COMPARATIVE ACTIVITIES OF SOME ANTIFUNGAL AGENTS AGAINST DERMATOPHYTE ISOLATES FROM SCHOOL CHILDREN WITH TINEA CAPITIS.

BY

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MSC/PHARM SCI/50590/2005 – 2006

(Msc/ Pharm sci/ 24101/2000-2001)

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY ZARIA. IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PHARMACEUTICAL MICROBIOLOGY

DEPARTMENT OF PHARMACEUTICS & PHARMACEUTICAL MICROBIOLOGY
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AHMADU BELLO UNIVERSITY, ZARIA

JUNE 2006

DECLARATION

I hereby declare that the work reported in this thesis was carried out by me under the supervision of Dr. J. O. Ehinmidu and Prof. J.A. Onaolapo, both of the Department of Pharmaceutics and Pharmaceutical Microbiology. It has not been presented in any previous application for degree. The work of other investigators are acknowledged and referred to accordingly.

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This thesis, entitled ''Comparative Activities of Some Antifungal Agents Against Dermatophyte Isolates from School Children with Tinea Capitis'' by Aisha Muhammad, meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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This work is dedicated to:

ALL THOSE WHO NEVER QUIT

ACKNOWLEDGEMENT

I wish to express my profound gratitude to my Supervisor, Dr. J.O. Ehinmidu for his immeasurable efforts, commitment and useful suggestions given during the course of this work.

My thanks go to Prof. J. A. Onaolapo, my co-supervisor for his care and dedication.

My sincere appreciation goes to the technical staff and the entire staff of the Department. My gratitude also goes to Mr. Michael Ainoje, Shehu Namadi and Mr. Tanko Yohana for their concern and care

Aliu Osigwe Yesufu (THE BEST HUSBAND EVER) you have been of tremendous support to me. You helped me through thick and thin and have always inspired me to do more. Amir and Aliyyah my sweet little darlings I am so proud of your support. Your wish to go to mummy's school when you finish your primary and nursery school respectively in more ways than one helped me to remain focused. Habiba Ali Ahmad what can one do without a friend to prod one on when one feel like giving up? You have been a true friend and your daughter Fatiha Ahmad has brought sunshine into our lives. She will be a source of joy to all those she comes in contact with.

To my parents Ahmad Tijjani Muhammad and Zainab A. T. Muhammad for giving me a solid foundation in education against all odds I would forever be grateful. My siblings, Hamida, Amina, Sani, Furaira, Abduljalil and Halliru you are simply the best. I have always known I could depend on you.

There are a lot of you whom this page cannot accommodate but you are all remembered in prayers and the bountiful God will reward each and every one of you abundantly.

ABSTRACT

Tinea capitis is a fungal infection of the scalp and hair caused by dermatophytes. It occurs in all age groups but predominantly found in children. The antifungal activities such as MIC, MFC and rate of kill of fluconazole, terbinafine, lauric acid and sodium propionate alone and in admixture against dermatophyte isolates from school children with tinea capitis in L. E. A. primary school, Mando, Kaduna, Nigeria were assessed. *T. mentagrophyte, T. tonsuran, T. rubrum*, Trichophyton species *M. canis, P*. *furfur. P. hortei* were isolated from the school children. The trichophyton species had the highest order of prevalence (56.67%) followed by *P. furfur* (20%) while *P. hortei* was (13.33%) and *M.canis* was (10%).

The Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) of fluconazole ranges were (0.5- 1.0mg/ml) and (1.00 - 8.00mg/ml) respectively against the test organisms. *T. mentagrophyte* (isolate number 18) was found to be the most resistant of the organisms that were isolated from the school children. The order of potent activity of the test antifungal agents was fluconazole, terbinafine, lauric acid and sodium propionate. The combination of the test antifungal agents investigated was found to be synergistic. Terbinafine and Sodium propionate combination produced marked synergistic action (FIC=0.57) against the most resistant dermatophyte isolate.

Terbinafine (10mg/ml) and Sodium propionate (200mg/ml) after 60mins contact time produced 5.2 and 4.3 log reduction of 1.025 x 10^8 spores/ml of resistant *T. mentagrophyte.* The combination of Terbinafine (10mg/ml) and Sodium propionate (200mg/ml) effected 100% kill of 1.02 x10^8 spores/ml of resistant *T. mentagrophyte* after 20 minutes contact time. Thus the use of terbinafine/ sodium propionate combination therapy for dermatophyte infection seem promising

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CHAPTER ONE

1.0 INTRODUCTION

The body normally hosts a variety of microorganisms including bacteria, mold-like fungi (dermatophytes) and yeast-like fungi (such as candida). Some of these are useful to the body. Others may under proper conditions multiply rapidly and cause infection. Fungal skin infections are caused by microscopic fungi that flourish on the human skin. Fungal infection has emerged as a significant clinical problem in recent years (NCCLS 1997). Due to the increasing frequency of fungal infections, mycology is today undergoing renaissance. The incidence of fungal infection has markedly increased in recent years. Several factors have contributed to this. These include greater use of immunosuppressive drugs, prolonged use of broad-spectrum antibiotics, widespread use of in dwelling catheter and the Acquired Immunodeficiency Syndrome (AIDS)

Fungal infection is divided into systemic infection and dermatophycoses. Recognition and appropriate treatment of these infections reduce both morbidity and discomfort and lessen the possibility of transmission (Cohn 1992).

Dermatophyte infections are classified according to the affected body site such as Tinea Capitis (scalp and hair), Tinea Barbae (beard area), Tinea Corporis (skin other than bearded area, scalp, groin, hands and feet) Tinea Cruris (groin perineal area and perineum), Tinea Pedis (feet), Tinea manuum (hands) and Tinea unguuim (nails). The estimated lifetime risk of acquiring a dermatophyte infection is between 10 and 20 percent (Drake et al 1996).

1.1 DERMATOPHYTES

Dermatophytes are fungi that can cause infections of the skin, hair, and nails due to their ability to utilize keratin. The organisms colonize the keratin tissues and inflammation is caused by host response to metabolic bye-products. These infections are known as ringworm or tinea, in association with the infected body part. Occasionally, the organisms do invade the subcutaneous tissues, resulting in kerion development (St Germain and Summerbell, 1996).

The dermatophytic causative organisms are transmitted by either direct contact with infected host (human or animal) or indirect contact with infected exfoliated skin or hair in combs, hairbrushes, clothing, furniture, theatre seats, caps, bed linens, towels, hotel rugs, and locker room floors (St Germain and Summerbell, 1996).

Depending on the species, the organisms may be viable in the environment for up to 15 months. There is an increased susceptibility to infection when there is a preexisting injury to the skin such as scars, burns, excessive temperature and humidity (St Germain and Summerbell, 1996).

Dermatophytes cause a variety of clinical conditions. They are among the most common infectious agents of humans. Collectively, the group of diseases is termed dermatophytosis. From the site of infection the fungal hyphae grow centrifugally in the stratum corneum. The fungus continues downward growth into the hair invading keratin as it is formed. The zone of involvement extends upward at the rate at which the hair grows and it is visible above the skin surface by days 12-14. Infected hairs

are brittle and by the third week broken hair are evident (St Germain and Summerbell, 1996).

The infection continues (for 8-10 weeks) to spread in the stratum corneum to involve other hairs at which point, the infected area is approximately 3.5-7.0 cm in diameter. The spontaneous cure of naturally occurring infection at puberty is a familiar clinical observation(St Germain and Summerbell, 1996).

Dermatophytes are classified as anthropophilic, zoophilic or geophilic according to their normal habitat.

- Anthropophilic dermatophytes are restricted to human hosts and produce a mild, chronic inflammation.
- Zoophilic organisms are found in animals and cause marked inflammatory reactions in humans who have contact with infected cats, dogs, cattle, horses, birds, or other animals. This is followed by a rapid termination of the infection.
- Geophilic species are usually recovered from the soil but occasionally infect humans and animals. They cause a marked inflammatory reaction, which limits the spread of the infection and may lead to a spontaneous cure but may also leave scars.

1.1.1 MORPHOLOGY AND IDENTIFICATION OF DERMATOPHYTES

They are classified into three genera: Epidermophyton, Microsporum and Trichophyton. In keratinized tissue, these form only hyphae and arthrospores. In culture, they develop characteristic colonies and conidia, by means of which they can be divided into species. Sexual spores of some species have been found. Most

dermatophytes are worldwide in distribution, but some species show a higher incidence in certain regions than in others (e.g. *Trichophyton schoenleinii* in the mediterraneian, *Trichophyton rubrum* in tropical climates).

Representative colonies form on sabouraud dextrose agar at room temperature. Conidia formation may be observed by means of slide cultures. Sabouraud medium is suitable for the isolation of dermatophytes with the addition of cycloheximide, which inhibits many common non-pathogenic fungi contaminants.

Characteristics of more commonly isolated Dermatophytes are shown in table 1.0

❖ TRICHOPHYTON

Trichophyton is a dermatophyte which inhabits the soil, humans or animals. Based on its natural habitats, the genus includes anthropophilic, zoophilic, and geophilic species. Some species are cosmopolitan. Others have a restricted geographic distribution. *Trichophyton concentricum*, for example, is endemic at Pacific Islands, Southeast Asia, and Central America. *Trichophyton* is one of the leading causes of hair, skin, and nail infections in humans (Arenas *et al* 1995).

The genus *Trichophyton* has several species. Most common are *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton schoenleinii*, *Trichophyton tonsurans*, *Trichophyton verrucosum*, and *Trichophyton violaceum. Trichophyton rubrum* is the commonest causative agent of dermatophytoses worldwide (Arenas *et al* 1995). *Trichophyton* species may cause invasive infections in immunocompromised hosts (Squeo *et al* 1998).

The growth rate of *Trichophyton* colonies is slow to moderately rapid. The texture is waxy, glabrous to cottony. From the front, the color is white to bright yellowish beige or

red violet. Reverse is pale, yellowish, brown, or reddish-brown (Dehoog *et al* 2000; Larone, 1995; St Germain and Summerbell 1996; Sutton *et al* 1998).

Trichophyton have septate, hyaline hyphae, conidiophores, microconidia, macroconidia, and arthroconidia. Chlamydospores may also be produced. Conidiophores are poorly differentiated from the hyphae. Miroconidia (also known as the microaleuriconidia) are one-celled and round or pyriform in shape. They are numerous and are solitary or arranged in clusters. Microconidia are often the predominant type of conidia produced by *Trichophyton*. Macroconidia (also known as the macroaleuriconidia) are multicellular (2- or more-celled), smooth-, thin- or thick-walled and cylindrical, clavate or cigar-shaped. They are usually not formed or produced in very few numbers. Some species may be sterile and the use of specific media is required to induce sporulation (Dehoog *et al* 2000; Larone, 1995; St Germain and Summerbell 1996; Sutton *et al* 1998). *Trichophyton* differs from *Microsporum* and *Epidermophyton* by having cylindrical, clavate to cigar-shaped, thin-walled or thick-walled, smooth macroconidia.



Microconidia and a terminal macroconidium of *T. rubrum*

❖ **MICROSPORUM**

Microsporum is a filamentous keratinophilic fungus included in the group of dermatophytes. The natural habitat of some of the *Microsporum* spp. is soil (the geophilic species), others primarily affect various animals (the zoophilic species) or human (the anthropophilic species). Some species are isolated from both soil and animals (geophilic and zoophilic). Most of the *Microsporum* spp. are widely distributed in the world while some have restricted geographic distribution. *Microsporum* is the asexual state of the fungus and the telemorph phase is referred to as genus *Arthroderma* (Caffara and Scagliarini, 1999; Pier and Morielli, 1998; St-Germain and Summerbell 1996).

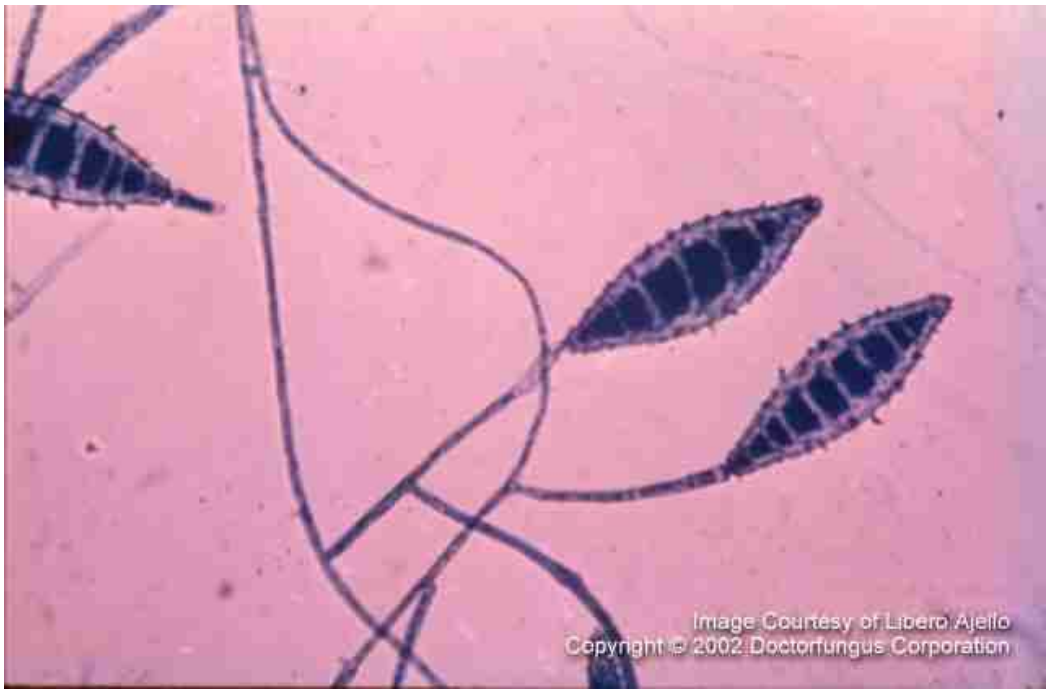
The genus *Microsporum* includes 17 conventional species. Among these, the most significant are: *M. canis, M. audouinii, M. nanum, M. gypseum, M. cookie, M. distortum, M. ferrugineum, M. gallinae*

Microsporum is one of the three genera that cause dermatophytosis. Dermatophytosis is a general term used to define the infection in hair, skin or nails due to any dermatophyte species. Notably, *Microsporum* spp. mostly infect the hair and skin, except for *Microsporum persicolor* which does not infect hair. Nail infections are very rare (Aly,1999; Collier *et al,* 1998; Elewski, 2000; Frieden, 1999; Romano, 1998).

Microsporum colonies are glabrous, downy, wooly or powdery. The growth on Sabouraud dextrose agar at 25°C may be slow or rapid and the diameter of the colony varies between 1 to 9 cm after 7 days of incubation. The color of the colony varies depending on the species. It may be white to beige or yellow to cinnamon. From the reverse, it may be yellow to red-brown (St-Germain and Summerbell 1996).

