MYCOSES ASSOCIATED WITH THE SKIN ON PATIENTS ATTENDING ALUPE KEMRI CLINIC

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Signature: …… Date: ……..

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This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

I dedicate this work to my family for the patience and moral support during the study period.

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
API	Analytical Profile Index
ATCC	American Type Culture Collection
CFU	Colony Forming Unit
CIPDCR	Centre for Infectious and Parasitic Disease Control Research
CLSI	Clinical Laboratory Standard Institute
DMSO	Dimethyl Sulfoxide
HIV	Human Immunodeficiency Virus
KEMRI	Kenya Medical Research Institute
KOH	Potassium Hydroxide
KPS	Key Populations
LMIC	Low and Middle Income Countries
LPCB	Lacto Phenol Cotton Blue
MIC	Minimum Inhibitory Concentration
MOPS	Morphine Propane Sulfonic Acid
NGO	No Growth Obtained
OFIS	Opportunistic Fungal Infections
OPD	Outpatient Department

PCR	Polymerase Chain Reaction
PV	Pityriasis Versiclor
RPMI	Roswell Park Memorial Institute
SDA	Sabouraud Dextrose Agar
SERU	Scientific Ethical Review Unit
SFI	Superficial Fungal Infections
SPSS	Statistical Package for the Social Sciences
SSC	Scientific Steering Committee
TB	Tuberculosis

ABSTRACT

Globally, over 300 million people are afflicted with serious mycoses infections and out of these; 25 million are at a high risk of dying. Estimates for the global burden of mycoses diseases are based on population and disease demographics; age, gender, HIV infection and occupation. The aim of this study was to determine the most common mycoses associated with the skin on patients attending Alupe, KEMRI clinic. A cross-sectional study was conducted in 371 patients comprising 42.9% (159) males and 57.1% (212) females, from Alupe Kemri clinic in Busia Western Kenya. Skin scrapings were collected in coded envelopes from the active edges of the affected skin and preliminary identification was done by placing skin scraps on a labeled slide then 20% potassium hydroxide (KOH) solution was added, put a cover slip over the preparation and gently heated over flame then observed at a magnification of 400. Samples were cultured in Sabouraud dextrose agar (SDA) with chloramphenicol and incubated at 30^{o}C for two weeks. Phenotypic identification was done based on macro-morphological, micro-morphological and physiological characteristics. Identification keys for species confirmation were done. By gender, 88.7% (141) males and 81.6% (173) females had fungal infections. The mycoses isolated were from the genera; *Alternaria* 3.8% (14), *Aspergillus* 12.1% (45), *Cladosporium* 2.2% (8) *Penicillium* 4.3% (16), *Rhizopus* 3.2% (12), y*east* 2.7% (10), *Trichopyton* 27.8% (103) and others 6.7% (25) respectively. No significant growth was obtained in 24.8% (92). The HIV status for respondents were; sero negative 49.3% (183), sero positive 6.2% (23), and unknown status 44.5% (165) respectively. Out of 23 HIV positive patients, 91.3% (21) had mycoses infection, while HIV negative patients 52.6% (183) had mycoses infections. The difference was statistically significant, $p<0.0023$. This shows that HIV patients are more vulnerable to mycoses infections than the HIV negative patients. This study observed that fungal isolates were resistant to clotrimazole, griseofulvin and terbinafine but susceptible to itraconazole drug. Among the infected sites were; whole body 100% (4/4), scalp 94% (47/50), hand 87.7% (50/57), neck, scalp, hand 86.9% (40/46), genitals 85.7% (6/7), neck 83.3% (48/40), trunk 81.8% (27/33), legs 79.5% (78/98) and face 78.6% (22/28). Regarding occupation, farmers were most susceptible to mycoses infections 35.6% (132) followed by unemployed 30.7% (114), business 18.3% (68), employed 11.6% (43) and other 3.8% (14) respectively. The mean age for the study was 31.0±20.0 with two groups being mostly affected; 5-14 years 91.4% (74/81) and 55-64 years 89.4% (34/38). The study confirmed that mycoses are associated with the skin on patients attending Alupe KEMRI clinic with *Trichophyton* species and *Cladosporium* species being the most and least isolated mycoses respectively and has no predilection , for neither gender nor age. The study revealed that HIV positive patients were more likely to be infected by mycoses, and the drug sensitivity testing will make improve the prescription of the drug that actually cure the infections.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Mycoses are fungal infection of animals or human beings caused by any fungus that invades tissues and can be divided into groups based on their intrusiveness. Mycoses that cause superficial infections of the, hair, nails and epidermis are called cutaneous mycoses. Mycoses that infiltrate the epidermis and the dermis to infect deeper tissues are known as subcutaneous mycoses while mycoses that spread throughout the body are known as systemic mycoses (Gupta, 1994; Kelly, 2012).

