonies are flat and suede-like in colour on the obverse and fast growing. Reverse pigmentation was deep red. Macroconidia resemble those of *M. gypseum* but are longer and more clavate and septate. Microconidia are pyriform (Plate IX).

4. 3.8 *Microsporum equinum*

Growth was relatively rapid and colonies had a downy texture. Obverse colony colour was cream colour while the reverse colour was reddish brown. Microconidia were pyriform – shaped and formed along the hyphae (Plate X).

4. 3.9 *Trichophyton soudanense*

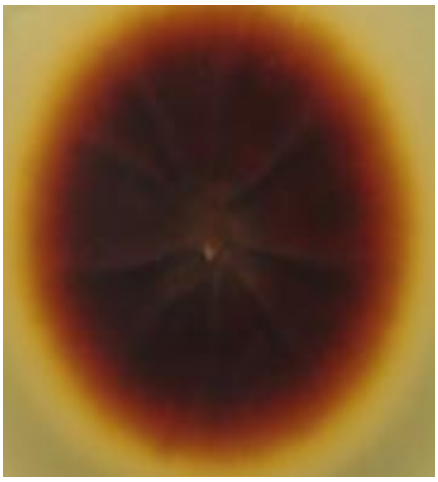
Growth rate was moderately rapid and texture of colonies was glabrous with filamentous fridge surrounding the colony. Both surface and reverse colony color were rusty brown (Plate XI).

4. 3.10 *Trichophyton concentricum*

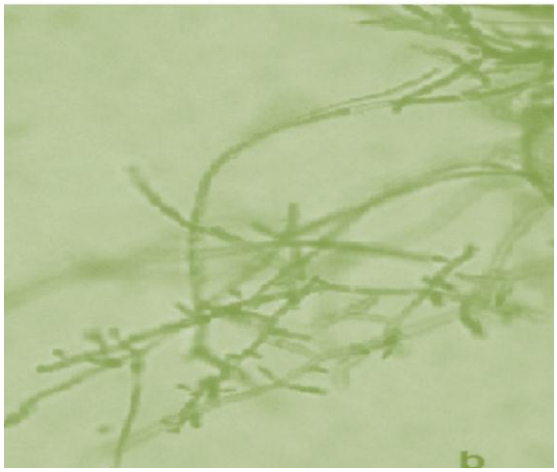
It showed slow growth rate and the colonies are glabrous colony which are highly folded. The obverse colony pigmentation was brown while the reverse was brown. Hyphae were were septate. Both macroconidia and microconidia were absent (Plate XII).

4. 3.11 *Trichophyton verrucosum*

Growth rate is very slow with colonies that are glabrous. Surface colony color is white or yellow while reverse is without any characteristic pigment. Macronidia are rare (Plate XIII).

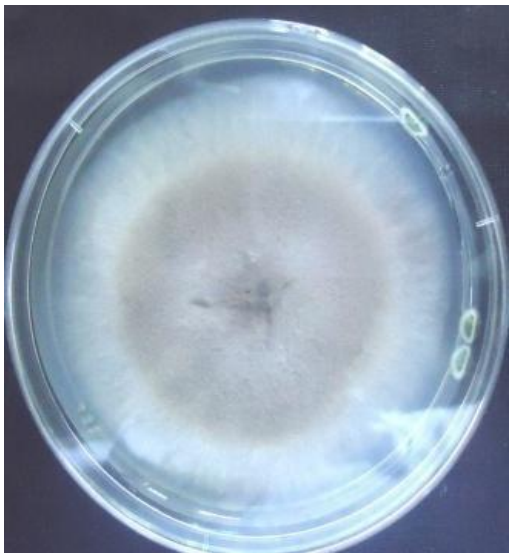
a   **b**

a. Sabouraud's dextrose agar culture plate of *Trichophyton rubrum* (Obverse view)
b. Sabouraud's dextrose agar culture plate of *Trichophyton rubrum* (Reverse view)

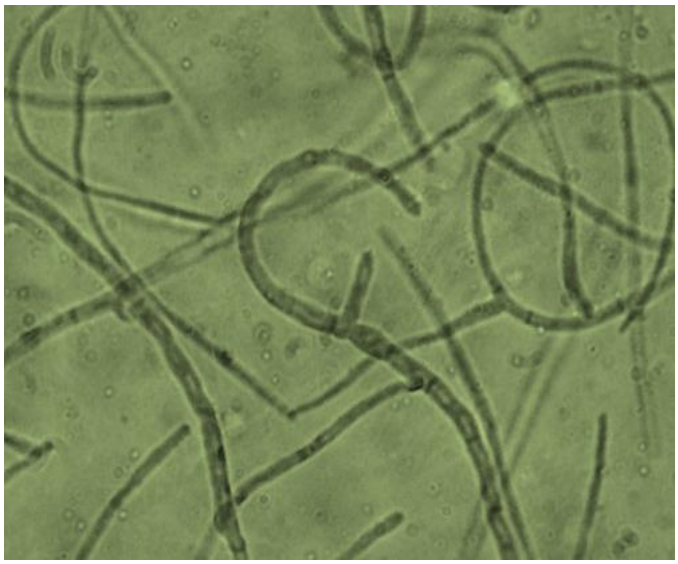
c 

c. Lactophenol cotton blue stain of *Trichophyton rubrum*

Plate III: ***Trichophyton rubrum***

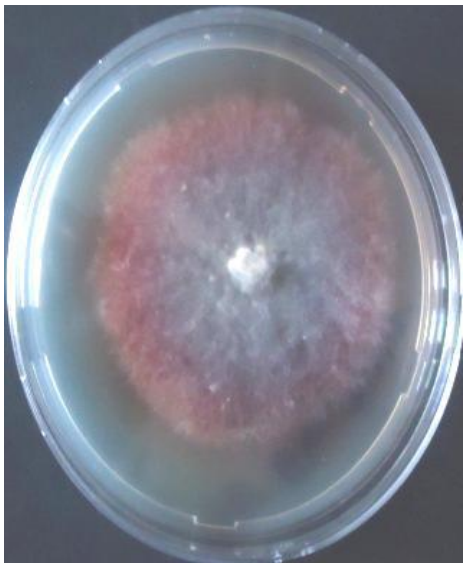
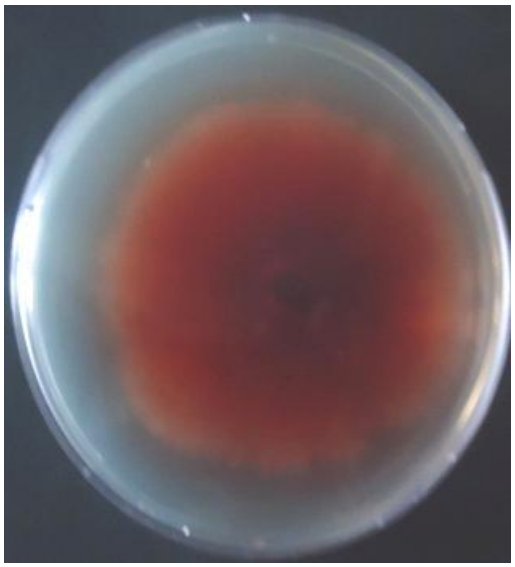
a  **b** 

a. Sabouraud's dextrose agar culture plate of *Trichophyton mentagrophytes* (Obverse view)
b. Sabouraud's dextrose agar culture plate of *Trichophyton mentagrophytes* (Reverse view)

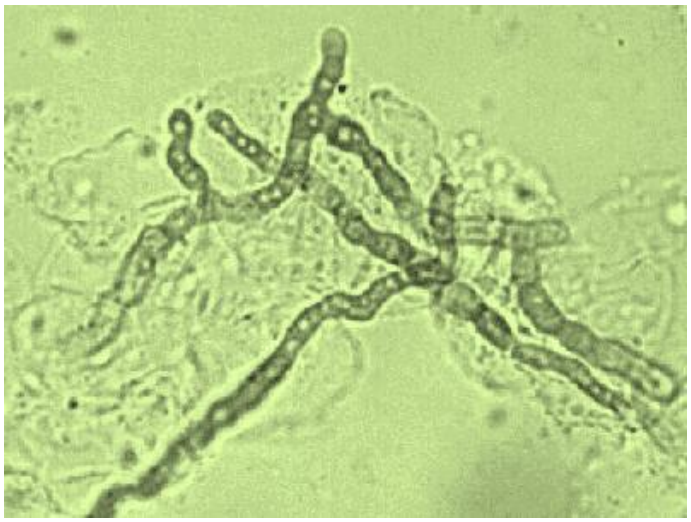
c 

c. Lactophenol cotton blue stain of *Trichophyton mentagrophytes*

Plate IV: *Trichophyton mentagrophytes*

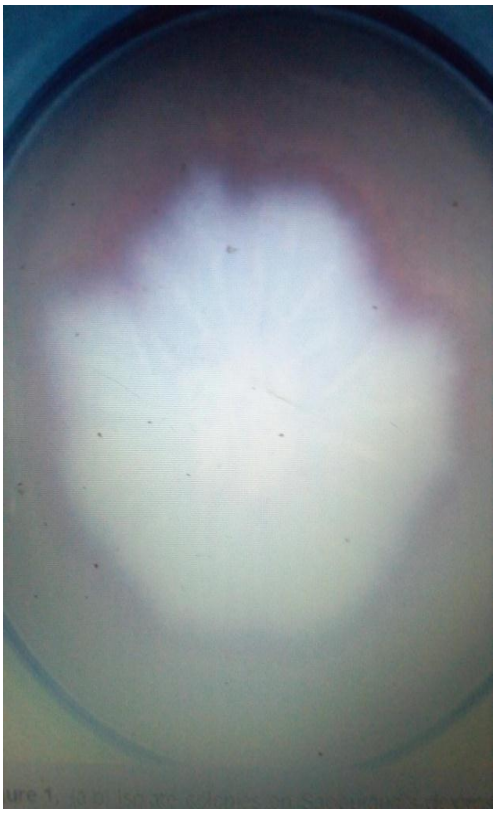
a   **b**

a. Sabouraud's dextrose agar culture plate of *Trichophyton tonsurans* (Obverse view)
b. Sabouraud's dextrose agar culture plate of *Trichophyton tonsurans* (Reverse view)