Microsporum spp. produces septate hyphae, microaleurioconidia, and macroaleurioconidia. Conidiophores are hyphae-like. Microaleuriconidia are unicellular, solitary, oval to clavate in shape, smooth, hyaline and thin-walled. Macroaleuriconidia are hyaline, echinulate to roughened, thin- to thick-walled, typically fusiform (spindle in shape) and multicellular (2-15 cells). They often have an annular frill. Inoculation on specific media, such as potato dextrose agar or Sabouraud dextrose agar supplemented with 3 to 5% sodium chloride may be required to stimulate macroconidia production of some strains (St-Germain and Summerbell 1996).

Microsporum differs from *Trichophyton* and *Epidermophyton* by having spindle-shaped macroconidia with echinulate to rough walls (St-Germain and Summerbell 1996).



Macroconidia of M. canis. The septal wall is thinner than the outer wall

❖ **EPIDERMOPHYTON**

Epidermophyton is a filamentous fungus and one of the three fungal genera classified as dermatophytes. It is distributed worldwide. Man is the primary host of *Epidermophyton floccosum*, the only species which is pathogenic. The natural habitat of the related but the nonpathogenic species *Epidermophyton stockdaleae* is soil (Dehoog *et al* 1998; Larone, 1995; Sutton *et al* 1998).

The genus *Epidermophyton* contains two species; *Epidermophyton floccosum* and *Epidermophyton stockdaleae*. *E. stockdaleae* is known to be nonpathogenic, leaving *E. floccosum* as the only species causing infections in humans.

The colonies of *E. floccosum* grow moderately rapidly and mature within 10 days. Following incubation at 25 °C on potato dextrose agar, the colonies are brownish yellow to olive gray or khaki from the front. From the reverse, they are orange to brown with an occasional yellow border. The texture is flat and grainy initially and become radially grooved and velvety by aging. The colonies quickly become downy and sterile (Dehoog *et al* 2000; Larone, 1995; St Germain and Summerbell 1996; Sutton *et al* 1998).

Septate, hyaline hyphae, macroconidia, and occasionally, chlamydoconidium-like cells are seen. Microconidia are typically absent. Macroconidia (10-40 x 6-12 µm) are thin walled, 3- to 5- celled, smooth, and clavate-shaped with rounded ends. They are found singly or in clusters. Chlamydoconidium-like cells, as well as arthroconidia, are common in older cultures (Dehoog *et al* 2000; Larone, 1995; St Germain and Summerbell 1996; Sutton *et al* 1998). *Epidermophyton floccosum* is differentiated from *Microsporum* and *Trichophyton* by the absence of microconidia.



Microscopic morphology of *E. flocosum* showing characteristic thin-walled macroconidia in clusters. No microconidia are formed

Table 1.0 Characteristic of Some Commonly Isolated Dermatophytes

DERMATOPHYTES	COLONIAL MORPHOLOGY	GROWTH RATE	MICROSCOPIC IDENTIFICATION
Microsporum audouinli	Downy white to salmon Pink colony.	2 week	Sterile hypae: terminal chlamydosporesm, favic chandeliers, and pectinate bodies; macroconidia rarely seen – bizarre shaped if seen; micronodia rare or absent
M. canis	Colony is usually membranous with feathery periphery; centre of colony is white to butt over orange –yellow or lemon yellow or yellow orange apron and reverse.	1 week	Thick walled, spindle shaped, multiseptate, rough walled, macroconidia some with macroconidia rarely seen

Microsporum gypscum	Cinnamon coloured, powdery, colony reverse light tan	1 week	Thick-walled, rough, elliptical, multisapate, macroconidia, microconidia few or absent.
Epidermophyton floccosum	Center of colony tends to be folded and is khaki green, periphery is yellow; reverse yellowish brown with observable folds	1 week	Macroconidia large, smooth-walled multisepate, clavate anf borne singly or in cluster of two or three microconidia not formed by this species.
Trichophyton mentagrophytes	Different colonial types; white or pinkish, granular and fluffly varieties; occasional light yellow periphery in younger cultures, reverse buff to reddish brown.	7-10 days	Many round to globose microconidia most commonly borne in grapelike cluster or laterally along the hyphea; spiral hyphae in 30% of isolates, macroconidia are thin-walled, smooth, club-shaped, and multisepate, numerous or are depending upon strain.
Trichophyton rubrum	Colonial types vary from white dowry to pink granular, rugal folds are common, reverse yellow when colony is young however, wine red colour commonly develop with age.	2 weeks	Microconidia usually teardrop, most commonly borne along sides of the hyphae, macroconidia usually absent, but when present are smooth thin walled and pencil-shaped.
Trichophyton tonsurans	White, tan to yellow or rust, suedelike to powdery; wrinkled with heaped or sunken center; reverse yellow to tan to rust red.	7-14 days	Microconidia are teardrop or club shaped with flat bottoms;vary in size but usually larger than other dermatophytes; macroconidia rare and balloon forms found when present
Trichophyton schoenleinii	Irregularly heaped, smooth white to cream colony with radiating grooves; reverse white.	2-3 weeks	Hyphae usually sterile; many antler-type hyphae seen (favic chandeliers)

Trichophyton violaceum	Port wine to deep violet colony, may be heaped or flat with waxy-glabrous-surface; pigment may be lost on subculture	2-3 weeks	Branched, tortuous hyphae that are sterile; chlamydospores commonly aligned in chains
Trichophyton verrucosum	Glabrous to velvety white colonies; rare stains produce yellow-brown colour; rugal folds with tendency to sink into agar surface	2-3 weeks	Microconidia rare; large and tear-drop when seen; macroconidia extremely rare, but forms characteristic 'rat-tail' types when seen; many chlamydospores seen in chains, particularly when colony is incubated at 37^0 C

(Koneman and Roberts1985).

1.2 TINEA CAPITIS

Tinea capitis (scalp ringworm) is a highly contagious infection of the scalp and hair caused by dermatophyte fungi such as M. canis, M. audounii. It occurs in all age group but predominantly children. It is endemic in some of the poorest countries (Gonzalez *et al* 2004).

1.2.1 PATHOPHYSIOLOGY OF TINEA CAPITIS

Tinea capitis is caused by species of Trichophyton and Microsporum. Tinea capitis is the most common pediatric dermatophyte infection worldwide. It affects mostly children of primary school age. The increased incidence of tinea among prepubertal children has been attributed to reduced fungistatic properties of the child's sebum. However comparison studies of sebum in prepubertal versus postpubertal children failed to reveal real fungistatic differences (Gorbach et al 1997).

Prepubertal infections by *Trichophyton tonsurans*, do not resolve at puberty, as do the infections by *Microsporum* (Bronson *et al* 1983). Kamalam and Thambiah, (1980) supposes that sebum is not of much value against the *Trichophyton species.*

1.2.2 EPIDEMIOLOGY OF TINEA CAPITIS

Tinea capitis, primarily a disease of children, (Aly 1999; Gupta and Summerbell 2000) is a public health problem in some countries because of increased incidence and epidemic transmission. Tinea capitis occur occasionally in other age groups. It is seen most commonly in children younger than 10 years. Peak age range is in patients aged 3-7 years (Mandell *et al* 1995).

Tinea capitis affects boys more than girls probably because short hairs help implantation of spores (Kanwar and Belhal, 1987). Although very rare after puberty, when it occurs, it is often associated with the infection simultaneously at another site (tinea corporis, tinea cruris, etc.), which is not so frequent in children (Kamalam and Thambiah, 1980).

In adults it affects mostly women (Bronson *et al* 1983) and the area of choice is the occiput. There is usually a trigger factor such as diabetes mellitus, pulmonary tuberculosis, immunodefficiency, malnutrition, drugs or some other factor that causes immunossupression (Kamalam and Thambiah, 1980). It is not infrequent in transplanted patients or in those with systemic lupus erythematosus (Barlow and Saxe, 1988).

Incidence of tinea capitis may vary by sex, depending on the causative fungal organism. In *M. audouinii*–related tinea capitis, boys are affected much more

commonly. The infection rate has been reported to be up to 5 times higher in boys than in girls; however, the reverse is true after puberty, possibly as a result of increased exposure to infected children by women and to hormonal factors. In infection by *M. canis*, the ratio varies, and the infection rate usually is higher in male children. Girls and boys are affected equally by *Trichophyton* infections of the scalp, but in adults, women are infected more frequently than are men.

The epidemiology of tinea capitis in the United Kingdom has recently changed dramatically, (Higgins *et al* 2000) reflecting a similar trend in the United States 20 years ago (Bronson *et al* 1983). In the United Kingdom it is becoming a major public health problem, and Afro-Caribbean children are particularly affected (Fuller *et al* 2003a). The predominant organism was *M. canis*, but now *T. tonsurans* causes 90% of cases in the United Kingdom and the United States (Higgins *et al* 2000). *T. tonsurans* is an anthropophilic fungus, which spreads from person to person. The reason for this change is unclear, but hairdressing practices such as shaving the scalp, plaiting, and using hair oils may increase the spread (Higgins *et al* 2000).

This variation in the epidemiology of tinea capitis reflects people's habits, standards of hygiene, climatic conditions and levels of education. Interestingly, increased education may increase the number of patients seeking medical attention for their scalp lesions, which in turn increase the diagnosed level of tinea capitis in a given area.

1.2.3 FREQUENCY OF TINEA CAPITIS

The frequency of tinea capitis compared to other types of dermatophytosis varies from one location to another. Tinea capitis is considered the most frequent cause of

dermatophytosis in the Islamic Republic of Iran and Jordan (Chadegani, 1987,Khosravi *et al* 1994 Shtayeh and Arda 1985) and the second most frequent form of dermatophytosis in Mosul (Iraq) after tinea corporis (Yehia, 1980). In contrast, there has been a marked decline in the incidence of tinea capitis in Mexico City, down from 31.0% of all cases of dermatophytosis between 1940 and 1950 to 1.6% between 1986 and 1992 (Gayosso, 1994).

Tinea capitis is widespread in some urban areas in North America, Central America, and South America. It is common in some parts of Africa and India. In Southeast Asia, the rate of dermatophytic infection has been reported to decrease dramatically from 14% (average of male and female children) to 1.2% in the last 50 years because of improved general sanitary conditions and personal hygiene. In northern Europe, the disease is sporadic (Gupta *et al* 1999).

1.2.4 GEOGRAPHIC DISTRIBUTION OF TINEA CAPITIS

The geographic distribution and prevalence of dermatophytes are not static but change under the influence of various forces such as climate, migration of people and developments in prophylaxis and therapy.

T. tonsurans is now the major cause of tinea capitis in the USA (Matsuoka and Gedz, 1982; Rebell and Tschen 1984) but until some years ago it was *M. canis* and *M. audouinii* (Matsuoka and Gedz 1982; Tschen, 1984). These fungi have been reported to be the major cause of tinea capitis infection in Chicago over the past 20 years (Bronson *et al* 1983). In New York, the predominantly infected children were reported to be black (30 cases out of 31) (Ravits and Himmerstein, 1983) and in Philadelphia since 1979 (Shockman and Urbach, 1983).

The incidence of the dermatophytes causing tinea capitis varies greatly. In Western Australia, the major causative agent is *M. canis* (McAleer, 1980) as it is in Umbria, Italy (Binazzi *et al* 1983) and Uruguay (Vignale *et al* 1983). In Madras, India, it is *T. violaceum* (Kamalam and Thambiah 1980) while in Tel Aviv, Israel, *T. schoenleinii* is the causative organism whereas in Ile-Ife, Nigeria, M. audouinii was found to be the major causative organism (Ajao and Akintunde 1985). However, in South Africa, *T. violaceum* was found to be the causative agents of Tinea capitis (Barlow and Saxe 1988).

Garcia-Perez & Moreno-Gimenez (1981), reviewing the literature on tinea capitis in adults, found 39.59% of the cases caused by *T. tonsurans*. In Japan only a few cases of *T. tonsurans* have been reported (Yamasaki *et al* 1982) and in Israel among 1000 cases of dermatophytosis not a single case of tinea capitis associated with *T. tonsurans* as infective organism could be found.

Furtado *et al (*1985), in Manaus, State of Amazon, found among 115 cases of tinea capitis, 91.7% was caused by *T. tonsurans*, out of these 91.7%, 13.9% were adults of which 52.2% were women. In Rio de Janeiro, Brazil, some cases of tinea capitis in adults due to *M. canis* and *T. tonsurans* have been reported (Severo and Gutierrez 1985; Miranda *et al* 1989).

1.2.5 CLINICAL MANIFESTATION OF TINEA CAPITIS

The clinical picture of tinea capitis varies greatly and depends mainly on the type of infective agent. In general, zoophilic species produce much more severe inflammation than those which are confined to humans (anthropophilic). In some cases, the

inflammation can be minimal with delicate scaling and inappreciable hair loss. In some individuals an asymptomatic carrier state occurs.

Tinea capitis causes patchy alopecia, but specific clinical patterns can be varied. Six main patterns are recognised as shown in Table 1.1

Table 1.1: Main clinical manifestation of tinea capitis according to occurrence.

Tinea capitis	Clinical Patterns
Grey type	Circular patches of alopecia with marked scaling
Moth eaten	Patchy alopecia with generalised scale
Kerion	Boggy tumour studded with pustules; lymphadenopathy usually present
Black dot	Patches of alopecia with broken hairs stubs
Diffuse scale	Widespread scaling giving dandruff-like appearance
Pustular type	Alopecia with scattered pustules; lymphadenopathy usually present

(Fuller *et al* 2003b)

There are different types of Tinea capitis. The first type is the ringworm of the scalp commonly associated with to *M. audouni.* Its hallmarks appear as patchy alopecia, scaling, and dull broken hairs (“gray patch”). In another type of scalp involvement, scattered individual hairs are affected. In children, the head is the most commonly affected area, but lesions may occur on any place on the body. The primary lesion is usually a small vesicle, although the most important characteristic of the lesion is lack of inflammatory response. The lesions usually involve small area on the scalp in which the hair is dull, and broken off about 1 to 2mm from the surface of the skin. The skin is scaly with little inflammation. Lesions may occur around the nape of the neck and occasionally the glabrous skin, and even the eyelids and eyelashes are involved.

A second type of tinea capitis is that caused by *M.canis*. The lesions are usually more inflammatory from the beginning than those produced by *M.audouni*. There are usually three to four small spots of the eruption in the scalp. The primary lesion is formed of minute vesicles, and the hair is usually broken off 1 to 2 mm from the skin surface. At times the hair may even be lost as a result of the inflammation around the hair follicle. The centre of the lesion is elevated, and the borders of the lesions are more inflamed than those seen with *M. audouni*. The vesicles are seen more readily around the actively advancing margin of the lesion. The glabrous skin is frequently infected. This form of the disease is frequently transmitted in young animals, such as kittens or puppies, to man.

A third type of tinea infection of the scalp is the so-called kerion formation, otherwise known as tinea profunda or the granulomatous disease of majocchi. This type of tinea is very inflammatory and is caused by a virulent fungus of either animal or human origin. The onset is rather acute, and the lesions usually remain localized to one spot. The inflammatory reaction is rather severe. The lesion is boggy and indurated, and the inflamed lesion is studded with broken or unbroken hairs, vesicles and pustules. The organisms usually causing kerion formation are *T. mentagrophytes, T. verrucosum, M. canis, and M. gypseum.*

A fourth type of tinea capitis is that produced by *T. tonsurans* and *T. violaceum,* commonly known as “black dot” ringworm. It is characterized by multiple bald patches on the scalp, with hairs broken at or below the surface of the scalp. Occasionally folliculitis may be noted, and the patients may actually develop permanent baldness. No fluorescence is noted. The organisms invade the hair, producing an endothrix type

of infection, causing the shafts of the shaft of the hair to break at or below the surface of the skin. The disease may persist many years, causing some degree of atrophy of the scalp, scarring and permanent alopecia.

1.2.6 DIAGNOSIS OF TINEA CAPITIS

Laboratory diagnosis of dermatophytosis depends on examination and culture of rubbings, scrapings, pluckings, or clippings from infected lesions. Infected hairs appearing as broken stubs are best for examination. They can be removed with forceps without undue trauma or collected by gentle rubbing with a moist gauze pad or toothbrush.

Selected hair samples are cultured or allowed to soften in 10-20% potassium hydroxide (KOH) before examination under the microscope. Examination of KOH preparations (KOH mount) usually determines the proper diagnosis if a tinea infection exists.

Microscopic examination of the infected hairs may provide immediate confirmation of the diagnosis of ringworm and establishes whether the fungus is small-spore or large-spore ectothrix or endothrix.

Culture provides precise identification of the species for epidemiologic purposes. Primary isolation is carried out at room temperature, usually on Sabouraud dextrose agar containing antibiotics (penicillin/streptomycin or choramphenicol) and cycloheximide (Acti-dione), which is an antifungal agent that suppresses the growth of environmental contaminant fungi. In cases of tender kerion, the agar plate can be inoculated directly by pressing it gently against the lesion. Most dermatophytes can be identified within 2 weeks, although *T. verrucosum* grows best at 37ºC and may have

formed only into small and granular colonies at this stage. Identification depends on cultural characteristics, gross colony and microscopic morphology.

Infected hairs and some fungus cultures fluoresce in ultraviolet light. The black light commonly is termed Wood lamp. Light is filtered through a Wood nickel oxide glass (barium silicate with nickel oxide), which allows only the long ultraviolet rays to pass (peak at 365 nm).

Hairs infected by *M. canis, M. audouinii,* and *M. ferrugineum* fluoresce a bright green to yellow-green colour. Hairs infected by *T. schoenleinii* may show a dull green or blue-white color, and hyphae regress leaving spaces within the hair shaft. *T verrucosum* exhibits a green fluorescence in cow hairs, but infected human hairs do not fluoresce .The fluorescent substance appears to be produced by the fungus only in actively growing infected hairs.Infected hairs remain fluorescent for many years after the arthroconidia have died. When a diagnosis of ringworm is under consideration, the scalp is examined under a Wood lamp. If fluorescent infected hairs are present, hairs are removed for light microscopic examination and culture. Infections caused by Microsporum species fluoresce a typical green color. The myriad debilitating effects of these manifested infective fungi necessitated the need for effective therapeutic agents.

1.3 ANTIFUNGAL AGENT

An antifungal agent is a drug that selectively eliminates fungal pathogens from a host with minimal or without toxicity to the host. The development of antifungal agents has lagged behind that of antibacterial agents. This is a predictable consequence of the cellular structure of the organisms involved. Bacteria are prokaryotic and hence offer numerous structural and metabolic targets that differ from those of the human host.

Fungi, in contrast, are eukaryotes, and consequently most agents toxic to fungi are also toxic to the host. Fungi generally grow slowly and often in multicellular forms so they are more difficult to quantify than bacteria. This difficulty complicates experiments designed to evaluate the *in vitro* or *in vivo* properties of a potential antifungal agent. Despite these limitations, numerous advances have been made in developing new antifungal agents and in understanding the existing ones.

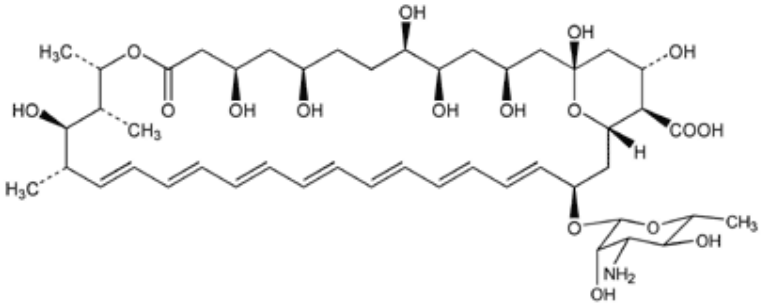
There are different classes of antifungal agents and they include the following:

a) Polyene Antifungal Drugs

The polyene compounds are so named because of the alternating conjugated double bonds that constitute a part of their macrolide ring structure. The polyene antibiotics are all products of *Streptomyces* species. These drugs interact with sterols in cell membranes (ergosterol in fungal cells; cholesterol in human cells) to form channels through the membrane, causing the cells to become leaky. The polyene antifungal agents include nystatin, amphotericin B, and pimaricin.

AMPHOTERICIN B

Amphotericin B is a polyene antifungal agent, first isolated from *Streptomyces nodosus* in 1955. It is an amphoteric compound composed of a hydrophilic polyhydroxyl chain along one side and a lipophilic polyene hydrocarbon chain on the other. Amphotericin B is poorly soluble in water (Terrell and Hughes 1992).



amphotericin B

Amphotericin B binds to sterols, preferentially to the primary fungal cell membrane sterol, ergosterol. This binding disrupts osmotic integrity of the fungal membrane, resulting in leakage of intracellular potassium, magnesium, sugars, and metabolites and then cellular death (Terrell and Hughes, 1992).

Amphotericin B has a very broad range of activity and is active against most pathogenic fungi e.g *Coccidioides immitis, Histoplasma capsulatum, Blastomyces dermatitidis* and *Paracoccidioides brasiliensis*. Notable exceptions include *Trichosporon beigelii* (Walsh *et al*, 1990), *Aspergillus terreus* (Sutton *et a*l 1999), *Pseudallescheria boydii* (Walsh *et al*, 1992), *Malassezia furfur* (Francis and Walsh, 1992), and *Fusarium spp* (Arikan *et al* ,1999). Among the *Candida spp*, isolates of *C. albicans, C. guilliermondii, C. lipolytica, C. lusitaniae C. norvegensis C. tropicalis C. glabrata*, and *C.krusei* have been reported to be relatively resistant to amphotericin B (Karyotakis and Anaissie, 1994; Karyotakis *et al,* 1993; Meyer, 1992 and Terrell and Hughes, 1992). Reduced susceptibility has been observed specifically at fungicidal levels for *C. parapsilosis*.

The most commonly observed infusion-related side effects of amphotericin B deoxycholate are fever, chills, and myalgia. These can be partially overcome by premedication with diphenhydramine and/or acetaminophen (Goodwin *et al* 1995).

Nephrotoxicity is the major adverse effect limiting the use of amphotericin B. The manifestations of nephrotoxicity are azotemia, decreased glomerular filtration, loss of urinary concentrating ability, renal loss of sodium and potassium, and renal tubular acidosis (Meyer 1992). The renal injury reduces erythropoietin production and leads to a normochromic normocytic anemia (Lin *et al,* 1990). Thrombophlebitis may occur at the site of infusion. Thrombocytopenia may rarely be observed (Chan *et al* 1982).

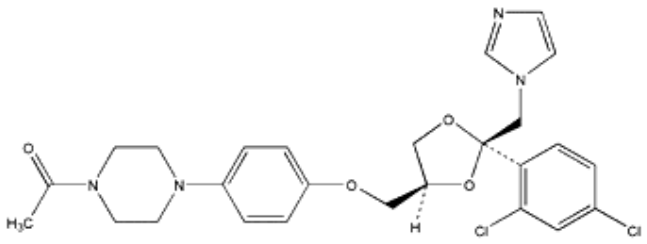
b) Azole Antifungal Drugs

The azole antifungal agents have five-membered organic rings that contain either two or three nitrogen molecules (the imidazoles and the triazoles respectively). The clinically useful imidazoles are clotrimazole, miconazole, and ketoconazole. Two important triazoles are itraconazole and fluconazole. The azoles inhibit fungal cytochrome P450 3A-dependent C14-α-demethylase that is responsible for the conversion of lanosterol to ergosterol. This leads to the depletion of ergosterol in the fungal cell membrane. The in-vitro antifungal activity of the azoles varies with each compound, and the clinical efficacy of each compound may not coincide exactly with in-vitro activity. The azoles are primarily active against *C. albicans, C. neoformans, C. immitis, H. capsulatum, B. dermatitidis, P. brasiliensis; C. glabrata, Aspergillus* spp., and *Fusarium* spp. and zygomycetes are resistant to currently available azoles.

KETOCONAZOLE

Ketoconazole is an imidazole antifungal agent. It has five-membered ring structures containing two nitrogen atoms. Ketoconazole is the only member of the imidazole class that is currently used for treatment of systemic infections.

Ketoconazole is a highly lipophilic compound. This property leads to high concentrations of ketoconazole in fatty tissues and purulent exudates. Expectedly, the distribution of ketoconazole into cerebrospinal fluid is poor even in the presence of inflammation. Its oral absorption and solubility is optimal at acidic gastric pH (Sheehan *et al*, 1999; Van der Merr *et al,* 1980).



ketoconazole

As with all azole antifungal agents, ketoconazole works principally by inhibition of cytochrome P450 14a-demethylase (P45014DM). This enzyme is in the sterol biosynthesis pathway that leads from lanosterol to ergosterol (Lyman and Walsh, 1992). The affinity of ketoconazole for fungal cell membranes is less compared to that of fluconazole an itraconazole. Ketoconazole has thus more potential to effect mammalian cell membranes and induce toxicity (Como and Dismukes, 1994).

Ketoconazole is active against *Candida spp* and *Cryptococcus neoformans* However, its activity is limited compared to that of fluconazole and itraconazole Furthermore, due to its limited penetration to cerebrospinal fluid, it is clinically ineffective in meningeal cryptococcosis. Its activity against the dimorphic moulds, *Histoplasma capsulatum, Blatomyces dermatitidis, Coccidioides immitis, Sporothrix schenckii, Paracoccidioides brasilliensis*, and *Penicillium marneffei* is favourable. However, fluconazole and itraconazole are at least as effective as ketoconazole against these fungi and are safer. Thus, ketoconazole remains as an alternative second-line drug for treatment of infections due to dimorphic fungi. Ketoconazole is not recommended for treatment of meningeal infections due to *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides immitis* due to its limited penetration to cerebrospinal fluid (Como and Dismukes, 1994).

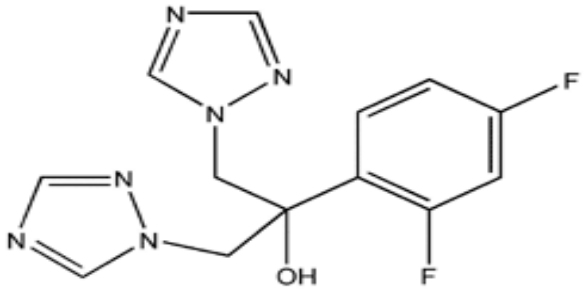
Ketoconazole is also active against *Pseudallescheria boydii* and is a good alternative for treatment of pseudallescheriasis (Sheehan *et al,* 1999). It is also effective in *Pityriasis versicolor* (Degreef and DeDoncker, 1994). Ketoconazole has practically no activity against *Aspergillus spp, Fusarium spp*, and *zygomycetes* order of fungi (Como and Dismukes, 1994).

The major drawbacks of ketoconazole therapy are from the occasionally seen adverse reactions. It may induce anorexia, nausea and vomiting (Como and Dismukes, 1994; Dismukes *et al,* 1983). Increase in transaminase levels and hepatoxicity may occur (Lewis *et al* 1984; Walsh *et al,* 1991). Ketoconazole may decrease testosterone and cortisol levels, resulting in gynecomastia and oligospermia in men and menstrual

irregularities in women (O'connor *et al,* 2002; Thomson and Carter, 1993).

FLUCONAZOLE

Fluconazole is a widely used bis-triazole antifungal agent. It has five-membered ring structures containing three nitrogen atoms.



fluconazole

Fluconazole works principally by inhibition of cytochrome P450 14a-demethylase (P45014DM). This enzyme is in the sterol biosynthesis pathway that leads from lanosterol to ergosterol (Lyman and Walsh, 1992; Marriot and Richardson, 1987; Odds *et al* ,1986).

Fluconazole is generally considered a fungistatic agent. It is principally active against *Candida spp.* and *Cryptococcus spp.* However, *Candida krusei* is intrinsically resistant to fluconazole. In addition, isolates of *Candida glabrata* often generate considerably high fluconazole MICs, with as many as 15% of isolates being completely resistant (Pfaller *et al*, 1999). Acquired resistance to fluconazole among *Candida albicans* strains has been reported particularly in HIV-infected patients (Bodey, 1992; Colin *et al*, 1999; Hoban *et al,* 1999; Rex *et al,* 1995).

Fluconazole has useful activity against *Coccidioides immitis* and is often used to suppress the meningitis produced by that fungus (Galgiani, 1993). It has limited activity against *Histoplasma capsulatum* (Wheat *et al,* 1997), *Blastomyces dermatitidis* (Pappas *et al,* 1997), and *Sporothrix schenckii* (Kauffman *et al,* 1996), and is sometimes used a second-line agent in these diseases. Fluconazole has no meaningful activity against *Aspergillus spp.* or most other mould fungi (Bodey, 1992; Denning *et al* 1992).

Carrillo-munoz *et al* (2003) studied the in vitro antifungal activity of sertaconazole against 114 dermatophytes with low susceptibility to fluconazole following the National Committee for Clinical Laboratory Standards for filamentous fungi (M38-P). However, several important factors such as the temperature (28 vs. 35°C) and time of incubation (4-10 days vs. 21-74 h), have been found to affect dermatophytes. Sertaconazole was active against 114 isolates of 12 fungal dermatophyte species, showing an overall geometric mean of 0.41 µg/ml with a minimum inhibitory concentration (MIC) range of 0.01-2 µg/ml against these isolates with reduced fluconazole susceptibility. Differences between both antifungals were significant ($p < 0.05$). MIC_{50} and MIC_{90} of sertaconazole were of 0.5 and 1 µg/ml, respectively, while the MIC of fluconazole was ≥16 µg/ml

For the *in vitro* susceptibility tests of fluconazole against some strains of Cryptococcus neoformans the MIC ranges changed from 0.5-16 µg/ml in RPMI 1640 medium and from 0.25 to 16 mg/ml in YNB-1 (Aves *et al,* 2002). Fluconazole has been shown to be

an effective alternative to amphotericin B in the treatment of cryptococcal meningitis and is the most commonly used antifungal agent in maintenance therapy of this disease (Powderly, 2000). The *C. neoformans* susceptibility to fluconazole could be an important predictor of treatment success and MICs can be useful to monitor possible development of resistance during therapy and to identify primary resistance (Amengou *et al*, 1996; Coker *et al,* 1991; Espinel-Ingroff *et al,* 1997; Orni-Wasserlauf *et al,* 1999; Paugam *et al,* 1994; Peetemans *et al,* 1993; Witt *et al,* 1996).

Fluconazole has been found to have MIC of 256–512 mg/L against isolates of *A. fumigatus A. terreus* and A*. flavus* which fell to 16–128 mg/L when combined with terbinafine (Mosquera et al 2002).