c 

c. Lactophenol cotton blue stain of *Trichophyton tonsurans*

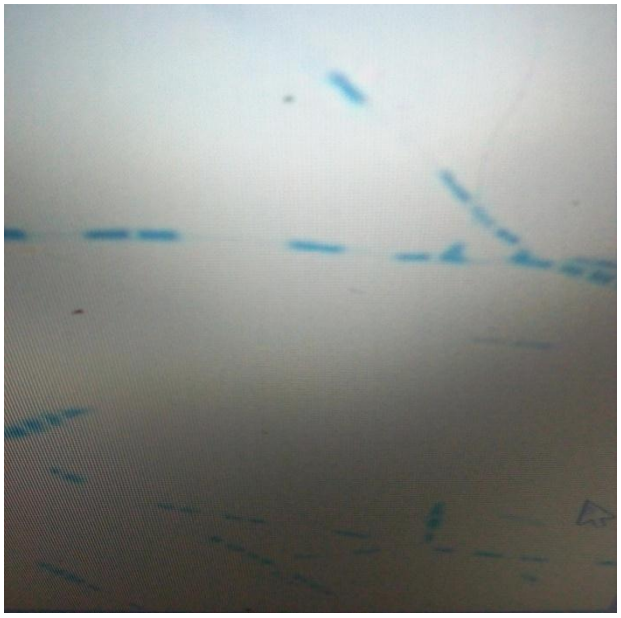
Plate V: *Trichophyton tonsurans*



a

b

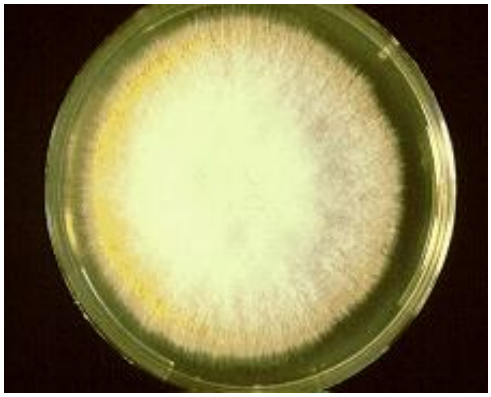
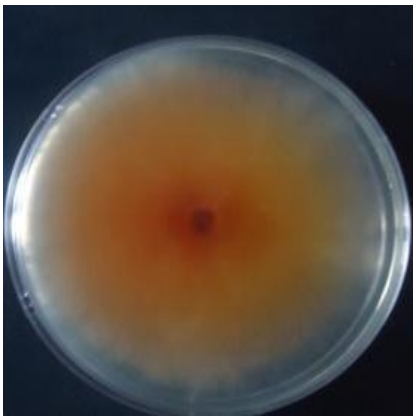
a. Sabouraud's dextrose agar culture plate of *Microsporum gallinae* (Obverse view)
b. Sabouraud's dextrose agar culture plate of *Microsporum gallinae* (Reverse view)



c

c. Lactophenol cotton blue stain of *Microsporum gallinae*

Plate VI: ***Microsporum gallinae***

a   **b**

a. Sabouraud's dextrose agar culture plate of *Microsporum canis* (Obverse view)
b. Sabouraud's dextrose agar culture plate of *Microsporum canis* (Reverse view)

c 

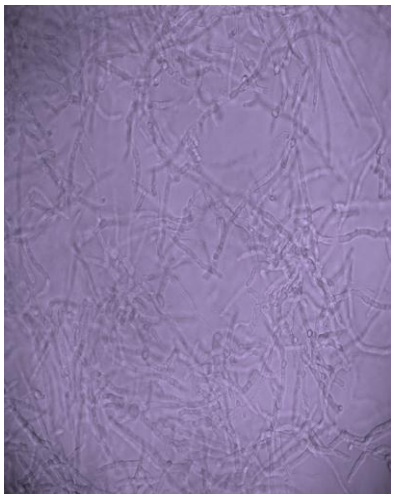
c. Lactophenol cotton blue stain of *Microsporum canis*

Plate VII: *Microsporum canis*

a 

 b

a. Sabouraud's dextrose agar culture plate of *Trichophyton violecium* (Obverse view)
b. Sabouraud's dextrose agar culture plate of *Trichophyton violecium* (Reverse view)

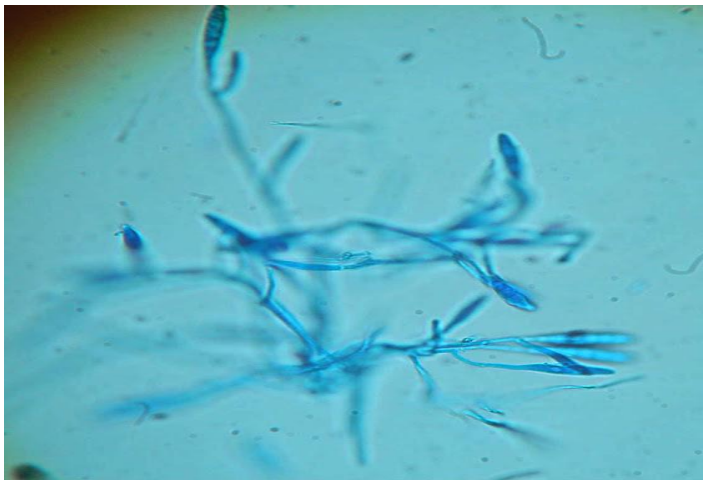
c 

c. Lactophenol cotton blue stain of *Trichophyton violecium*

Plate VIII: *Trichophyton violecium*

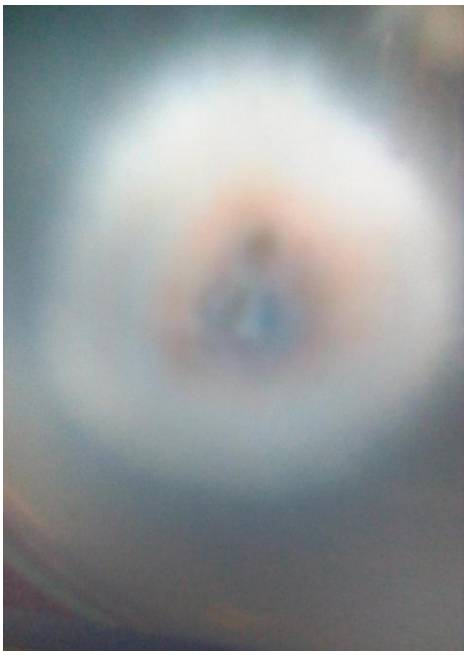
a  **b** 

a. Sabouraud's dextrose agar culture plate of *Microsporum fulvum* (Obverse view)
b. Sabouraud's dextrose agar culture plate of *Microsporum fulvum* (Reverse view)