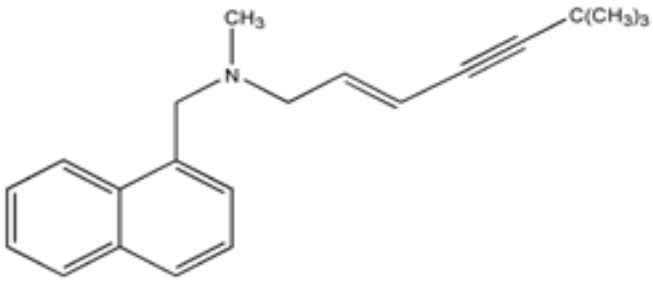
Fluconazole is generally quite well tolerated. In common with all azole antifungal agents, fluconazole may cause hepatotoxicity. Fluconazole has both oral and intravenous formulations. Fluconazole is a very widely used antifungal agent. It is one of the first-line drugs, particularly in treatment of infections due to *Candida* spp. other than *Candida krusei* and some *Candida glabrata* isolates. Fluconazole is commonly used also for prophylaxis in transplant patients (Patel, 2000; Wolff *et al* 2000).

C) Allylamine and Morpholine Antifungal Drugs

Allylamines (naftifine, terbinafine) inhibit ergosterol biosynthesis at the level of squalene epoxidase. The morpholine drug, amorolfine, inhibits the same pathway at a later step.

TERBINAFINE

Terbinafine is an allylamine structurally related to naftifine. It is a synthetic antifungal agent. It is highly lipophilic in nature and tends to accumulate in skin, nails, and fatty tissues (Elewski, 1998; Roberts, 1994).



terbinafine

Terbinafine inhibits ergosterol biosynthesis via inhibition of squalene epoxidase. This enzyme plays a vital role in the fungal sterol synthesis pathway that enhances the production of sterols needed for functional fungal cell membrane.

Terbinafine is mainly effective on a specific group of fungi such as dermatophytes. The *in-vitro* activity of terbinafine has been tested against various dermatophytes.

Terbinafine yields lower MICs compared to fluconazole, itraconazole and griseofulvin (Jessup *et al,* 2000b), an indication of likely better performance.

Terbinafine has better *in-vitro* activity also against most *Candida spp, Aspergillus spp, Sporothrix schenckii* (Jessup *et al,* 2000a), *Penicillium marneffei* (McGinnis *et al,* 2000), *Malassezia furfur* (Petranyi *et al,* 1987), *Cryptococcus neoformans* (Ryder *et al,* 1998), *Trichophyton spp.* and *Blastoschizomyces* (Ryder, 1999).

The *in-vitro* antifungal susceptibilities of six clinical *Trichophyton rubrum* isolates obtained sequentially from a single onychomycosis patient who failed oral terbinafine therapy (250 mg/day for 24 weeks) were determined by broth microdilution and macrodilution methodologies (Mukherjee *et al,* 2003).

The MICs of terbinafine for these resistant strains were >4 µg/ml, whereas they were <0.0002 µg/ml for the susceptible reference strains. Consistent with these findings, the minimum fungicidal concentrations (MFCs) of terbinafine for all six strains were >128 µg/ml, whereas they were 0.0002 µg/ml for the reference susceptible strains. The MIC of terbinafine for the baseline strain (cultured at the initial screening visit and before therapy was started) was already 4,000-fold higher than normal, suggesting that this is a case of primary resistance to terbinafine. The results obtained by the broth macrodilution procedure revealed that the terbinafine MICs and MFCs for sequential isolates apparently increased during the course of therapy. RAPD analyses did not reveal any differences between the isolates. The terbinafine-resistant isolates exhibited normal susceptibilities to clinically available antimycotics including itraconazole, fluconazole, and griseofulvin (Mukherjee *et al,* 2003).

Soares and Curry (2001) evaluated the *in vitro* activity of antifungal and antiseptic agents against dermatophytes isolated from patients with tinea pedis. The spore population per ml used was 10^6 cells/ml. The MICs of terbinafine for the strains were 0.007µg/ml or 0.015µg/ml. Most strains of *T. rubrum* (16; 72.7%), *T. mentagrophytes* (24; 72.7%) and *E. floccosum* (2; 50%) were inhibited at concentration of 0.007µg/ml. The MFC ranged from0.03 µg/ml to > 4 µg/ml. This antifungal agent was lethal to two strains of *E. floccosum* at the concentration of 0.03µg/ml, and at 0.5 µg/ml it was lethal to the other two strains. The fungicidal concentration for 13 (59.1%) strains of *T. rubrum* was up to 0.25 µg/ml, and for 20 (60.6%) strains of *T. mentagrophytes* it was up to 0.5 µg/ml. Only 2 and 6 strains of *T.rubrum* and *T. mentagrophytes*, respectively, were not killed by concentrations up to 4 µg/ml

In order to develop new approaches for the chemotherapy of invasive infections caused by *Scedosporium prolificans*, the *in-vitro* interaction between itraconazole and terbinafine against 20 clinical isolates was studied using a checkerboard microdilution method. Itraconazole and terbinafine alone were inactive against most isolates, but the combination was synergistic against 95 and 85% of isolates after 48 and 72 h of incubation, respectively. Antagonism was not observed. The MICs obtained with the terbinafine-itraconazole combination were within levels that can be achieved in plasma. (Meletiadis *et al,* 2000).

The MICs of terbinafine and itraconazole based on 50% reduction of growth for *P. variotii* were 0.125 and 0.25 µg/ml, respectively. Itraconazole was inactive in vitro

against most isolates, with the MIC at which 90% of the isolates were inhibited being >32 µg/ml after both 48 and 72 h of incubation. An attempt was made to establish the exact MIC of itraconazole by an agar dilution method. Serial dilutions ranging from 32 to 512 µg of itraconazole per ml were made in RPMI 1640 agar. The growth of none of the *S. prolificans* isolates was inhibited by any of these concentrations after 48 h of incubation. Therefore, a MIC of 64 µg/ml was chosen for calculations for those isolates that grew in the wells that contained the highest concentration of itraconazole. The MIC of terbinafine at which 90% of the isolates were inhibited was 2 µg/ml after 48 h but increased to 64 µg/ml after 72 h. Synergism was found for 19 of 20 (95%) of the *S. prolificans* isolates after 48 h and for 17 of 20 (85%) of the isolates after 72 h of incubation. For three isolates the effect of the combination appeared to be indifferent after 72 h of incubation, and antagonism was not observed (Meletiadis *et al,* 2000).

Mock *et al* (1998) studied the sensitivity of different species of dermatophytes towards terbinafine and itraconazole, and compared the results with a retrospective study on 35 immunocompetent patients with tinea capitis who were treated with terbinafine (Lamisil®). Each tested species of dermatophyte was sensitive to terbinafine and itraconazole at different concentration ranges. The MIC for terbinafine ranged from 0.005 to 0.5 µg/ml and for itraconazole from 40 to 80 µg/ml. *Microsporum canis* was the dermatophyte least sensitive to terbinafine. The study showed that the cure rate was excellent for *Trichophyton violaceum* and *T. soudanense*, variable for *T. mentagrophytes* and poor for *M. canis* and *M. langeronii.*

Adverse reactions to terbinafine are in general transient and mild. The incidence of

these reactions has been found to be 10.5% in a large scale study. These adverse reactions are mostly with gastrointestinal system and the skin (Hall *et al,* 1997). Reversible agranulocytosis has been reported as a rare side effect (Ornsteins and Ely, 1998).

Terbinafine is one of the mainstays of treatment of dermatophytosis. Compared to the previously existing antifungal agent, griseofulvin, it is more effective, as well as being significantly less toxic. Moreover, the required duration of therapy is also shorter with terbinafine.

This property is of interest, particularly in cases of onychomycosis where prolonged courses of therapy are needed (Arenas *et al,* 1995). Terbinafine is a safe and efficacious agent in treatment of onychomycosis (Drake *et al,* 1997; Hecker, 1997), as well as other dermatophytosis. It appears to be similarly or more effective than its alternative, itraconazole and fluconazole (DeBacker *et al,* 1998; Roberts, 1994; Havu *et al,* 2000).

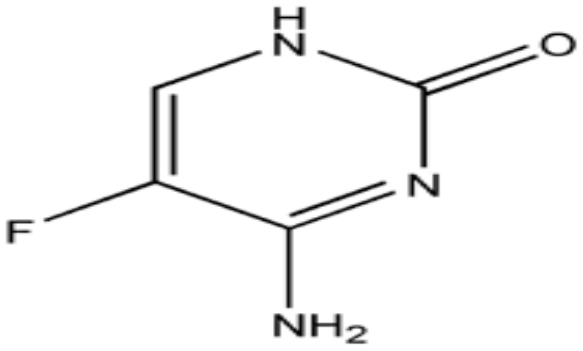
Terbinafine, when combined with fluconazole, has occasionally been successful in treatment of oropharyngeal infections due to fluconazole-resistant *Candida* spp. (Ghannoun and Elewski, 1999). A report has suggested a possible role for terbinafine as drug of choice against azole-resistant oropharyngeal infections (Vazquez *et al,* 2000)

d) Antimetabolite Drugs

5-Fluorocytosine acts as an inhibitor of both DNA and RNA synthesis via the intracytoplasmic conversion of 5-fluorocytosine to 5-fluorouracil.

FLUCYTOSINE

Flucytosine (5-fluorocytosine; 5-FC; 4-amino-5-fluoro-2-pyrimidone) is an antimetabolite type of antifungal drug. It is chemically a pyrimidine. It is activated by deamination within the fungal cells to 5-fluorouracil.



flucytosine

Flucytosine is the the only available antimetabolite drug having antifungal activity. It inhibits fungal protein synthesis by replacing uracil with 5-flurouracil in fungal RNA. Flucytosine also inhibits thymidylate synthetase via 5-fluorodeoxy-uridine monophosphate and thus interferes with fungal DNA synthesis. Flucytosine is active against *Candida spp*, *Cryptococcus neoformans*, *Aspergillus spp.* and the

dematiaceous fungi, *Phialophora spp* and *Cladosporium spp.* causing chromoblastomycosis.

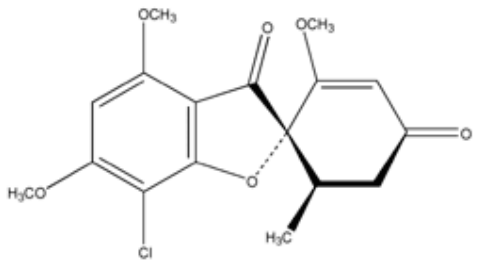
While flucytosine is in clinical use for few specific indications, its use alone in treatment frequently results in emergence of resistance. This resistance has been ascribed to mutations in cytosine permease or cytosine deaminase enzymes. Thus, flucytosine is always administered with amphotericin B (Dismukes *et al,* 1983) or fluconazole (Mayanja-Kizza *et al,* 1998) or with both amphotericin B and fluconazole together (Diamond *et al*, 1998; Just-Nubling *et al*, 1996) as combination therapy. Amphotericin B and flucytosine combination has proven to be favorable in treatment of cryptococcal meningitis (Dismukes *et al,* 1987). Primary resistance to flucytosine by Candida strains has also been speculated as a possibility (Barchiesi *et al,* 2000).

The adverse side effects of flucytosine has been reported to include gastrointestinal intolerance and bone marrow depression. Rash, hepatotoxicity, headache, confusion, hallucinations, sedation and euphoria have also been observed

Since flucytosine is commonly combined with amphotericin B, the renal impairment caused by amphotericin B has been speculated to probably increase the flucytosine levels in the body and thus potentiate its toxicity. The increase in toxicity of flucytosine is presumably ascribed to 5-fluorouracil produced from flucytosine released by bacteria in gut lumen.

e) OTHER ANTIFUNGAL AGENTS

GRISEOFULVIN



griseofulvin

Griseofulvin is effective on a specific group of fungi such as dermatophytes. The *in-vitro* activity of griseofulvin has been tested against various dermatophytes. Griseofulvin yields higher MICs compared to terbinafine and itraconazole when tested against *Trichophyton rubrum* isolates (Jessup *et al,* 2000; Korting *et al,* 1995). It is also less active than voriconazole against most dermatophytes (Wildfeuer *et al,* 1998). The *in-vitro* activity of griseofulvin against *Trichophyton mentagrophytes* has been reported to be less compared to that displayed by *Trichophyton rubrum* (Korting *et al*,1995).

Adverse reactions of griseofulvin are uncommon. Nausea, diarrhea, headache, skin eruptions and photosensitivity are occasionally observed. Hepatotoxicity and neurological side effects are rarely observed (Korting *et a,l* 1993; Montero-Gei, 1998).

Griseofulvin has been the first-line drug for treatment of dermatophytosis for many years. However, following the emergence of alternatives such as itraconazole and

terbinafine, its use, has been limited. The major advantages of these newer agents over griseofulvin are their reduced toxicity, enhanced efficacy, and shorter duration of therapy (Hecker, 1997; Roberts, 1994).

1.5.3 SODIUM PROPIONATE

This chemical antifungal agent is also known as propionic acid or sodium salt. It has a chemical formula as follows CH_3CH_2COONa. It is transparent crystal, granular, deliquescent in moist air, neutral to slightly alkaline in reaction to litmus. One gram dissolves in approximately one ml of water, in approximately 24 ml of alcohol. It is most active at an acid pH (Budavari 1996). Propionic acid occurs naturally as the result of metabolic prosesses, and can be obtained through fermentation of Propionibacterium.

Sodium propionate is used as a fungicide and for mold prevention. It is commonly used as a food additive, particularly in baked goods, confections, and gelatine. It is also used in cosmetics. It is used as a topical antifungal agent in livestock, and also as a preservative for hay and silage.

Propionic acids and its salts, including sodium propionate, is toxic to molds and certain bacteria based on the inability of the affected organisms to metabolize the three-carbon chain(Budavari, 1996).

1.5.4 LAURIC ACID

Lauric acid has a molecular formular of $C_{12}H_{24}O_{2.}$ It is a medium-length long-chain fatty acid. In its solid form, the compound occurs as white or colorless needlelike crystals that melt at approximately 44^0C. Lauric acid is extremely irritating to the gastrointestinal system in its pure state. It is found in the form of glycerides in a number of natural fats and oils, especially those of the coconut and palm kernel. Lauric acid has the additional beneficial function of being formed into monolaurin in the human or animal body (Rouse *et al*, 2005).

Monolaurin, help in inactivating viruses such as measles, herpes, vesicular stomatitis and cytomegalovirus (CMV). Monolaurin, of which the precursor is lauric acid, disrupts the lipid membranes of envelope viruses and also inactivate bacteria, yeast and fungi. The action attributed to monolaurin is that of solubilizing the lipids in the envelope of the virus causing the disintegration of the virus envelope. Other pathogens inactivated by monolaurin include HIV, measles, vercular stomatitis virus (VSV), herpes simplex virus (HSV-1), visna, cytomegalovirus (CMV), Influenza virus, pneumonovirus, Syncytial virus and rubeola. Some bacteria inactivated by monolaurin include Listeria, S*taphylococcus aureus, Streptococcus agalactiae*, Groups A, B, F and G streptococci. It is active against gram-positive and gram-negative organisms, if they are treated with chelator (Rouse *et al*, 2005).

Due to increasing mupirocin resistance, alternatives for *Staphylococcus aureus* nasal decolonization are needed. Lauric acid monoesters combined with lactic, mandelic, malic, or benzoic acid are being evaluated as possible alternatives. The *in-vitro* activity of 13 lauric acid monoester (LAM) formulations and mupirocin were determined

against 30 methicillin-susceptible *S. aureus* (MSSA) isolates and 30 methicillin-resistant *S. aureus* (MRSA) isolates. A murine model of MRSA nasopharyngeal colonization was then used to compare the *in-vivo* activity of mupirocin with three LAM formulations. MSSA and MRSA MIC_{90} values were 0.25 µg/ml for mupirocin and ≤4 µl/ml for all LAM formulations tested. (Rouse *et al* 2005)

1.4 TREATMENT OF TINEA CAPITIS

Griseofulvin has been the treatment of choice for 40 years, with good evidence of efficacy in infections caused by *T. tonsurans* and *M. canis* (Caceres-Rios *et al,* 2000; Fuller *et al,* 2001 and Guptal *et al,* 2001)

Griseofulvin taken orally at a dose of 15-25 mg/kg/day is still the treatment of choice for tinea capitis. As a fatty meal enhances griseofulvin absorption, it should be taken during a meal or directly after food. Treatment for 6-8 weeks usually suffices but it is recommended to perform cultures every few weeks and to continue the treatment until the culture is negative.

Itraconazole has been found to be very effective in tinea capitis. Itraconazole should be dosed according to body weight at about 3 to 5 mg/kg/day. A continuous therapy with itraconazole (100 mg/day) for 4-6 weeks has been reported as very effective (Legendre and Esola-Macre, 1990; Greer, 1996). Availability of itraconazole in a liquid formula permits the administration of a more precise dose than using the non-divisible capsules. Gupta *et al* (2001) used intermittent pulse therapy, 5 mg/kg/day; each pulse lasted one week, with two weeks off the drug between the first and second pulses and

three weeks off between the second and third pulses. The third pulse was not necessary in every patient. It has been reported that itraconazole was found to be effective and resulted in both clinical and mycologic cure with intermittent pulse therapy. In itraconazole pulse therapy the drug is largely eliminated from the plasma within 7 to 10 days, thereby reducing the potential for adverse effects. Itraconazole should not be used with terfenadine or other non-sedating antihistamines owing to potential combined cardiac toxicity.

Fluconazole, another azole anti-fungal, was also found to be a promising drug for tinea capitis (Mercurico *et al*, 1998). A continuous treatment with a dose of 5 mg/kg/day for 4 weeks was found effective in cases of tinea capitis caused by *Trichophyton species*. Availability of fluconazole in oral suspension makes it a useful alternative in paediatric patients.

Terbinafine, a member of the allylamine family, is also a useful agent in tinea capitis. It appears very effective in infections with *T. violaceum*. The dose of 62.5-250 mg/kg/day for 4-6 weeks, depending on body weight, is usually required in infections caused by this fungus. *M. canis* usually requires higher doses and a longer period of administration, 10 to 12 weeks. Terbinafine, initially considered free of any side-effect potential, lost a bit of its innocuous image since, with wide use; several unwanted effects have been reported (most often blood dyscrasisias and hepatotoxicity). It is never-the less a safe drug and there do not seem be to any absolute contra-indications.

1.6 DRUG RESISTANCE IN FUNGI

In spite of the availability of effective drugs and vaccines, the battle against infectious diseases is far from being over. Not only do they continue to cause a large number of infections and deaths, particularly in developing countries, but the emergence and spread of antimicrobial resistance is now threatening to undermine the ability to treat infections and save lives.

In general, antimicrobial agents act by interfering with specific processes that are essential for the growth and/or replication of a microorganism. Agents are commonly separated into groups based on their specific pharmacologic mechanisms of action: inhibitors of cell wall synthesis (penicillins, cephalosporins, vancomycin, cycloserine, fosfomycins); inhibitors of normal cytoplasmic membrane structure and function (polymyxins, polyenes, imidazoles); direct and indirect inhibitors of nucleic acid synthesis (nitroimidazoles, quinolones, sulfonamides, trimethoprim); and inhibitors of protein synthesis or ribosome function (aminoglycosides, tetracycline, chloramphenicol, erythromycin, clindamycin). In addition, antimicrobial agents are classified as either bactericidal /fungicidal (those that kill the target bacterium or fungus) or bacteriostatic/fungistatic (those that inhibit the microorganism's growth). Although bactericidal agents are more efficient, bacteriostatic agents can also be extremely beneficial, since they permit normal defenses of the host to destroy the microorganisms.

1.6.3. PREVENTION AND CONTROL OF ANTIFUNGAL RESISTANCE

Strategies to avoid and suppress the emergence of antifungal resistance have not been defined. However, approaches analogous to those recommended for antibacterials (Cohen, 1992; Levy, 1990; Shales *et al,* 1997) could be suggested. These measures include (i) prudent use of antifungals, (ii) appropriate dosing with special emphasis on avoiding treatment with low antifungal dosage, (iii) therapy with combinations of existing agents, (iv) treatment with the appropriate antifungal (in cases where the etiological agent is known), and (v) use of surveillance studies to determine the true frequency of antifungal resistance. It should be emphasized that data supporting the use of the suggested measures is largely lacking, and ongoing studies may provide some specific guidelines in the near future. Additionally, advances in rapid diagnosis of fungi may be helpful in reducing the use of inappropriate antifungals to treat organisms that are resistant to a particular agent. Unfortunately, progress in developing diagnostic methods specific to fungi has been slow. The recent approval of a reference method for the antifungal susceptibility testing of yeast (NCCLS, 1997) is encouraging and provides a means for performing surveillance studies.

1.7.5 METHOD FOR STUDYING ANTIFUNGAL COMBINATIONS

Calculation of the fractional inhibitory concentration (FIC) index (FICI) by the use of the Checkerboard method has long been the most commonly used way to characterize the activity of antimicrobial combinations in the laboratory (Eliopolos and Moellering, 1991). The FICI represents the sum of the FICs of each drug tested, where the FIC is

determined for each drug by dividing the Minimum Inhibitory concentration (MIC) of each drug when used in combination by the MIC of each drug when used alone. Stated in terms of the Loewe additivity model, the FICI model assumes that indifference is seen when this equation is true: $1 = (MIC_{drug\ A\ in\ combination}/MIC_{drug\ A\ alone})+ (MIC_{drug\ B\ in\ combination}/MIC_{drug\ B\ alone})$. When the equation <1 synergism occurs and antagonism is observed when the equation >1

1.8 AIMS AND OBJECTIVES

Tinea capitis a highly contagious infection of the scalp and hair which occurs predominantly in children is endemic in some of the poorest countries (Gonzalez *et al* 2004). Nigeria is no exception. It is a public health problem because of its increased incidence and epidemic transmission. Ajao and Akintunde found a prevalence rate for clinical infection among school children in Ile-Ife of 14.02% (Ajao and Akintunde, 1981). Tinea capitis is the most common paediatric dermatophyte infection worldwide. It affects mostly children of primary school age. The available drugs for treatment are expensive with long duration of treatment and high level of toxicity (Ghannoun and Rice, 1999). The main objective was to come up with an alternative that could be cheaper with reduced toxicity enhanced efficacy and shorter duration of therapy.

This study, therefore aims at

i. Isolating and identifying the organisms associated with Tinea capitis.

ii. Testing the susceptibility of the isolated organisms to the selected compounds.

iii. Determining the rate of kill of the most resistant isolate.

iv. Testing Antifungal combination using rate of kill of resistant fungal spore isolate to determine if there is synergistic or additive effect.

CHAPTER TWO

2.0 MATERIALS AND METHODOLOGY

2.1 MATERIALS

2.1.1 ORGANISMS USED

a) Trichophyton tonsurans

b) Pityriasis furfur

c) Trichophyton mentagrophyte

d) Philaspora hortei

e) Trichophyton rubrum

f) M. canis

g) Trichophyton spp

The above mentioned dermatophyte isolate were obtained from school children infected with Tinea capitis in L.E.A. Primary School Mando, Kaduna

2.1.2 CULTURE MEDIA AND SUSPENDING MEDIA

a) SABOURAUD DEXTROSE AGAR (BIOTEC)

b) SABOURAUD DEXTROSE LIQUID MEDIUM (OXOID)

c) HARVESTING DILUENT

2.1.3 ANTIFUNGAL AGENTS TESTED

a) FLUCONAZOLE obtained from Pfizer (Nigeria)

b) TERBINAFINE obtained from Novartis (Nigeria)

c) SODIUM PROPIONATE (BDH)

d) LAURIC ACID (BDH)

2.1.4 OTHER MATERIALS

70% EHANOL (BDH)

TWEEN 80 (KOCH)

LACTOPHENOL (BDH)

STREPTOMYCIN from RIKA PHARMA (Nigeria)

PENICILLIN from DOYIN (Nigeria)

CYCLOHEXIMIDE (BDH)

DISTILLED WATER

2.2 METHODOLOGY

2.2.1 PREPARATION OF MEDIA

For the culture medium, 62g of sabouraud deXtrose agar was weighed (for double strength 124g was weighed) and dissolved in 1 litre of distilled water. It was heated to dissolve and 10mls dispensed in universal bottles and sterilized by autoclaving at 121^0C for 15 minutes and stored until required for use.

For the recovery broth, 30g of sabouraud dextrose liquid medium powder was weighed and dissolved in 1 litre of distilled water. It was heated to dissolve and 5ml dispensed into universal bottles and sterilized by autoclaving at 121^0C for 15 minutes.

For suspending medium 9g of sodium chloride was weighed and dissolved in 1 litre of distilled water and 0.05% v/v of Tween 80 was added. This was then sterilized by autoclaving at 121^0C for 15 minutes.

2.2.2 COLLECTION OF SAMPLES AND ISOLATION OF TEST FUNGI SPORE.

The verbal consent of L.E.A. Primary School, Mando Kaduna was obtained in order to collect samples from affected school pupils. Samples were collected in bottles of 10

samples made up of 5 males and 5 females each at a time with age ranging from 5 to 12 years. Pre-adolescent children are the primary victims of scalp ringworm (Tinea Capitis). Not until puberty do glands secrete oil containing medium chain fatty acids that help protect scalp from skin fungus.

The samples were collected by scrapping the affected part of the head with sterile scalpel and the dust collected on a clean sheet of paper. These samples were stored in an air-tight and dust free container.

Sabouraud dextrose agar supplemented with 4µg/ml of streptomycin, 20 i:u/ml of penicillin, 0.5%w/v of cycloheximide were prepared. The streptomycin and penicillin inhibits the growth of bacteria while cycloheximide prevents the growth of fungi other than dermatophytes. This preparation was dispensed into 10ml bottles and sterilized by autoclaving at 121^{o}C for 15 minutes. Each bottle of the prepared SDA was aseptically poured into sterile plate and allowed to set.

2.2.3 **INOCULATION OF TINEA CAPITIS SAMPLES ON SDA**

A sterile cotton swab dipped in sterile normal saline with 0.05% Tween 80 was rolled on the sample collected from the school children and spread on the SDA plates. These plates were then incubated at 30^{o}C for seven days. Suspected isolates were subcultured onto SDA slants repeatedly to get pure culture. From these slants, subsequent subculturing were done at regular intervals and stored in the refrigerator at 4^{0}C till required.

2.2.4 **MICROSCOPY AND IDENTIFICATION**

Slides were prepared from the plates showing fungi spores. A drop of cotton blue lactophenol was put on the slide. A loopful of the organism from the portion that showed fungi growth was cut and spread on the slide with the aid of an inoculating pin. This was then mounted on the microscope and the morphological characteristics examined and identified.

2.3 **PREPARATION OF SPORE SUSPENSION**

Sterile beads of medium sizes were added to the slant culture of organisms. Ten-milliliter aliquots of normal saline containing 0.05% v/v Tween 80 was added to the same agar slant and shaken to harvest the spores. The harvested spores were aseptically washed with 10ml of sterile harvesting medium eight times to ensure reasonable population density of the spores. The spores suspension was then stored in the refrigerator at 4^0C for subsequent use.

2.4 **PREPARATION OF ANTIFUNGAL AGENTS**

2.4.1 **FLUCONAZOLE**

The solution was prepared by weighing 4g of the compound and dissolving in 100ml of ethyl-alcohol and the volume made up to 250ml with sterile distilled water. This gave a concentration of 16.0mg/ml. 10ml of this solution was added to 10ml of sterile distilled water to obtain a concentration of 8.0mg/ml. Subsequent dilutions were made with

10ml of sterile distilled water to obtain concentrations ranging from 0.0078mg/ml – 16.0mg/ml

2.4.2 **TERBINAFINE**

The solution was prepared following the same procedure as for fluconazole.

2.4.3 **LAURIC ACID**

The solution was prepared by weighing 4g of the compound and dissolving in 250ml of ethylalcohol. This gave a concentration of 16.0mg/ml. 10ml of this solution was added to 10ml of sterile distilled water to obtain a concentration of 8.0mg/ml. Subsequent dilutions were made with 10ml of sterile distilled water to obtain concentrations ranging from 0.00 78mg/ml –16.0mg/ml

2.4.4 **SODIUM PROPIONATE**

The solution was prepared by weighing 60g of the compound and dissolving in 250ml of sterile distilled water. This gave a concentration of 240.0mg/ml. 10ml of this solution was added to 10ml of sterile distilled water to obtain a concentration of 120.0mg/ml. Subsequent dilutions were made with 10ml of sterile distilled water to obtain concentrations ranging from 25.0mg/ml –240.0mg/ml

2.5 **DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF TEST ANTIFUNGAL AGENTS.**

For the minimum inhibitory concentration (MIC), graded concentrations of the test antifungal agents were prepared and aseptically mixed with 10ml double strength

SDA. These were aseptically poured into petri dishes and allowed to set firmly at room temperature. 10µl of standardised spores suspensions containing 10^4 cfu were inoculated on equidistantly placed sterile membrane filter disc. The plates were allowed to stand for one hour and then incubated at 30^0C for 5 days. The lowest concentration of the agent that inhibited visible growth of the test organism was taken as the MIC.

For the MIC determination of the antifungal agents in admixture, graded concentrations of the two antifungal agents at different ratios were prepared with one antifungal agent at sub-inhibitory concentration fixed and the second one at sub-inhibitory concentration varied and mixed to 10ml volume. This was poured with 10ml of melted double strength SDA into sterile plates aseptically, 10µl spores suspensions containing 10^4 cfu were inoculated on equidistantly placed sterile membrane filter disc. The plates were allowed to stand for one hour and then incubated at 30^0C for 5 days. The lowest concentration of the combined agents that inhibited visible growth of the organism was taken as the combined MIC.

2.6 **DETERMINATION OF MINIMUM FUNGICIDAL CONCENTRATION (MFC)**

The membrane filter disc showing no visible growth from the determination of minimum inhibitory concentration were removed and placed in drug free sabouraud dextrose liquid medium supplemented with 5%v/v Tween 80 and incubated at 30^oC for 5 days. The lowest concentration of the antifungal agents that killed the test fungal organism which showed no growth was taken as the MFC.