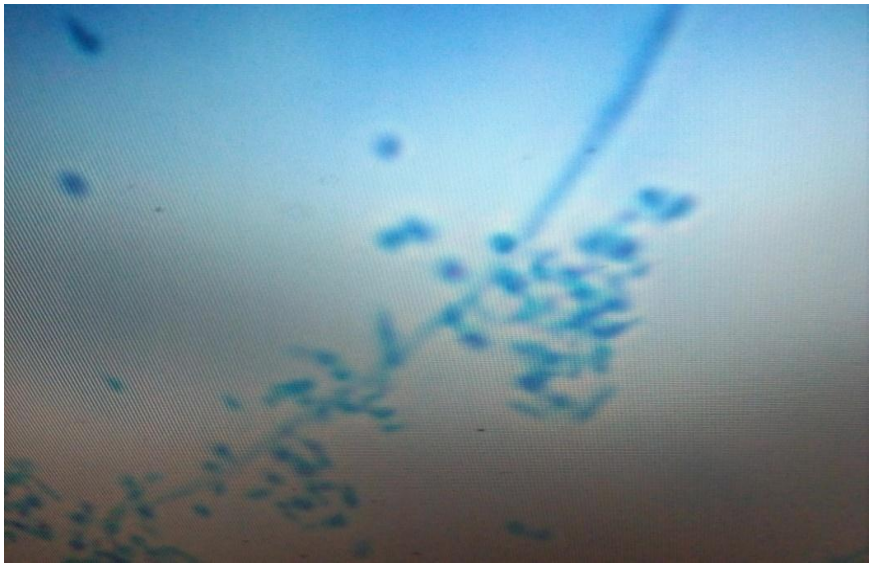
c 

c. Lactophenol cotton blue stain of *Microsporum fulvum*

Plate IX: *Microsporum fulvum*

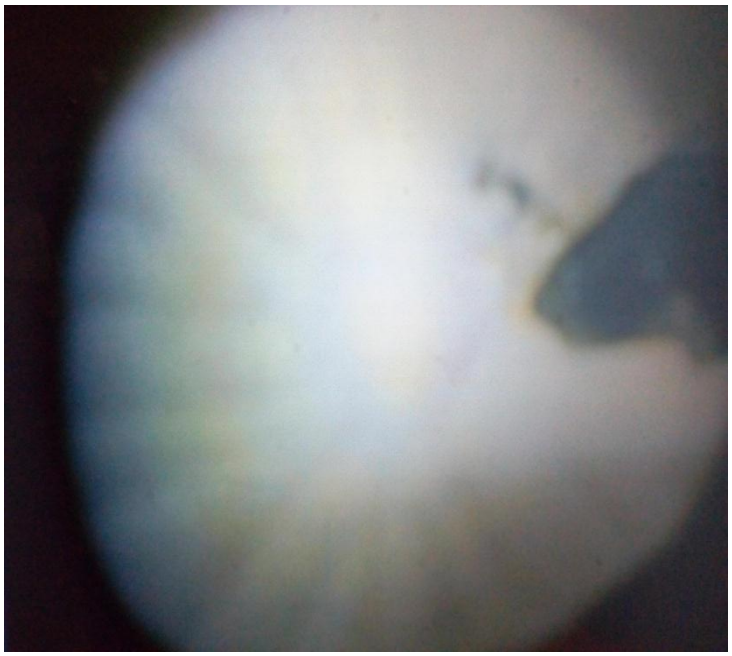
a  **b** 

a. Sabouraud's dextrose agar culture plate of *Microsporum equinum* (Obverse view)
b. Sabouraud's dextrose agar culture plate of *Microsporum equinum* (Reverse view)

c 

c. Lactophenol cotton blue stain of *Microsporum equinum*

Plate X: *Microsporum equinum*



.

a. Sabouraud's dextrose agar culture plate of *Trichophyton soudanense* (Obverse view)
b. Sabouraud's dextrose agar culture plate of *Trichophyton soudanense* (Reverse view)

Plate XI: *Trichophyton soudanense*

a  **b** 

a. Sabouraud's dextrose agar culture plate of *Trichophyton concentricum* (Obverse view)
b. Sabouraud's dextrose agar culture plate of *Trichophyton concentricum* (Reverse view)

c 

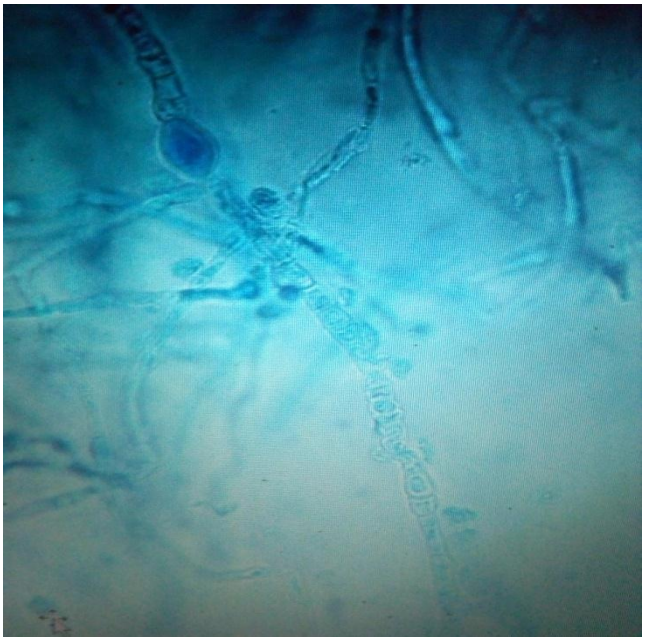
c. Lactophenol cotton blue stain of *Trichophyton concentricum*

Plate XII: *Trichophyton concentricum*

a 

b 

a. Sabouraud's dextrose agar culture plate of *Trichophyton verrucosum* (Obverse view)
b. Sabouraud's dextrose agar culture plate of *Trichophyton verrucosum* (Reverse view)

c 

c. Lactophenol cotton blue stain of *Trichophyton verrucosum*

Plate XIII: *Trichophyton verrucosum*

4.4.1 Distribution of Dermatophytes Based on the Age Groups of *Almajirai.*

The distribution of dermatophytes infection of *almajirai* in Makarfi L.G.A. based on their age shows that the highest infection occurred in the *almajirai* that are between the 5 and 7years (54.67%), followed by 8 - 10 years (51.13%), then 11-13 years (25%) respectively. Those that are of the age 14 - 16 years had an infection rate of 21.82% while the least infection occurred in those between 17- 19years (17.50%). The overall prevalenceof infection was 37.50%. There is significant difference in infection of dermatophytes among the different age groups of *almajirai* (χ^2 =41.292, P< 0.00001, df=4) (Figure II).

4.4.2 Distribution of Dermatophytes based on the Occupation of Parents/Guardians.

Dermatophytes infection based on occupation of parents or guardians shows highest infection in *almajirai* who are children of farmers (59.83%), traders (49.12%), casual workers (14.58%), unemployed parents (14.29%), permanent salaried (13.89%) while those whose parents' occpations constituted other occupations apart from the ones mentioned had an infection rate of 18.42% (χ^2 =, P< , df=) (Figure III).

4.4.3 Distribution of Dermatophytes Based on Number of Rooms in the House.

The percentage of infection based on the number of rooms in the house shows that those in houses with one to two rooms had the highest prevalence of 31.25%, followed by five rooms and above (29.29%) while those that live in houses with three to four rooms had the least infection of 28.57% (χ^2 =0.0547, P=0.973 , df=2). The result is *not* significant at $p < 0.05$ (Table 4.2).

4. 4.4 Distribution of Dermatophytes Based on their Nature of Residence

Figure III shows percentage of dermatophytes infection based on nature oof residence. The highest infection occurred in *almajirai* that do not reside with their parents or guardians (30.29%) while those that reside with their parents or guardians had an infection rate of 29.17% (χ^2 =2.7373, P<0.09803, df=1). The result is *not* significant at $p < 0.05$.

4. 4.5 Distribution of Dermatophytes Based on Type of Living Quarters

The percentage of dermatophytes infection based on type of living quarters shows that those living in buildings with cemented walls and floors had the least infection of 22.64%, followed by those that live in buildings with mud walls with earthen floors (26.67%) while the highest infection occurred in those that live in those that live in buildings with cemented floors and earthen walls (33.93%). The result is *not* significant at $p < 0.05$ (χ^2 =3.5012, P=0.1737, df=3) (Table 4.2).

4. 4.6 Distribution of Dermatophytes Based on Number of adults in the house.

The rate of infection based on the number of adults living in the house shows that those living in houses with five adults or more had the highest infection rate of 29.83%, followed by those in houses with one to two adults (28.57%) while those in houses with three to four adults had the least infection rate of 28.44%(χ^2 =0.0777, P=0.9619, df=2). The result is *not* significant at $p < 0.05$ (Table 4.2).

4. 4.7 Distribution of Dermatophytes Based on Number of *Almajirai* in the House.

The result of infection rate based on the number of *almajirai* living in the house shows that houses with one to two *almajirai* had the least infection rate of 25% while those with three to four *almajirai* had the highest rate of 30.11% and those with five *almajirai* and above had the infection rate of 28.99%. The result is *not* significant at $p < 0.05$ (χ^2 =0.1117, P=0.9457, df=2) (Table 4.2).

4.4.8 Sharing of Beddings

Those that share beddings had the highest infection of 31.17% and those that do not share beddings had an infection rate of 31.12% (χ^2 =0.0001, P=0.9931, df=1). The result is not statistically significant at $p < 0.05$. The rate of infection based on the number of *almajirai* that share bed or beddings show no infection recorded among those that do not share bed or beddings. Highest infection rate occurred among participants that share bed or beddings with two (2) other *almajirai* (31.90%), followed by those that share with only one *almajiri* (29.67%) and the least infection occurred in that that share with three or more *almaijirai* (25.00%)(χ^2 =5.8579, P=0.1187 , df=3). The result is *not* significant at $p < 0.05$ (Table 4.2).

4.4.9 Type of Bed

The distribution of infection based on the type of bed shows that those that sleep on mattresses had the highest infection of 34.43%, followed by those that sleep on iron/wooden beds (32.56%) and the least infection occurred in those that sleep on mats (27.63%)(χ^2 =1.4029, P= 0.4959, df=2). The result is *not* significant at $p < 0.05$ (Table 4.2).

4.4.10 Participants Bathing Frequency

The distribution of dermatophytes infection based on participants' bathing frequencyshows that highest infection rate was recorded among *almajirai* that had their baths occasionally (33.78%), followed by those that had their baths daily (31.78%) while the least infection occurred among those that had their baths weekly (23.79%)(χ^2 = 3.9896, P= 0.1360 , df=2). The result is *not* significant at $p < 0.05$ (Table 4.2).

4.4.11 Frequency of Soap Use in Bathing

Infection based on their use of soap in bathing showed that the highest rate of infection occured in those that do not use soap at all to have their baths (33.33%), followed by those that do not always use soap in bathing (29.70%) and the least occurrence was in those that always use soap to have their baths (28.95%)(χ^2 = 0.0463, P= 0.9771 , df=2). The result is *not* significant at $p < 0.05$ (Table 4.2).