2.7 DETERMINATION OF THE RATE OF KILL

One mililitre of standardized culture of resistant *Trichophyton Mentagrphyte* (10^8 spores per ml) was inoculated to the 9ml volume of aseptically prepared chemical agents. Samples were taken at 10, 20, 30, and 60 minutes intervals and diluted by ten-fold dilution protocol with sterile normal saline containing 5% tween 80. These were mixed with melted (40^oC) Sabouraud Dextrose Agar and plated in duplicates aseptically. These plates were incubated at 30^0C for five days. The colonies were counted using a colony counter.

CHAPTER THREE

3.0 RESULT

3.1 FUNGI ISOLATES

The dermatophytic fungi isolated from the heads school children are as shown in Table 3.0. The *Trichophyton spp* had the highest order of prevalence in this study followed by *P. furfur* with 6 isolates while *Philaspora hortei* and *M. canis* had 4 and 3 isolates respectively.

TABLE 3.0

INFECTIVE FUNGI ISOLATED FROM SCHOOL CHILDREN WITH CLINICAL SYMPTOMS OF TINEA CAPITIS IN KADUNA CITY, NIGERIA.

ORGANISM	NO OF ISOLATES	%FREQUENCY
1. *T.mentagraphytes*	6	20.00
2. *T. tonsurans*	2	6.67
3. *T.rubrum*	2	6.67
4. *Tricophyton spps*	7	23.33
5. *Pityriasis furfur*	6	20.00
6. *Philaspora hortei*	4	13.33
7. *M. canis*	3	10.00
TOTAL ISOLATES	30	100.00

3.2 MINIMUM INHIBITORY CONCENTRATION (MIC) & MINIMUM FUNGICIDAL CONCENTRATION (MFC) OF THE TEST ANTIFUNGAL AGENTS AGAINST ISOLATES FROM THE SCHOOL CHILDREN

The MICs of the test antifungal agents on the test organism are as shown in Table 3.1. The MICs of fluconazole against *T. mentagrophytes Trichophyton spps, Pityriasis furfur, Philaspora hortei* and *M. canis* isolates were found to be the same range of 0.5-1.00 mg/ml while the MIC of fluconazole against *T. tonsurans* and *T. rubrum* were found to be 0.25-0.50mg/ml and 1.00mg/ml respectively. That of terbinafine against *M. canis* and *Pityriasis furfur* was also found to be the same range of 0.50-1.00mg/ml while that of *T. mentagrophyte* and *Philaspora hortei* were found to range from 0.5-2.00 mg/ml, but *T. tonsurans* and *T. rubrum* had the same MIC of 1.00mg/ml while *Trichophyton spps* had MIC range of 0.25-1.00mg/ml

The MICs of Lauric acid against the isolates was found to be the same (1.00-2.00mg/ml) except against *M.canis*, which was found to be 1.00mg/ml. The MICs of Sodium propionate against *T. mentagrophyte, Pityriasis furfur, Philaspora hortei* and *M. canis* were found to be the same 80-100mg/ml while that against *T. tonsurans, T. rubrum* and *Trichophyton spp* were 80, 40-80 and 40-100 mg/ml respectively

The MFC of the test antifungal agents on the isolates are shown in Table 3.2. The MFC of both fluconazole and Terbinafine ranged from 1.00-8.oomg/ml while the MFC of Lauric acid ranged from 2.00-8.00mg/ml. The MFC of sodium propionate ranged from 80-120mg/ml.

Table 3.1: MIC OF THE TEST ANTIFUNGAL AGENTS AGAINST THE FUNGAL ISOLATES (1.56 x 10^6 spores/ml)

Test organism isolated	Frequency	Terbinafine mg/ml	Fluconazole mg/ml	Lauric acid mg/ml	Sodium propionate mg/ml
T. mentagrophyte	6	0.50-2.00	0.50-1.00	1.00-2.00	80.00-100.00
T. tonsurans	2	1.00	0.25-0.50	1.00-2.00	80.00
T. rubrum	2	1.00	1.00	1.00-2.00	40.00-80.00
Trichophyton spp	7	0.25-1.00	0.50-1.00	1.00-2.00	40.00-100.00
Pityriasis furfur	6	0.50-1.00	0.50-1.00	1.00-2.00	80.00-100.00
Philaspora hortei	4	0.50-2.00	0.50-1.00	1.00-8.00	80.00-100.00
M. canis	3	0.50-1.00	0.50-1.00	2.00	80.00-100.00

TABLE 3.2 MFC OF THE TEST ANTIFUNGAL AGENTS AGAINST THE FUNGAL ISOLATES (1.56 x 10^6 spores/ml)

Test organism isolated	Frequency	Terbinafine mg/ml	Fluconazole mg/ml	Lauric acid mg/ml	Sodium propionate mg/ml
T. mentagrophyte	6	4.00-8.00	1.00-8.00	2.00-8.00	100.00-120.00
T. tonsurans	2	1.00-8.00	1.00	2.00	80.00-120.00
T. rubrum	2	4.00	2.00	2.00	100.00-120.00
Trichophyton spp	7	1.00-4.00	1.00-8.00	2.00-8.00	100.00-120.00
Pityriasis furfur	6	2.00-8.00	8.00	2.00-4.00	80.00-120.00
Philaspora hortei	4	1.00-4.00	1.00-4.00	2.00-8.00	100.00-120.00
M. canis	3	1.00-2.00	1.00-2.00	2.00	120.00

T. mentagrophyte (isolate number 18) was selected as the most resistant of the 30 isolates that were isolated from the school children. The MIC and MFC of the test antifungal agents needed against the *T. mentagrophyte* were the highest.

3.3 DETERMINATION OF MIC & MFC OF THE TEST ANTIFUNGAL AGENTS IN ADMIXTURES

The result of the combination of Terbinafine and Sodium propionate (T/S) against resistant *T.mentagrophyte* showed that a concentration of 0.8mg/ml and 3mg/ml respectively inhibited the test organism while a concentration of 0.2mg/ml terbinafine and 12mg/ml sodium propionate inhibited the same test organism. The FIC was found to be synergistic (0.57) see table 3.4 below.

Table 3.3: FRACTIONAL INHIBITORY CONCENTRATION (FIC) OF TERBINAFINE AND SODIUM PROPIONATE AGAINST RESISTANT *T. mentagrophyte*

Concentration of Terbinafine in Admixture (mg/ml)	FIC	Concentration of Sodium propionate in Admixture (mg/ml)	FIC	∑FICs
0.8	0.8	3.0	0.03	0.83.
0.6	0.6	6.0	0.06	0.66
0.4	0.4	6.0	0.06	0.46
0.2	0.2	12.0	0.12	0.32
				2.27
MIC of Terbinafine 1mg/ml		MIC of Sodium propionate 100mg/ml		

Means of sum FIC =2.27/4=0.57

Synergism = $MIC_{AB} < MIC_A + MIC_B$

The combination of Terbinafine and Sodium propionate showed the most synergistic effect against the resistant isolate while the combination of Terbinafine and Lauric acid was the least synergistic see table 3.4 below.

Table3.4: SUMMARY OF THE FICs OF THE TEST ANTIFUNGAL AGENTS IN COMBINATION AGAINST RESISTANT *T. mentagrophyte*

Combination of Antifungal Agents	Mean of FICs	Inference
Terbinafine/Lauric acid	0.80	SYNERGISTIC
Terbinafine/sodium propionate	0.57	SYNERGISTIC
Fluconazole/Lauric acid	0.75	SYNERGISTIC
Fluconazole/Sodium propionate	0.60	SYNERGISTIC

3.4 RATE OF KILL OF TEST ANTIFUNGAL AGENTS ALONE AND IN ADMIXTURE AGAINST RESISTANT *T. mentagrophyte*

The rate of kill obtained for the test agents are shown in fig.3.5-3.9. The fungal spores log reduction after 30 minutes contact time was 4.4, 4.2, 4.1 and 3.9 for lauric acid, terbinafine, fluconazole, and sodium propionate respectively. Lauric acid gave the highest log reduction of the spores. However at the end of the 60 minutes contact time log reduction of the spores was 5.3, 5.2, 5.0 and 4.3 for fluconazole, terbinafine, lauric acid and sodium propionate respectively with fluconazole giving the highest log reduction of the spores.

The exponential death phase of the test fungal spores was in the first 30 minutes and this was followed by a drastic reduction in the rate of kill as shown in fig 3.5. Ten

milligram per ml (10mg/ml) of terbinafine effected 4.2 log kill of the resistant *T. mentagrophyte* within the first 30 minutes while not more than 1.0 log kill was effected within the next 30 minutes an indication of decrease in death rate. Also 200mg/ml of sodium propionate effected 3.9 log kill of the resistant *T. mentagrophyte* within the first 30 minutes while not more than 0.4 log kill of the resistant *T. mentagrophyte* was effected in the next 30 minutes.

Figs 3.2-3.5 show the rate of kill of the antifungal agents alone and when in combination against the resistant *T. mentagrophyte*. 10mg/ml of terbinafine and 200mg/ml of sodium propionate after 30 minutes contact time effected 4.2 and 3.9 log kill of the test spore respectively. When the two antifungal agents were combined there were no surviving spores after 20 minutes contact (fig 3.2). Similarly 10mg/ml of terbinafine and 10mg/ml of lauric acid after 30 minutes contact time effected 4.2 and 4.4 log kill of the test spores respectively. However when the two antifungal agents were combined there were no surviving spores after 30 minutes (fig 3.4).

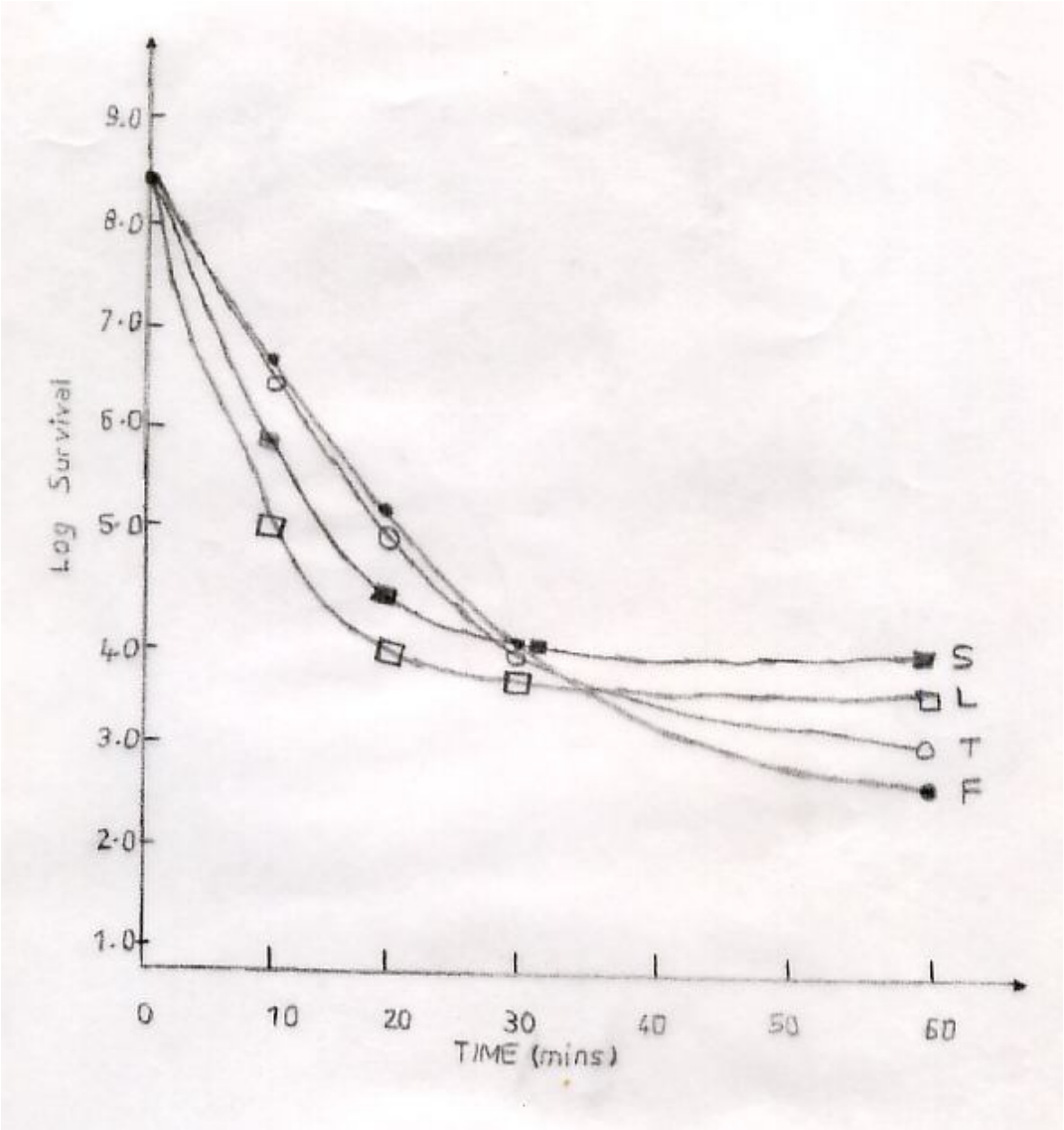


Fig. 3.1 Survival of Resistant *T*. *Mentagrophyte* Spores Suspension with fixed concentration of Test Antifungal Agents at Different Time Intervals.

KEY

○	T	-	10.0mg/ml Terbinafine
●	F	-	10.0mg/ml Fluconazole
□	L	-	10.0mg/m Lauric Acid
■	S	-	200.0mg/ml Sodium Propionate

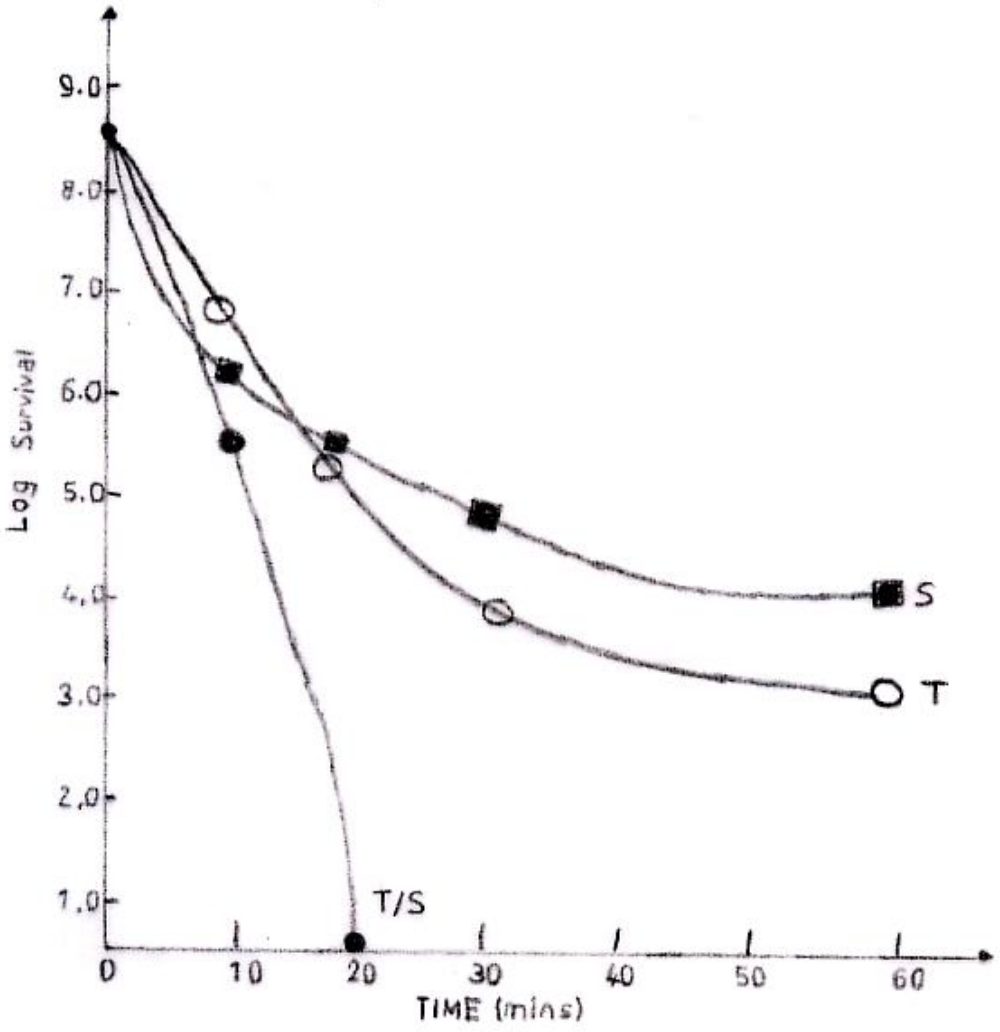


Fig.3.2. Survival of Resistant *T. Mentagrophyte* Spores Suspension with Fixed Concentration of Test Antifungal Agents (alone & in admixture) at Different Time Intervals.

KEY

●	T/S	-	10.0mg/mlTerbinafine/200mg/mlSodium Propionate
○	T	-	10.0mg/ml Terbinafine
■	S	-	200.0mg/ml Sodium Propionate

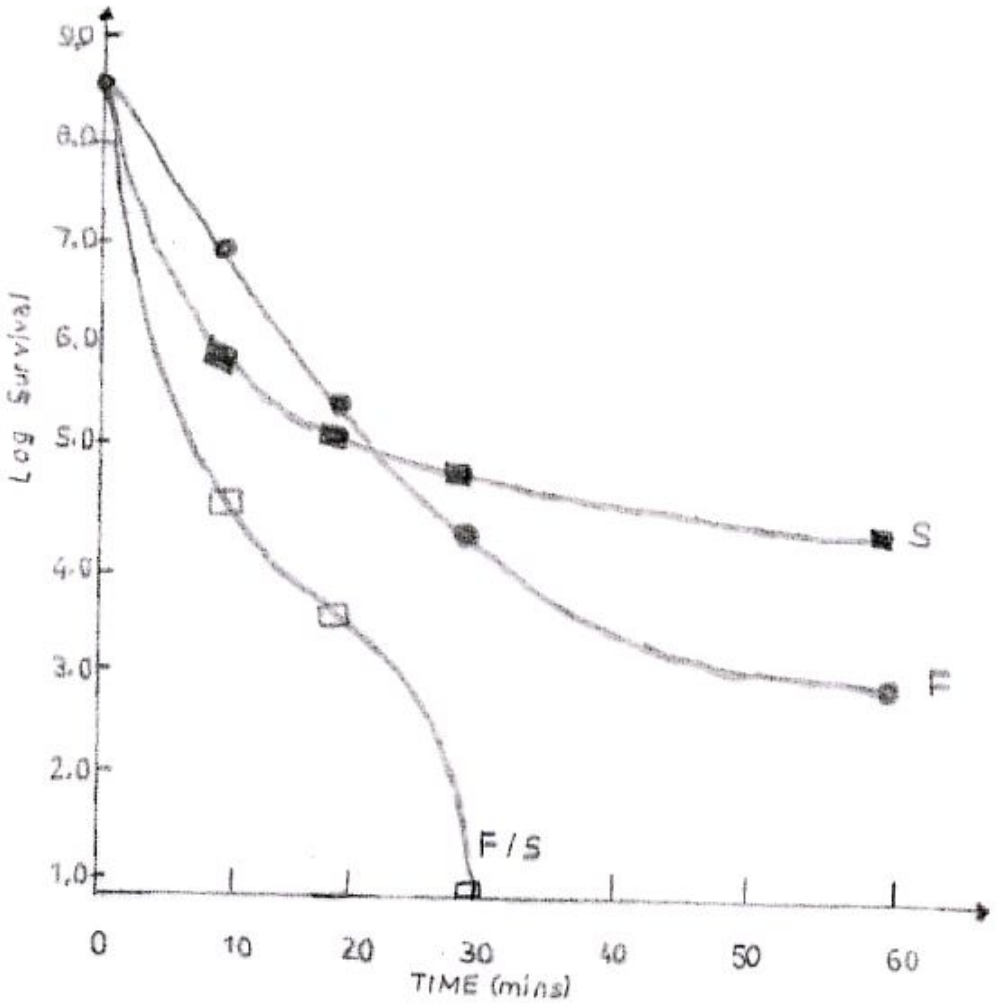


Fig.3.3. Survival of Resistant *T*. *Mentagrophyte* Spores Suspension with Fixed Concentration of Test Antifungal Agents (alone & in admixture) at Different Time Intervals.

KEY

□	F/S	10.0mg/mlFluconazole/ 200mg/mlSodium Propionate
○	F	10.0mg/ml Fluconazole
■	S	200.0mg/ml Sodium Propionate

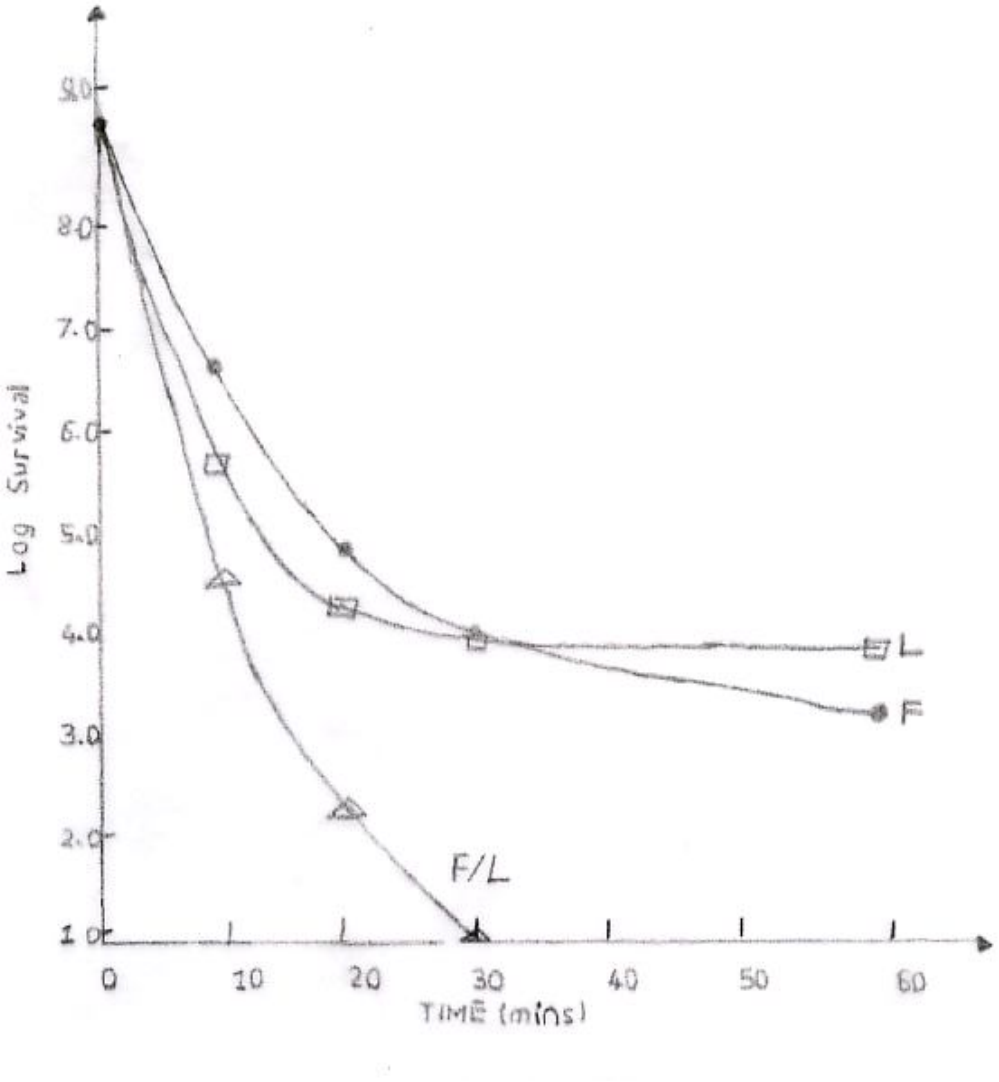


Fig.3.4. Survival of Resistant *T. Mentagrophyte* Spores Suspension with Fixed Concentration of Test Antifungal Agents (alone & in admixture) at Different Time Intervals.

KEY

△	F/L	-	10.0mg/mlFluconazole/ 10.0mg/mlLauric Acid
●	F	-	10.0mg/ml Fluconazole
□	L	-	20.0mg/ml Lauric Acid

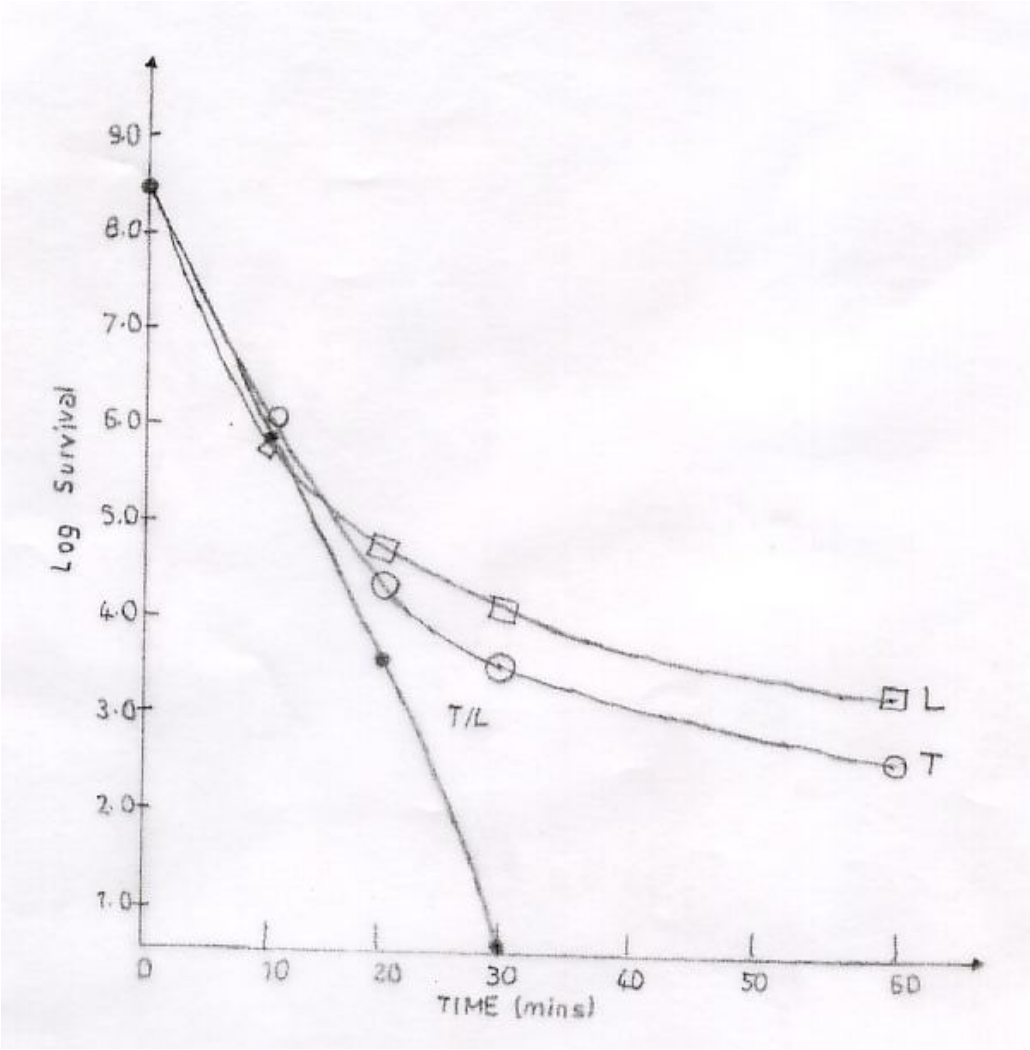


Fig.3.5. Survival of Resistant *T. Mentagrophyte* Spores Suspension with Fixed Concentration of Test Antifungal Agents (alone & in admixture) at Different Time Intervals.

KEY

●	T/L	-	10.0mg/mlTerbinafine /10.0mg/mlLauric Acid
○	T	-	10.0mg/ml Terbinafine
□	L	-	10.0mg/ml Lauric Acid

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1 GENERAL DISCUSSION

Tinea capitis (scalp ringworm) is a highly contagious infection of the scalp and hair caused by dermatophyte fungi such as M. canis, M. audounii. It occurs in all age group but predominantly children. It is endemic in some of the poorest countries (Gonzalez *et al* 2004).

One of the greatest problems hindering the eradication and prevention of Tinea capitis is the presence of healthy, asymptomatic carriers. It has been reported that asymptomatic carriers might be equal to symptomatic sufferers. As many as 14% of asymptomatic children have been found to be carriers of causative dermatophyte for tinea capitis in a primary school in Philadelphia (Williams *et al*, 1995). Without therapy, 4% developed symptoms of infection, 58% remained culture positive, and 38% became culture negative within an average 2-3-month follow-up period.

Asymptomatic carriers, who demonstrate neither signs nor symptoms of skin infection, such as adults and siblings in the family of patients with tinea capitis, patient caretakers and playmates, require active treatment, since they may act as a continuing source of infection.

4.2 Prevalence of Dermatophytic Infection in L.E.A. Primary School Mando, Kaduna

Tinea capitis is caused by fungal species of Trichophyton and Microsporum. It is the most common pediatric dermatophyte infection worldwide. It affects mostly children of primary school age (Gonzalez 2004).

The result of the isolation studies showed the presence of Trichophyton and Microsporum species. Trichophyton species were the most prevalent isolated

dermatophyte with about 56.67% of the isolated organisms. This is consistent with other reported works. *T. tonsurans* was found to be the major cause of tinea capitis in the USA (Matsuoka and Gedz, 1982; Tschen 1984) but until some years ago it was *M. canis* and *M. audouinii* (Matsuoka and Gedz 1982; Tschen, 1984).