4.4.12 Frequency of Shaving

The highest rate of infection occurred in those that shave their hair occassionally (33.12%), followed by those that shave their head weekly (30.89%) and the least infected are those that always shave their heads (30.53%)(χ^2 = 0.261, P= 0.8777 , df=2). The result is *not* statistically significant at $p < 0.05$ (Table 4.2).

4.4.13 Place of Shaving

Those that shave at home had the highest infection (31.37%) and those that shave at the barber's shop had the least infection (27.45%)(χ^2 = 0.3215, P= 0.5707, df=). The result is *not* significant at $p < 0.05$ (Table 4.2).

4.4.14 Relationship with Pets and Livestock

The participants that were in contact with pets and livestock had the highest infection rate of 29.35% while those that were not in contact with pets and livestock had an infection rate of 28.70%(χ^2 =0.0172, P=0.8957 , df=1). The result is *not* significant at $p < 0.05$ (Table 4.2).

4.4.15 Type of Pet or Livestock Kept

Those participants that kept cats in their residences had an infection rate of 25.58%. Those that kept sheep/goats had infection rate of 29.20%, poultry 34.37%, while those that do not keep any pets/livestock in their places of residence had 28.97% infection rate (χ^2 =0.6898, P=0.8756 , df=3). The result is *not* significant at $p < 0.05$ (Table 4.2).

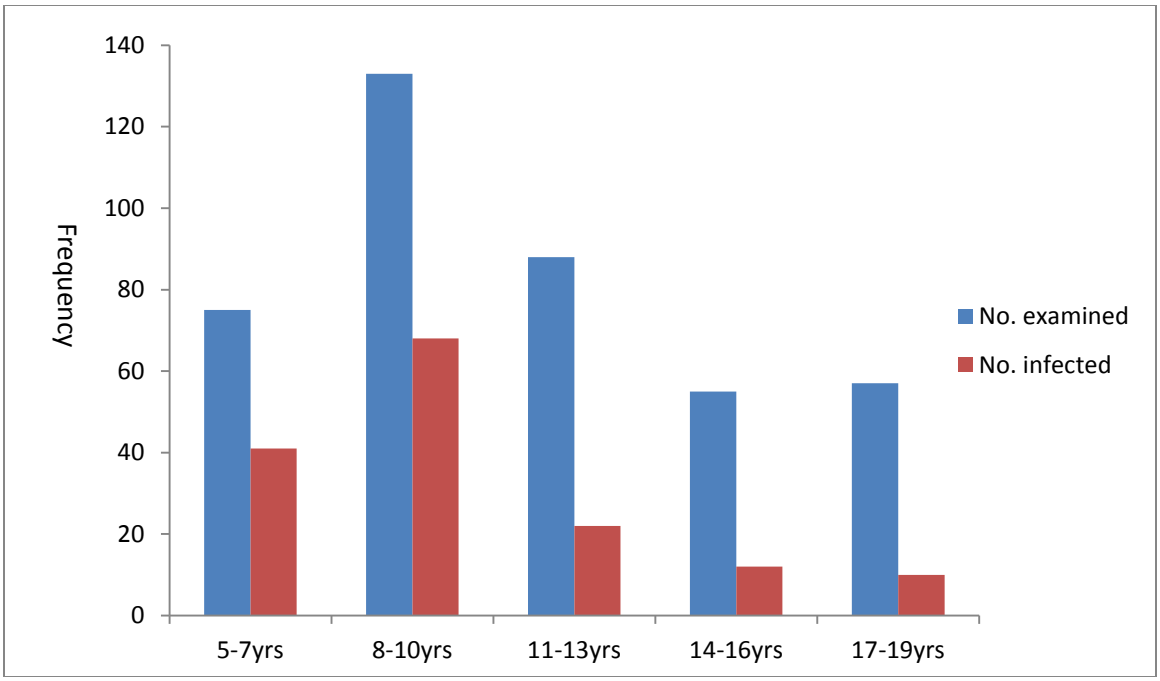


Figure III: Distribution of Dermatophytes based on the Age Groups of *Almajirai*

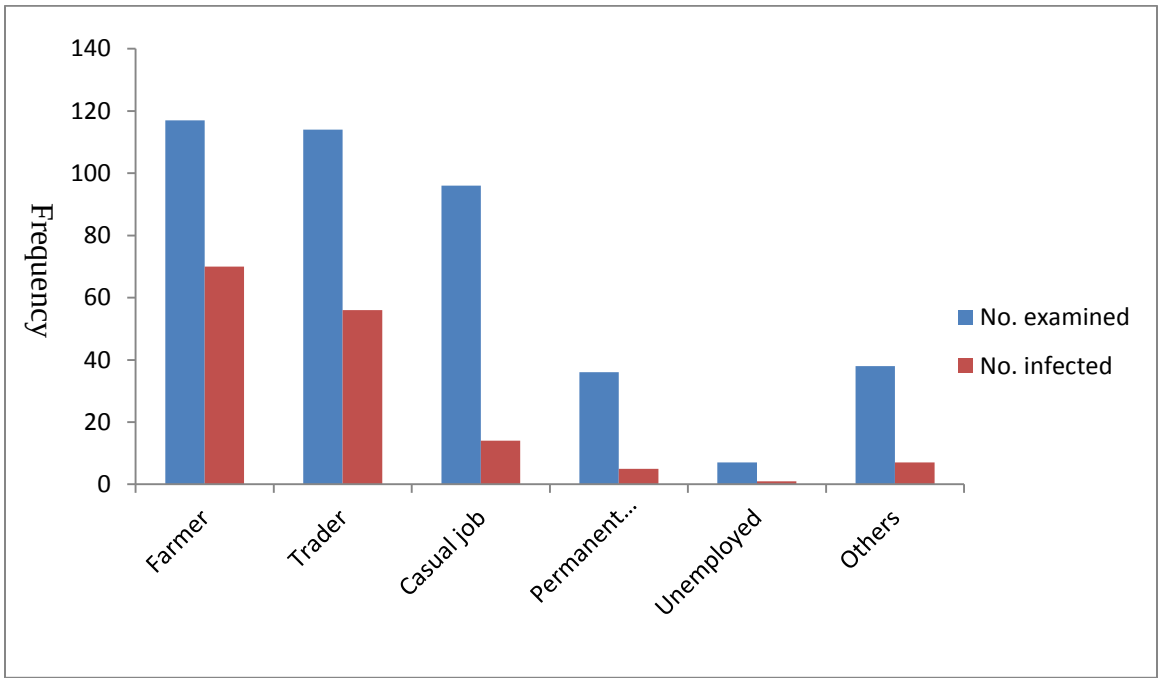


Figure Iv: Distribution of Dermatophytes based on Occupation of Parents/Guardians

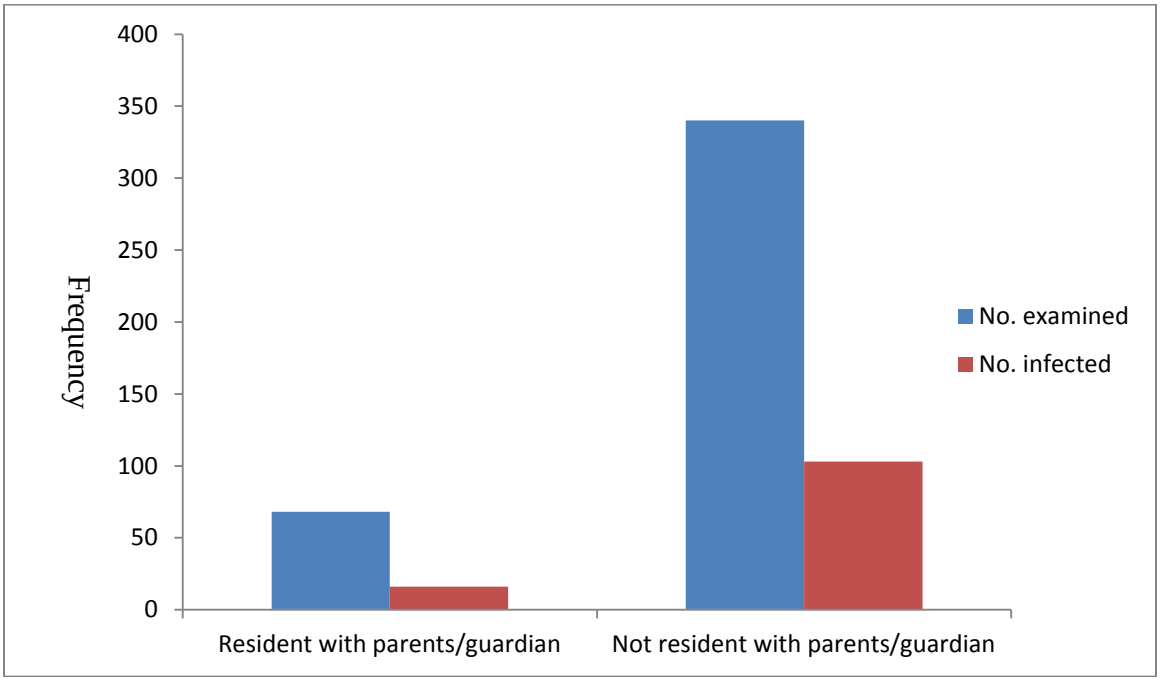


Figure V: Distribution of Dermatophytes based on their Nature of Residence

Table 4.2: Distribution of dermatophytes infection among *almajirai* in Makarfi L. G. A. in Respect to Risk factors

Variable	Number examined	Number (%) infected	χ^2	P-value

Type of living quarters	Cemented walls and floors	53	12 (22.64)	3.5012	0.1737
	Cemented floors and earthen walls	280	95 (33.93)		
	Mud walls with earthen floors	75	20 (26.67)		
Number of rooms in the house	1-2	16	5 (31.28)	0.0547	0.973
	3-4	112	32 (28.57)		
	5 and above	280	82 (29.29)		
Number of adults in the house	1-2	14	4 (28.57)	0.0777	0.9619
	3-4	109	31 (28.44)		
	5 and above	285	85 (29.83)		
	No	115	33 (28.70)		
Number of *almajirai* in the house	1-2	8	2 (25.00)	0.1117	0.9457
	3-4	93	24 (25.81)		
	5 and above	307	93 (30.29)		
Sharing of beddings	Sharing	77	24 (31.17)	0.0001	0.9931
	Not sharing	331	103 (31.12)		
Number sharing beddings	Non	10	0 (0)	5.8579	0.1187
	1	91	27 (29.67)		
	2	163	52 (31.90)		
	3 or more	144	36 (25.00)		
Type of bed	Iron/wooden	43	14 (32.56)	1.4029	0.4959
	Mattress	61	21 (34.43)		
	Mat	304	84 (29.17)		
Bathing frequency	Daily	107	34 (31.78)	3.9896	0.1360
	Weekly	227	54 (23.79)		
	Occasionally	74	25 (33.78)		
Use of soap in bathing	Always	304	88 (28.95)	0.0463	0.9771
	Not always	101	30 (29.70)		
	Not at all	3	1 (33.33)		
		408	119 (29.17)		
Frequency of Shaving	Always	131	40 (30.53)	0.261	0.8777
	Sometimes	154	51 (33.12)		
	Weekly	123	38 (30.89)		
Place of shaving	Home	357	112 (31.37)	0.3215	0.5707
	Barber's shop	51	14 (27.45)		
Contact with pets / livestock	Yes	293	86 (29.35)	0.0172	0.8957
	No	115	33 (28.70)		
Type of pet or livestock kept	Cat	43	11 (25.58)	0.6898	0.8756
	sheep/goats	226	66 (29.20)		
	Poultry	32	11 34.37)		
	None	107	31 (28.97)		

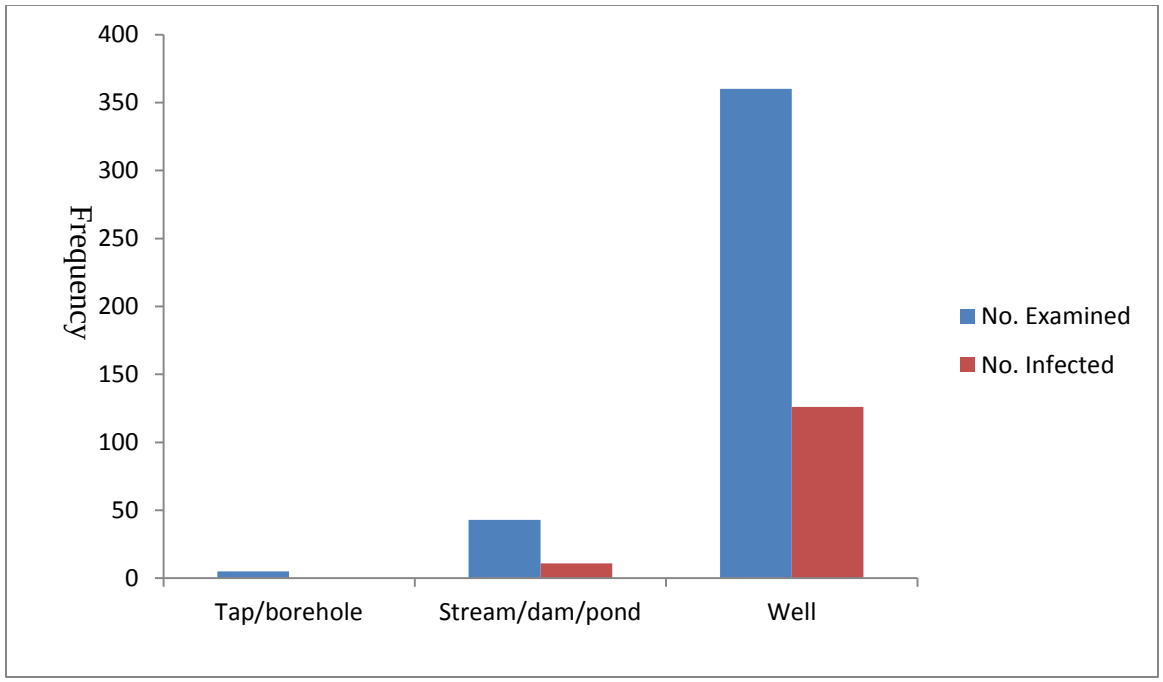


Figure VI: Distribution of Dermatophytes based on their source ot water for Domestic use

4.5 Distribution of Dermatophyte Species based on the Ages of *Almajirai*

The species identified based on the age of the *almajirai*. *Trichophyton mentagrophytes* was present among all the age groups. The age group 5-7years accounted for 2(2.67%) infection rate, 8-10 years had 6(4.51%), 11-13 years had 3(3.41%) while 14-16 years had 1(1.75%) and 17-19 years had 1(1.75%). The age group 5-7years accounted for 6(8.00%) infection rate, 8-10 years had 12(9.02%), 11-13 years had 318(20.45%) while 14-16 years had 9(16.36%). *Trichophyton soudanense, Trichophyton quickeanum, Trichophyton megninii, Trichophyton verrucosum* were found only among *almajirai* between 8-10 years which accounted for 2(1.50%), 1(0.75%), 1(0.75%), 1(0.75%), infection rates respectively. *Trichophyton tonsurans* was not detected in age groups 14-16 years and 17-19 years but present in 5-7 years 1(1.33%), 8-10 years 8(6.02%) and 11-13 years 8(9.09%). *Trichophyton violaceum* was present in age groups 5-7 years accounting for 1(1.33%), 11-13 years 1(1.14), 14-16 years1(1.82%), while none observed in 8-10 years and 17-19 years age groups. *Trichophyton concentricum* was not detected in *almajirai* of 5-7 years but detected in 8-10 years 1(0.75%), 11-13years 1(1.14%), 14-16years 1(1.82%), and 17-19years 1(1.75%). *Microsporum nanum* and *Microsporum audounii* were absent in all age groups except 5-7 years and 11-13 years with infection rate of 1(1.33%) and 1(1.14%) respectively. *Microsporum fulvum* was found in 8-10 years 2(1.50%) and 11-13 years 5(5.68%) but absent the other age groups. *Microsporum equinum* was detected in the age groups 5-7 years 1(1.33%), 8-10 years 2(1.50%), and 11-13 years 2(2.27%) but absent in 14-16 years and 17-19 years. *Microsporum canis* was not detected among *almajirai* of ages between eleven to thirteen years but found among the other age groups 5-7 years 2(2.67%), 8-10 years 1(0.75%), 14-16 years 1(1.82%) and 17-19 years 1(1.75%). *Microsporum gallinae* was present in 8-10 years 1(0.75%), 11-13 years 4(4.55%), 14-16 years 4(7.27%), 17-19 years 1(1.75%) and absent in 5-7 years age group (Table 4.3).

Table 4.3: Distribution of dermatophyte species based on the age of participants

Dermatophyte species	Age (Years)					
	5-7	8-10	11-13	14-16	17-19	Total
	No. infected (%)	No. infected (%)	No. infected (%)	No. infected (%)	No. infected (%)	
Trichophyton mentagrophytes	2(2.67)	6(4.51)	3(3.41)	4(7.27)	1(1.75)	16
Trichophton rubrum	6(8.00)	12(9.02)	18(20.45)	9(16.36)	-	45
Trichophyton violaceum	1(1.33)	-	1(1.14)	1(1.82)	-	03
Trichophyton soudanense	-	2(1.50)	-	-	-	02
Trichophyton tonsurans	1(1.33)	8(6.02)	8(9.09)	-	-	17
Trichopuyton concentricum	-	1(0.75)	1(1.14)	1(1.82)	1(1.75)	04
Trichophyton quickeanum	0(0.00)	1(0.75)	-	-	-	01
Trichophyton megninii	-	1(0.75)	-	-	-	01
Microsporum canis	2(2.67)	1(0.75)	-	1(1.82)	1(1.75)	05
Microsporum fulvum	-	2(1.50)	5(5.68)	-	-	07
Microsporum gallinae	-	1(0.75)	4(4.55)	4(7.27)	1(1.75)	10
Microsporum audounii	-	-	1(1.14)	-	-	01
Microsporum equinum	1(1.33)	2(1.50)	2(2.27)	-	-	05
Trichophyton verrucosum	-	1(0.75)	-	-	-	01

Microsporum nanum	1(1.33)	-	-	-	-	01
TOTAL	14(18.67)	38(28.57)	43(48.86)	20(36.36)	4(7.02)	119

4.6 MOLECULAR IDENTIFICATION OF DERMATOPHYTE SPECIES

4.6.1 Identification of Dermatophyte Species using PCR

Results of PCR analysis from dermatophyte specific primer (ITS 1 and 2), the 18S ribosomal RNA primer set and 28S ribosomal RNA primer set are shown in Plates I, II, and III. Bands were obtained for 5 strains – *Microsporum canis, Microsporum audouinii, Trichophyton rubrum, Trichophyton tonsurans and Trichophyton mentagrophytes.*which showed similar band sites on PCR using a dermatophyte specific primer (ITS1-2). An approximately 500 base pairs band on ITS1-2 was observed in *M. canis, T. rubrum, and T. tonsurans* while band patterns of 560 base pairs band on ITS1-2 were observed in *M. audoinii* and *T. mentagrophytes. M. canis, M. audouinii, T. violaceum, T. rubrum, T. mentagrophytes and T. verrucosum* were visible around 200 baase pair long band with the 18S ribosomal RNA primer PCR. A 300 base pair long band was identified with the 28S ribosomal RNA primer PCR on *M. canis, T. violaceum, T. mentagrophytes T. tonsurans, T. verrucosum and M. gypseum.* An approximately 300 base pair-long was observed in *M. audouinii, T. rubrum and M. fulvum* on the 18S ribosomal RNA primer.