The high prevalence rate of tinea capitis has been reported in many poor countries. In a school survey of tinea capitis in Benghazi a rate of 4.49% was observed (Al-Mosawi *et al* 1993). This is even low compared to the studies of Ajao and Akintunde who found a prevalence rate for clinical infection among schoolchildren in Ile-Ife of 14.02% and in urban and rural schools in Lusaka the prevalence rate was found to be 16.8% (Ajao & Akintunde 1985, Simpanya, 1989).

The prevalence of Tinea capitis can be attributed to a lot of factors. Many studies offer explanations for the prevalence of tinea capitis in children. It was reported that deficiency in sebum, which acts as a fungistatic factor, would favour infection. Martinez suggested that the presence of dermatophytes on a healthy scalp may be due to commensalism and that factors such as high blood sugar levels (which are favourable to skin fungi) and the presence of fatty acids in the skin (which are unfavourable) determine the presence of these organisms and explain their gradual decline with advancing age (Martinez 1980), with improved personal hygiene.

Matinez, studying 1146 people with no clinical lesions of dermatophytoses anywhere on the body, found that only 4.6% of samples from the scalps of individuals with a clean appearance tested positive, compared with 14.8% from individuals with an unclean appearance (Martinez 1980). As 12.6% of these cases were in children under 13 years of age, it seems that unclean children are the prime target of tinea capitis

and serve as the chief vehicle of transmission. This is consistent with what was observed in this study. Most of the affected children from whom samples were taken were unclean.

Poor personal hygiene is a reflection of a low standard of living and a low level of education within the family. A high level of parental education appears to be an important contributing factor in lowering the prevalence of tinea capitis. Furthermore maternal education may also play an important role in this regard, because children of uneducated mothers, in particular, may have high risk of infection in an unhealthy environment. Conversely, maternal literacy or even simple education may contribute to reducing the prevalence of the infection, irrespective of the quality of the environment in which the child lives. Contact with animals is also considered a risk factor for tinea capitis. Sehgal *et al,* (1985) found that animals played a significant role in the prevalence of tinea capitis, with 18% of infected children involved in rearing animals (Sehgal *et al* 1985).

Tinea capitis infection is also linked to overcrowding. This link between crowded living conditions and the prevalence of tinea capitis was observed by Sehgal *et al*, (1985) who noticed that 85% of school children affected with tinea capitis were from families with four or more members, all living in a single-roomed house (Sehgal *et al* 1985).

Epidemiological studies of tinea capitis have demonstrated that poor hygiene, low levels of education, proximity to livestock and overcrowding are interrelated and all contribute to the frequent transmission of the infection. Intrafamilial infection was reported in 27.5% of total cases of tinea capitis. This reflects the highly communicable nature of dermatophyte infection.

4.3 MICs and MFCs of the Test Antifungal Agents Against Isolates

The increase in dermatophytoses and the high level of therapeutic failure warrant the search for new therapeutic strategies (Tatsumi *et al* 1986). Griseofulvin has been the treatment of choice for tinea capitis for 40 years, with good evidence of efficacy in infections caused by *T. tonsurans* and *M. canis* (Caceres-Rios *et al* 2000; Fuller *et al* 2001 and Guptal *et al* 2001)

In a recent survey of griseofulvin treatment of tinea capitis, approximately 40% of patients did not respond to the drug and required additional treatment (Abdel-Rahman *et al* 1997). The goal of a new therapy for tinea capitis would be to reduce treatment duration while maintaining good efficacy and safety profiles (Schuster and Ryder 1990; Goodfield 1992).

Four antifungal agents namely Fluconazole, Terbinafine, Lauric Acid and Sodium Propionate were used in this study.

The MIC and MFC values obtained in this study showed that Fluconazole, Terbinafine, Lauric acid and Sodium propionate was the potent order of antifungal activities of the test agents. The results showed that Sodium propionate was the least effective test antifungal. Sodium propionate has been reported as a preservative in some hair cosmetic products, hence the observed high value of both MIC and MFC obtained. Terbinafine, fluconazole are new generation antifungal drugs recently introduced into the Nigerian market with high cost. The high cost of procuring these antifungal drugs probably reduced their abuse, hence the high susceptibility of these dermatophyte fungal isolates to these drugs. Lauric acid has not been widely reported in the treatment of dermatophytic infection. This may probably account for its high activity against the fungal isolates in this study. The antifungal activity of lauric acid

has been reported to be due to monolarium solubilization of the lipids in the spores' cells envelopes of the fungi leading to the disintegration of the intergrity of the fungal cell envelope.

The high resistance of dermatophyte isolates in this study to sodium may explain the frequent failure rate of the management of dermatophyte infections in Kaduna city Nigeria. Development of resistance to many antifungals by some pathogenic fungi is a major cause of concern to health workers. Indiscriminate sale of antifungals as over-the-counter (OTC) drugs have also been reported to contribute to widespread resistance development to various antifungal agents. In Nigeria, there is indiscriminate and rampant use of drugs including antifungals by general populace. The drugs are available from patent medicine shops, street drug vendors and in open markets where adequate storage conditions for the drugs are usually not observed. Most often physicians or pharmacist are never consulted before drugs are procured and used. Thus a significant proportion of the populace or parents of these school children infected with Tinea capitis might have bought antifungal creams from alleged conduits. It is also possible that inadequate regimen and extremely short duration courses were used. The contributory effect of this self-medication practice on the emergence of multiple antifungal resistance if unabated.

4.4 Fungicidal Effects of The Test Agents in Admixtures

The results in this work showed that the test antifungal agenys possess fungicidal activity. There is a slow initial kill of susceptible members of the population. This is

followed by a faster linear rate of kill showing a similar pattern to first order kinetics. This is followed by a slower death rate of resistant members. Fluconazole produced the most potent fungicidal effect singly and Terbinafine plus Sodium propionate produced the most fungicidal effect in combination. Sodium propionate produced the least fungicidal effect singly while Terbinafine plus Lauric acid produced the least fungicidal effect in admixture. The high fungicidal effect of fluconazole to the test fungal isolate may be due to low level of usage. Fluconazole is an expensive drug and so it is less abused by users.

Combination therapy has been shown to be beneficial for several difficult-to-treat infections associated with human immunodeficiency virus and mycobacterial infections which do not respond well to single-drug therapy, either due to lack of efficacy or rapid emergence of resistance (Horsburgh *et al* 2000).

The result from this study shows that the combination produced synergistic and fungicidal effect on the resistant test isolate. This is most likely due to the ability of the antifungal agents to inhibit cell processes at different levels of development. The fungicidal activity observed was rapid and generally concentration dependent.

There is no single concentration of the agents at which all cells in a suspension would be killed instantaneously. The process of killing occurs chiefly as a function of time within a range of concentrations and this probably explains the increased lethal activities of higher concentrations of these agents above the minimum effective concentration.

The antifungal agents studied have shown activities against the resistant *T. mentagrophyte* singly and in combination. On the assumption that further toxicity tests would indicate reasonable level of safety, the combination of terbinafine and Sodium propionate may prove to be a promising antifungal agent for the treatment of tinea capitis.

CONCLUSION

This study appears to represent the first report that proved:

1. Trichophyton spp are the most prevalent infective fungi in Tinea capitis among school children in L. E. A. primary school Mando, Kaduna.
2. The test antifungal agents viz: Fluconazole, Terbinafine, Lauric acid and Sodium Propionate proved effective in inhibiting isolated test dermatophytic spores.
3. Admixtures of test chemical compounds produced desirable synergistic result.
4. The rate of kill of test fungi spores by the investigated test antifungal agents was first order kinetic.
5. The result of admixtures from this study has shown a probable solution that can limit emergence of dermatophytic fungi and spores resistance to any of these test compounds.

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APPENDIX I

MIC AND MFC OF THE TEST ANTIFUNGAL AGENTS AGAINST THE FUNGAL ISOLATES

S/n	Test Organism	Terbinafine		Fluconazole		Lauric acid		Sodium propionate	
		MIC (mg/ml)	MFC (mg/ml)	MIC (mg/ml)	MFC (mg/ml)	MIC (mg/ml)l	MFC (mg/ml)	MIC (m*g/ml)*	MFC (mg/ml)
1	*Trichophyton tonsurans*	1	1	0.25	1	1	2	80	120
2	*Pityriasis furfur*	1	2	0.5	8	1	4	100	120
3	*Trichophyton specie*	1	4	0.5	8	1	8	100	120
4	*Trichophyton mentagrophytes*	1	4	0.5	8	1	2	100	100
5	*Trichophyton tonsurans*	1	8	0.5	1	2	2	80	80
6	*Trichophyton mentagrophytes*	2	8	1	2	2	2	100	120
7	*Trichophyton specie*	0.5	4	1	8	2	8	40	100
8	*Trichophyton mentagrophytes*	0.5	4	1	8	2	8	80	120
9	*Philaspora hortei*	2	4	1	1	1	2	80	120
10	*Trichophyton specie*	0.5	1	1	2	1	4	100	120
11	*Pityriasis furfur*	0.5	8	0.5	8	1	2	80	800
12	*Pityriasis furfur*	1	8	0.5	8	2	2	80	120
13	*Philaspora hortei*	0.5	1	1	1	1	8	80	120
14	*Trichophyton rubrum*	1	2	1	2	2	2	40	100
15	*Microsporum canis*	1	2	1	2	1	2	80	120
16	*Microsporum canis*	0.5	1	1	2	1	2	100	120
17	*Trichophyton mentagrophytes*	1	4	1	1	1	2	100	100
18	*Trichophyton mentagrophytes*	1	8	1	8	2	8	100	120
19	*Trichophyton mentagrophytes*	1	8	0.5	1	1	2	100	120
20	*Trichophyton rubrum*	1	4	1	2	1	2	80	120

21	*Trichophyton specie*	1	2	1000	1	1	2	100	100
22	*Pityriasis furfur*	1	8	500	8	1	4	80	120
23	*Pityriasis furfur*	1	2	1000	8	1	2	80	120
24	*Trichophyton specie*	0.25	2	500	1	1	2	80	120
25	*Trichophyton specie*	1	2	500	1	1	2	100	120
26	*Philaspora hortei*	1	2	1000	1	2	2	100	100
27	*Pityriasis furfur*	0.5	2	0.5	2	1	2	100	100
28	*Microsporum canis*	0.5	2	0.5	1	1	2	100	120
29	*Trichophyton specie*	0.5	2	0.5	1	1	2	100	100
30	*Philaspora hortei*	0.5	4	0.5	1	1	2	100	100

APPENDIX II

FRACTIONAL INHIBITORY CONCENTRATION (FIC) OF TERBINAFINE AND SODIUM PROPIONATE

Concentration of Terbinafine in Admixture mg/ml	FIC	Concentration of Sodium propionate in Admixture mg/ml	FIC	∑FICs
0.8	0.8	3	0.03	0.83.
0.6	0.6	6	0.06	0.66
0.4	0.4	6	0.06	0.46
0.2	0.2	12	0.12	0.32
				2.27
MIC of Terbinafine 1mg/ml		MIC of Sodium propionate 100mg/ml		

Means of sum FIC =2.27/4=0.57

APPENDIX III

FRACTIONAL INHIBITORY CONCENTRATION (FIC) OF TERBINAFINE AND LAURIC ACID

Concentration of Terbinafine in Admixture mg/ml	FIC	Concentration of Lauric acid in Admixture mg/ml	FIC	∑FICs
0.8	0.8	0.2	0.1	0.9.
0.6	0.6	0.6	0.3	0.9
0.4	0.4	0.8	0.4	0.8
0.2	0.2	0.8	0.4	0.6
				3.2
MIC ofTerbinafine1mg/ml		MIC of Lauric acid 2mg/ml		

Means of sum FIC =3.2/4=0.8

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APPENDIX IV

FRACTIONAL INHIBITORY CONCENTRATION (FIC) OF FLUCONAZOLE AND SODIUM PROPIONATE

Concentration of Fluconazole in Admixture mg/ml	FIC	Concentration of Sodium propionate in Admixture mg/ml	FIC	∑FICs
0.8	0.8	3	0.03	0.83.
0.6	0.6	9	0.09	0.69
0.4	0.4	12	0.12	0.52
0.2	0.2	15	0.15	0.35
				2.39
MIC of Fluconazole 1mg/ml		MIC of Sodium propionate100mg/ml		

Means of sum FIC =2.39/4=0.60

APPENDIX V

FRACTIONAL INHIBITORY CONCENTRATION (FIC) OF FLUCONAZOLE AND LAURIC ACID

Concentration of Fluconazole in Admixture mg/ml	FIC	Concentration of Lauric acid in Admixture mg/ml	FIC	∑FICs
0.8	0.8	0/2	0.1	0.9
0.6	0.6	0.4	0.2	0.8
0.4	0.4	0.6	0.3	0.7
0.2	0.2	0.8	0.4	0.6
				3.00
MIC of Fluconazole 1mg/ml		MIC of Lauric acid 2mg/ml		

Means of sum FIC =3.00/4=0.75

APPENDIX VI

T. mentagrophyte SPORES SURVIVAL IN DIFFERENT CONCENTRATIONS OF TEST ANTIFUNGAL AGENTS

Time (Mins)	Sodium propionate 200mg/ml	Fluconazole 10mg/ml	Terbinafine 10mg/ml	Lauric acid 10mg/ml
0.0	$4.82x10^8$ cfu/ml	$4.82x10^8$cfu/ml	$4.82x10^8$cfu/ml	$4.82x10^8$cfu/ml
10.0	$1.40x10^7$cfu/ml	$1.06x10^8$cfu/ml	$1.06x10^8$cfu/ml	$1.05x10^8$cfu/ml
20.0	$7.50x10^5$ cfu/ml	$6.0x10^6$ cfu/ml	$7.20x10^6$cfu/ml	$4.90x10^6$cfu/ml
30.0	$6.00x10^4$ cfu/ml	$4.0x10^4$ cfu/ml	$3.0x10^4$ cfu/ml	$1.30x10^4$cfu/ml
60.0	$2.30x10^4$ cfu/ml	$2.5x10^3$ cfu/ml	$3.0x10^3$ cfu/ml	$5.0x10^3$ cfu/ml

APPENDIX VII

T. mentagrophyte SPORES SURVIVAL IN TEST ANTIFUNGAL AGENTS ADMIXTURES

Time (Mins)	Terbinafine/Lauric acid 10/10mg/ml	Terbinafine/Sodium propionate 10/200mg/ml	Fluconazole/lauric acid 10/10mg/ml	Fluconazole/Sodium propionate 10/200mg/ml
0.0	$1.025x10^6$cfu/ml	$1.025x10^6$cfu/ml	$1.025x10^6$cfu/ml	$1.025x10^6$cfu/ml
10.0	$4.0x10^5$cfu/ml	$2.5x10^5$cfu/ml	$2.0x10^5$cfu/ml	$1.0x10^3$cfu/ml
20.0	$2.0x10^5$cfu/ml	$0.0x10^0$cfu/ml	$4.2x10^2$cfu/ml	$5.0x10^2$cfu/ml
30.0	$0.0x10^0$cfu/ml	$0.0x10^0$cfu/ml	$0.0x10^0$cfu/ml	$0.0x10^0$cfu/ml
60.0	$0.0x10^0$cfu/ml	$0.0x10^0$cfu/ml	$0.0x10^0$cfu/ml	$0.0x10^0$cfu/ml