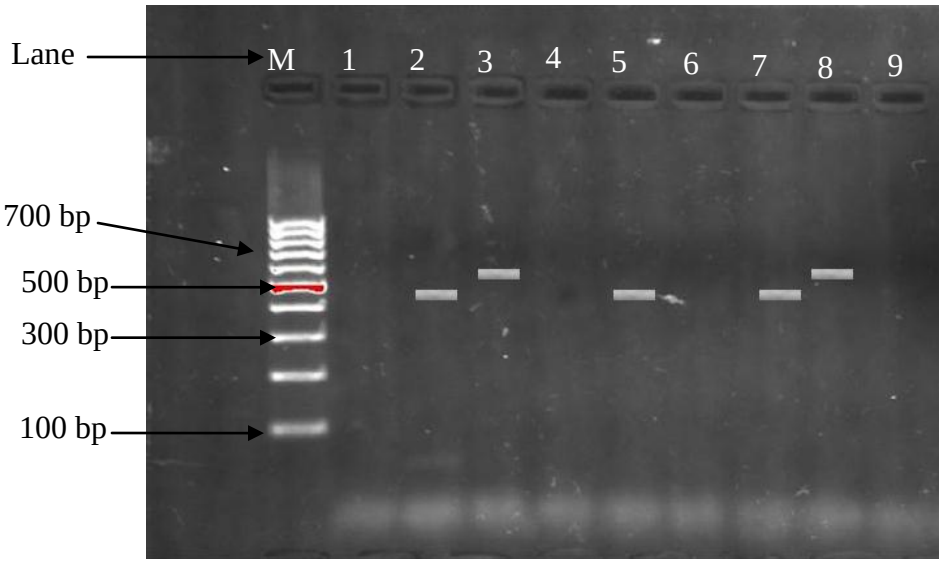


Plate XIV: PCR amplification from dermatophyte strains with the ITS1-2 region. Lane M, 100 bp DNA ladder; Lane 1, negative; Lane 2, *Microsporum canis*; Lane 3, *Microsporum audouinii;* Lane 4, Negative; Lane 5, *Trichophyton rubrum;* Lane 6, negative; Lane 7, *Trichophyton tonsurans;* Lane 8, *Trichophyton mentagrophytes*; Lane 9, negative control.

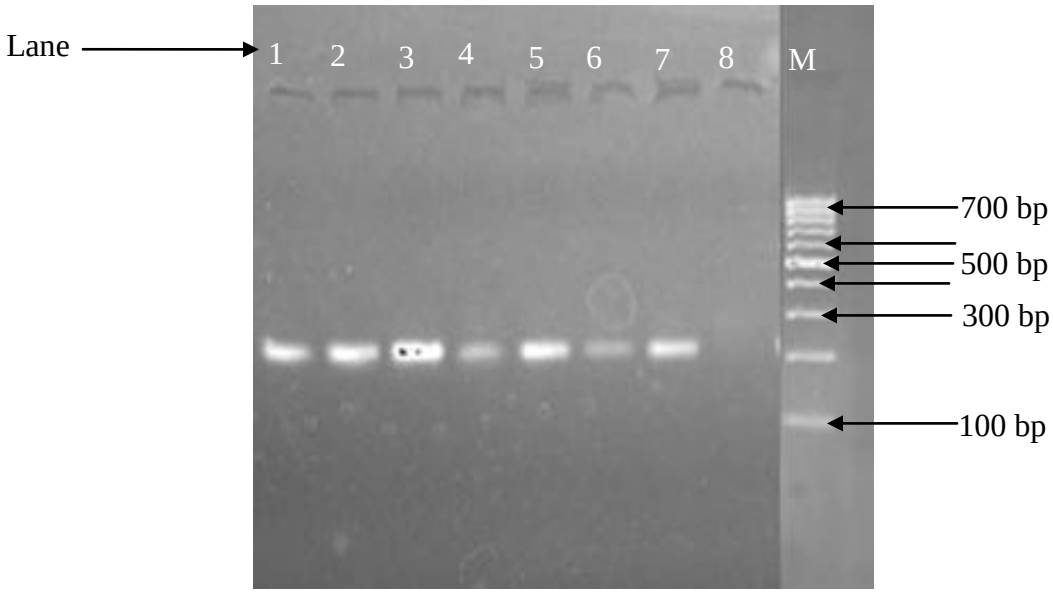


Plate XVI: PCR amplification from dermatophyte strains with the 18S ribosomal RNA region. Lane M, 100 bp DNA ladder; Lane 1, *Microsporum canis*; Lane 2, *Microsporum audouinii*; Lane 3, *Trichophyton violaceum*; Lane 4, *Trichophyton rubrum*; Lane 5, *Trichophyton mentagrophytes*; Lane 6, *Trichophyton tonsurans*; Lane 7, *Trichophyton verrucosum*; Lane 8, negative control.

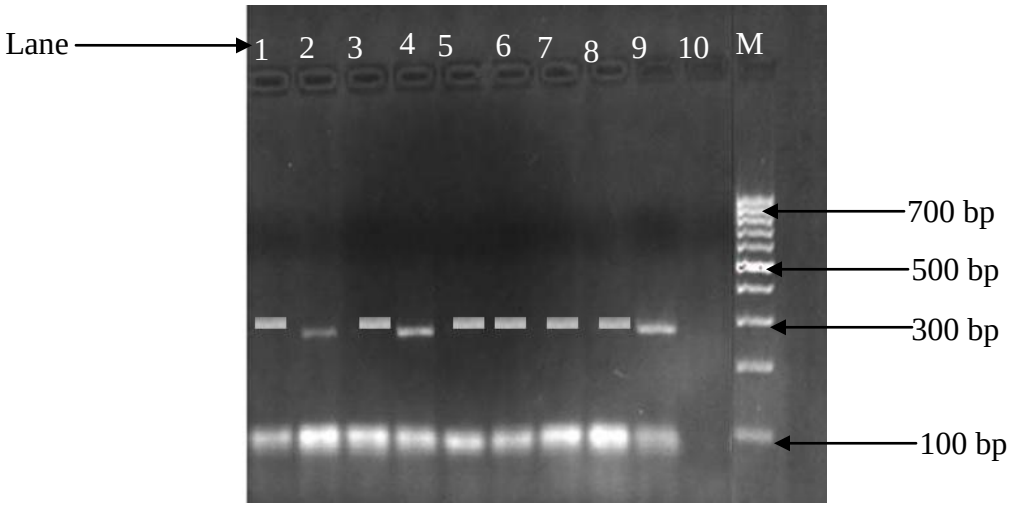


Plate XV: PCR amplification from dermatophyte strains with the 28S ribosomal RNA region. Lane M, 100 bp DNA ladder; Lane 1, *Microsporum canis*; Lane 2, *Microsporum audouinii*; Lane 3, *Trichophyton violaceum*; Lane 4, *Trichophyton rubrum*; Lane 5, *Trichophyton mentagrophytes*; Lane 6, *Trichophyton tonsurans*; Lane 7, *Trichophyton verrucosum*; Lane 8, *Microsporum gypseum;* Lane 9, *Microsporum fulvum* and Lane 10, negative control.

CHAPTER FIVE

5.0 DISCUSSION

In epidemiological studies, fungal infections of the skin and scalp represent a relatively common problem especially in the tropical and subtropical regions of the world where warm and humid climate provides a favourable environment for fungi. Fungi are everywhere and no geographical area or group of people is spared by these organisms. The infections caused by pathogenic fungi, dermatophytes, thus become a significant health problem affecting children, adolescents and adults (Chepchirchir *et al*., 2009). *Tinea capitis* has been documented as a major health challenge in primary school children in Africa and all over the world, and this should be taken into consideration by health authorities concerned when planning for school health (Ayanlowo *et al*., 2014). They are transmitted from person to person directly by means of contact or via fomites contaminated with infected skin scales or hairs. They can also be acquired by humans from infected animals and by direct exposure to infected soils (Cohen and Powdery, 2004). These diseases remain endemic in Nigeria, largely because of lack of information on its prevalence and absence of control measures (Anosike *et al*., 2005). However, accurate assessment of the prevalence of etiologic agents is useful to estimate the size of the problem and to prevent transmission.

5.1 Prevalence of Dermatophytes Infection

Dermatophytes infections are common and remain an important public health problem in Nigeria and other parts of the world. This is can be seen in this study where prevalence of 37.5% was recorded among 408 *almajirai* randomly sampled within Makarfi Local Government Area of Kaduna State. This is similar to the 35% observed in Superficial Fungal Infections among school

children in Ile-Ife, South-Western Nigeria (Olaide *et al.,* 2014); 21% observed in a study in Ebonyi State, South-Eastern Nigeria (Anosike *et al*., 2005); 31.1% in Libya (Ellabib and Khalifa, 2001); 31.6% (Chukwu *et al.,* 2011); 19.52% (Sanuth and Efuntoye, 2010). This figure is higher than 4.6% found in Ibadan, South- Western Nigeria (Ogunbiyi *et al.,* 2005); 4.7% in Abakaliki, South-Eastern Nigeria (Uneke *et al.,* 2006); 2.7% in Iraq (Fathi and Al-Samarai, 2000); 7.4% in Egypt (Fathi and Al-Samarai, 2000); 6.6% in Anyigba town (Enemuor and Amedu, 2009). But lower than 58.2% prevalence of dermatophytic infections among randomly sampled school children within Nassarawa Local Government Area of Kano State (Ndako *et al.,* 2012); 57.7% in Mowe, Ogun State, Nigeria (Ayorinde *et al*., 2013); 40.5% in Awka South Local Government Area of Anambra State (Ogbu *et al*., 2015). The high prevalence obtained in this study could be due to low standard of living, lack of health education, overcrowding, poor personal hygiene, close personal contact, contact with animals, lack of medical facilities, sharing of beds or beddings, and poor environmental sanitation. Akinboro *et al.* (2013) suggested that differences in the prevalence in different regions could be due to variation in climatic and environmental conditions of the areas being studied. Factors such as the host socioeconomic characteristics (age, gender, family size, individualistic and communal life style, over-crowding, geography, level of hygiene practice, nature of school infrastructure and amenities, locality, climate, affinity for contact sports, frequent contact with domesticated animals and nature of health care system that facilitate the transmission of dermatophytic infections (Nweze, 2010b). Further more, prevailing climate at the time of study, inadequate classroom seats which forced some of the children to learn sitting on mats or bare floor, and the children play pattern which promote person-to-person transmission and create epidemiological circumstances for re-infection of human hosts by dermatophytes may also largely account for the high prevalence recorded

(Ndako *et al*., 2012). *Trichophyton rubrum* 45(29.4%), *Trichophyton mentagrophytes* 16(10.5%) and *Trichophyton tonsurans* 17(11.1%) constituted the predominant representative dermatophytic species recorded during the study.

The result of this study shows that dermatophyte infection was most prevalent among *almajirai* between 5-7 years of age and the difference is statistically significant ($P > 0.05$). This concurs with the findings of Ajao and Akintunde (1985) who reported that the infection was more frequent among primary school children usually between 5 and 10 years and Ayorinde *et al.* (2013) reported dermatophytes infection to be more frequent among children 5-7 years of age. While Oloide *et al*. (2014) on the other reported 9-12 years to have the highest prevalence among school Children in Ile-Ife, South-Western Nigeria while Ogbu *et al*. (2015) reported 10-14 years of age to have more prevalence of superficial fungal infections among primary school pupils in Awka South Local Government Area of Anambra State. Adeleke *et al*. (2008) in their study of 2150 Qur'anic school children examined in Kano State reported that the age group mostly affected was 10-14 years old children. The difference in infection among the various age groups could be due to poor hygiene and the children play pattern which promote person-to-person transmission and create epidemiological circumstances for re-infection of human hosts by dermatophytes may also largely account for the high prevalence recorded in this study.

5.2 Isolation and Identification of Dermatophyte Species

The dermatophytes isolated belong to the genera *Trichophyton* and *Microsporum* but no member of the genus *Epidermophyton* was isolated in this study. *Trichophyton* species were implicated in 58.82% (90/153) cases while *Microsporum* species was detected only in 18.95% (29/153) cases. This agrees with the findings of Ayorinde *et al*. **(**2013) in Mowe, Ogun State, Nigeria; as well as findings of Chukwu *et. al.* (2011) and Dike-Ndudim *et al*. (2013). It also agrees with studies

carried out in other parts of Africa and Asia such as the ones carried out by Chepchirchir *et al.* (2009) in Kibera slums, Kenya; Ewaldo *et al.* (2010) in Dar es Salaam and Thakur (2013) who studied Tinea capitis in Botswana. In India, Bhatia and Sharma (2014) carried out Epidemiological studies on Dermatophytosis in human patients in Himachal Pradesh, India showed similar result. *Trichophyton rubrum* was isolated at the highest frequency (29.4%; n = 45), which is an anthropophilic species. It can be deduced that *T. rubrum* is the major aetiologic agent of dermatophytoses amongst *almajirai* in this area. This was also reported by Srinivasan *et al.* (2012); Oyeka and Eze (2008); Adefemi *et al.* (2011); and Enemuor and Amedu (2009). *Trichophyton tonsurans* was the second most occurring species to *T. rubrum*. Similar findings were previously reported among children in the Anambra state (Nweze and Okafor, 2005). *Microsporum audouinii* is mainly a human pathogen but occasionally it infects animals and has been observed to resolve as children approach puberty thus, in this study, it was only found among the age group 11-13 years while in the study carried out by it was observed among the age group 4–11 years. Lynn, 2011 in Bauchi State reported thhat *Microsporum audouinii, Trichophyton rubrum, T. soudanense* and *T. violaceum* are anthropophilic in nature affected pupils of all age groups. It was observed in this study that the more the number of adults and *almajirai* in a household, the higher the rate of infection. Households with five adults and above had the highest prevalence of 29.83% while in the case of *almajirai,* highest rate of infection was found in households that had 5 *almajirai* or more (30.29%). Therefore the larger the family size, the higher the incidence of dermatophytoses. This agrees with the studies carried by Fathi and Al-samarai, 2000 and Yazdanfar, 2010. The distribution of dermatophytes among *almajirai* showed that those who had contact with birds were more at risk of acquiring the infection as compared to those living in homes with other domestic animals. It is in contrast with the findings

of Ndako *et al.* (2012). It is a common practice for most homes to keep poultry and other domestic animals. However, the interview conducted with the questionnaire it was noted some *almajirai* have in their residences domestic animals such as cats, goats and sheep which could be the source of zoophilic organisms such as *T. mentagrophytes* and *T. verrucosum.* The occurrence of *T. megninii* and *T. verrucossum* in this study could be as a result of direct interaction with soil either on farm, in their *Tsangaya* where due to inadequate facilities compel them to learn sitting on mats or bare floor and their play pattern which promote person-to-person transmission and create epidemiological circumstances for re-infection of human hosts by dermatophytes as well as walking without shoes may also largely account for the escalated value of prevalence recorded in this study. Dike-Ndudim *et al*. (2013) reported that poor infrastructure such as houses and classrooms may be a contributing factor to the high prevalence of dermatophytes amongst school children in Isu Local Government Area of Imo State, since these four rural schools investigated lacked standard studying accommodation as well as recreational facilities because some children who sit on uncemented floors can contract the infection from the soil. Poor garbage disposal has been associated with the presence of zoophilic type of dermatophytes owing to the presence of rodents that is, rats. This is a common phenomenon in slum characterized by heaps of garbage around living houses. Rodents scavenging into living houses in search of food may be reservoirs for fungal pathogens. Such mammals harbor dermatophytes like *T. mentagrophytes* which was a significant isolate in this study. *T. mentagrophytes* was the third most prevalent species isolated. Most parents/guadians had low paying jobs regardless of whether they were on permanent or casual basis and this corroborated with the findings of Chepchirchir *et al.,* 2009. Most of the participants' living in structures with cemented floors and earthen walls had the highest rate of infection. However the type of living structure was not a significant factor for dermatophyte

infections among the *almajirai* in this study. Although not significant, high prevalence was recorded among those that shave at home that those that at the Barber's shop (P= 0.5707). This could aid the spread the infection since only one person cuts their hair although a razor blade is used per individual. At the same time, direct contact with barber's instrument has been implicated in the report of Aliero *et al.* (2013) to aid the spread of these mycotic infections. The occurrence of *Microsporum canis* which is zoophilic is probably as a result of interaction with animals since people in the area keep domestic animals and some of the *almajirai* take care of the animals for their *Mallams'* wives or for other people in the community and they being paid or given food in return. *T. concentricum* was scarcely reported in many related studies from other parts of Nigeria but was isolated in this study and it was also reported by Ndako *et al*. (2012). It was noted that most of the infected children do not have their hair cut regularly. This creates humid conditions that favour the growth of the dermatophytes spores. This finding is in agreement with the result of Ameh and Okolo (2004) that tinea capitis was more frequent among children between ages 5 and 10 years. Hygiene status of an individual's skin has been cited as one of the risk factors associated with the acquisition of dermatophyte infections. It was observed that there was a poor personal hygiene in which some of the *almajirai* had their baths on a weekly basis (55.64%), some occasionally (18.14%) and only 26.23% had their bath at least once daily and in some cases not with soap. On the contrary, Chepchirchir *et al.* (2009) reported that over 75% of the participants reported bathing at least once daily and high frequency of bathing reduces rate of colonisation of the skin by the fungal agents following contact with infective agents. Their clothes were hardly washed at frequent intervals and when they do, some do not use laundry soap. This can be an important point source of the infection among all age groups. Another way of spreading infection is sleeping arrangement in close proximity to one

another thus leading to a probability of coming in contact with infection during sleeping (Chepchirchir *et al.*, 2009; Lynn, 2011). In this study, it was noted that *almajirai* in the same room or sharing bed or bedding had similar type of infection which could be attributed to contagious nature of the dermatophytes associated with overcrowding and proximity. Most of the *almajirai* (83.33%) do not reside with their parents or guardians and there was higher infection prevalence (30.29%) compared to those that were resident with their parents or guardians (23.53%) although the difference was not significant at $p < .05$ (p=0.098031). As a result of this, they lack parental care. It was observed that there was higher rate of infection (29.35%) among those that had contact with pet or livestock compared to those that did not (28.70%) although the difference was not significant $p < .05$ (p=0.895669). The interactions of the sampled population with different types of domestic animals in this study revealed that those who had contact with poultry had the highest incidence of 34.37%, while the least prevalence was observed in those that had contact with cats (25.58%). This is in contrast with the findings of Ndako *et a*l. (2012) who recorded 35.5% incidence among school children that had contact with cats while Nweze, (2011) recorded 53.2%.

5.3 Identification of Dermatophyte Species using Polymerase Chain Reaction (PCR)

The traditional method of identifying dermophytes was carried out by examining the shape of colony and morphological features of hyphea and spores on microscopy. However, morphology of dermatophytes may vary according to culture conditions especially when subculture is performed for a long time and some features may be lost. The problems are solved using different molecular methods such as restriction fragment length polymorphism (RFLP), nested PCR, random amplification of polymorphic DNA (RAPD) and base-sequencing have been

reported (Yang *et al*., 2008). PCR method is highly sensitive specific (Lim *et al.,* 2008). Selection of primer and targeting of DNA to be amplified are important when using the PCR method. Three sets primers targetting ITS1-2, 18S ribosomal RNA and 28S ribosomal RNA. Analysis of PCR results on the ITS1-2 region appeared on base pairs between 400 and 600. On the 18S ribosomal RNA and 28S ribosomal RNA gave a more definitive identification dermatophyte species.

CHAPTER SIX

6.0 CONCLUSION

The study established a dermatophyte prevalence of 37.50% among *almajirai* in Makarfi Local Government Area. There is significant difference between age of the *almajirai* and the disease. The age bracket with highest prevalence was 5-7 years (54.67%). This may be attributed to poor living and sanitary conditions. The study did not establish any statistically significant association with risk factors. Fifteen species of dermatophytes were isolated in this study with frequencies varying between 29.4% for *T. rubrum* and 0.7% for *M. audouini.* Which was confirmed by the amplification of the

6.1 RECOMMENDATIONS

1. The *Mallams,* parents/guardians as well as the *almajirai* should be educated on maintaining adequate personal, community and environmental hygiene.
2. Contact with pets and livestock should be minimised.
3. There should be provision of good infrastructure and upgrading of the informal settlements by the government so as to improve the living conditions of the *almajirai*.
4. Overcrowding at home and in the *Tsangaya* should be avoided.
5. Provision of health care services and regular water supply.
6. Treatment using full course appropriate antifungal drugs without any gap or negligence.

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APPENDIX I

QUESTIONNAIRE

1. Age (years)

5-10 [ ] 11-15 [ ] 16-20 [ ]

2. Residence with parents yes/no

Parents [ ] Relatives [ ]

3. Occupation of custodian

Farmer [ ] Trader [ ] Casual jobs [ ] Permanent salaried [ ]

Unemployed [ ] Others [ ]

4. Type of living quarters

Cemented walls and floors [ ] Cemented floors and earthen walls [ ]

Mud walled with earthen floors [ ] Uncompleted building [ ] Others [ ]

5. Number of living rooms in house

1-2 [ ] 3-4 [ ] 5 or more [ ]

6. Number of adults in house

1-2 [ ] 3-4 [ ] 5 or more [ ]

7. Number of children in the house

1-2 [ ] 3-4 [ ] 5 or more [ ]

8. Number sharing bed/ beddings with participant

None [ ] 1 [ ] 2 [ ] >2 [ ]

9. Type of bed

Iron/ Local [ ] Matress [ ] Mat [ ] Bare floor [ ]

10. Sharing of beddings

Sharing [ ] Not sharing [ ]

11. Source of water for domestic use

Tap/ Borehole [ ] stream /dam/pond [ ] Well [ ] Others [ ]

12. Participants' bathing frequency

Once daily [ ] Weekly [ ] Occasionally [ ]

13. Do you use soap in bathing?

Yes [ ] No [ ]

14. If yes, how often?

Always [ ] Weekly [ ] Sometimes [ ]

15. How often do you wash your clothes?

Always [ ] Weekly [ ] Sometimes [ ] Not at all [ ]

16. How often do you shave?

Always [ ] Weekly [ ] Sometimes [ ] Not at all [ ]

17. Place of shaving hair

Home [ ] Barbers' shop [ ]

18. Contact with pet and/or livestock.

Yes [ ] No [ ]

19. Type of pet/livestock kept

Cat [ ] Dog [ ] Cattle/Goats/Sheep [ ] Poultry [ ] Nil [ ]

APPENDIX II

MINISTRY OF HEALTH, KADUNA STATE

All Communication to be addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Telephone: 234-248048
Website: ttp://www/moh.kd.gov.ng.
Email: info@moh.kd.gov.ng.

Independence Way.
P.M/:B 2014
Kaduna.
Kaduna State, Nigeria.

Health Research Ethical Committee Kaduna state ministry of Health.

MOH/ADM/744/VOL.1/209 6th January, 2015.

To.....

Ministry of Health Research Ethical Clearance

RE:-EPIDEMIOLOGICAL STUDIES AND MOLECULAR CHARACTERIZATION OF DERMATOPHYTES ON *ALMAJIRAI* IN SOME SELECTED LOCAL GOVERNMENT IN KADUNA STATE

Name of investigator:-	Aisha Abdullahi Ahmed
Date of Receipt of Application:-	11th December, 2014
Date of Ethical Approval	6th January, 2015
Research Period	6 months

You are kindly requested to give research maximum cooperation. However, it is mandatory for research to summit his findings to the ministry please.

F.A. Kurah (Mrs)

Secretary Research Ethical Committee

APPENDIX III

Cocktail Preparation:

25 reaction mix was prepared:

Master mix: 12.5 µl x 16 = 200 µl

Forward primer: 1 µl x 10 = 16 µl

Reverse primer: 1 µl x 10 = 16 µl

Nuclease free water: 3.5µl x 16= 56 µl

Total 18 x 16 = 288 µl

18 µl was distributed to each pcr tube

7 µl of the DNA template was thereafter added

Making a total of 25 µl reaction mix

APPENDIX IV

Distribution of dermatophytes infection among *almajirai* in Makarfi L. G. A. by age.

Age Group (Years)	Number examined	Number infected	% Infected
5-7	75	41	54.67
8-10	133	68	51.13
11-13	88	22	25.00
14-16	55	12	21.82
17-19	57	10	17.54
Total	408	153	37.50

P= 0.00001 $\chi^2 =$ 41.292 **Df = 4**

APPENDIX V

Distribution of dermatophytes infection among *almajirai* in Makarfi L. G. A. by type of residency.

Residency	**Number examined**	**Number infected**	**% Infected**
With parents/guardians	68	16	23.53
Not with parents/guardians	340	103	30.29
Total	408	119	29.17

P= 0.0980 χ^2 = 2.7373 **Df = 1**

CONTRIBUTION TO KNOWLEDGE

1. The study established a dermatophyte prevalence of 37.5% among *almajirai* in Makarfi Local Government Area.

2. Age is directly associated with prevalence of dermatophytosis in Makarfi Local Government Area.

3. *Trichophyton rubrum* 45(29.4%), *T. mentagrophytes* 16(10.5%), *T. violaceum* 3(2.0%), *T. soudanense* 2(1.3%), *T. tonsurans* 17(11.1%*) T. concentricum* 4(2.6%), *T. quickeanum* 1(0.7%), *T. megninii* 1(0.7%), *T. verrucosum* 1(0.7%), *Microsporum canis* 5(3.3%), *M. fulvum* 7(4.6%), *M. gallinae* 10(6.5%), *M. audouinii* 1(0.7%), *M. equinum* 5(3.3%) and *M. nanum* 1(0.7%) have been identified as dermatophytes associated with dermatophytosis among *almajirai* in Makarfi Local Government Area.

4. *Microsporum canis* (500 base pairs), *Microsporum audouinii* (560 base pairs), *Trichophyton rubrum* (500 base pairs), *Trichophyton tonsurans* (500 base pairs) and *Trichophyton mentagrophytes* (560 base pairs) were confirmed using the ITS 1-2 sequence . 5’ ATCATTAACGCGCAGGC3’ and 5’ TGGCCACTGCTTTTCGG3’

5. *T. violaceum* (200 baase pair), *T. verrucosum* (200 baase pair), *T. verrucosum* (300 base pair) were confirmed using 5’AAGTTGGGTCAAACTCGGT 3’ and 5’ TGATCCTTCCGCAGGTT 3’. Primer set.

6. *M. gypseum* (300 base pair) *and M. fulvum* (300 base pair) were confirmed using the primer sequence 5’ ACAGGGATTGCCCCAGTA 3’ and 5’ CTTGTTCGCTATCGGTCTC 3’.