Mycoses are common and variety of ecological and physiological conditions that contribute to the progress of fungal diseases and are ubiquitous in nature. They are found in water, soil, air, in animal and human tissues (Garcia-Solache *et al.,* 2010). Mycoses are caused by a diversity of fungal, and with a diversity of clinical indicators. Some fungal are pathogenic to humans which cause fungal infections and can be categorized according to the genera *Trichophyton, Microsporum* or *Epidemophyton* and by primary habitation which comprise geophillic, anthropophilic and zoophilic (de Hoog *et al*., 2017; Tedersoo *et al.,* 2014). Some of these mycoses cause superficial cutaneous fungal infections (Gupta, 1994; Kelly, 2012). Superficial cutaneous fungal infections are the most common causes of skin infections in patients with low immunity (Guto *et al.,* 2016; Nweze, 2010).

Mycoses infections causes 1.5 million deaths yearly (Brown *et al*., 2012) and the total number of patients disposed to upsurge of these infections is on the increase (Wickes & Wiederhold ;2018; Arastehfar *et al*., 2019). The shortage of clinically effective antifungal agents together with the rise of multi-drug-resistant organisms and new mycoses species are main community health fears (Healey & Perlin ; 2018). A widening spectrum of mycoses species affecting individuals and species-specific susceptibility to antifungals accentuate the importance of precise identification to the types and, for few fungi, sub-species level. However, reliable diagnostic tools are not accessible in some countries (Kathuria *et al*., 2015).

Mycoses are categorized as superficial, cutaneous, subcutaneous, or systemic infections subject to the variety and degree of tissue associated and the host response to the pathogen. (Arenas *et al*., 2012).

Mycoses are common opportunistic infection in immunocompromised patients (Minamoto & Rosenberg, 1997). They include various *Tinea* species, *pityriasis* and fungal *keratitis*. These can be detected in the skin scales, nails and hair microscopically (Cheesbrough, 2000).

Some mycoses are neglected diseases of medical history. The infections, however, are part of a different setup. These infections, for most of recorded history as well as the majority of the last era, have remained rare or had a little impact on human healthiness. Approximately one billion people have skin mycoses, which makes this infection slightly less common on the world than dental and headaches caries. Fungal spores contribute to significant reactive airway diseases in over 10 million individuals. It is estimated that over 300 million people of all ages suffer from a serious mycoses each year worldwide (Rodrigues & Nosanchuk, 2020).

Mycoses are main cause of morbidity and mortality in infected patients .It is estimated that more than one million eyes go blind every year due to mycoses and more tha 1.5 million of these patients are likely to die from their mycoses (Rodrigues & Nosanchuk, 2020).

Mycoses are hidden killers causing a significant morbidity and mortality in vulnerable people. Though, their effect is not widely recognized as compared to other infections. A great disparity of access to fungal diagnostic and treatment services in resource constrained countries is evident mainly in sub-Saharan Africa. Furthermore, despite efficient diagnostic tests and safe effective drugs on fungal infections, research on skin fungal infections in comparison to other pathogens is fairly neglected. (Guto *et al.,* 2016).

Fungal disease kills more than 1.5 million and affects more than a billion people. However, it is a neglected disease even though most deaths from fungal diseases are

avoidable. Severe skin fungal infections occur as a result of immuno-suppression in patients (Bongomin *et al*., 2017).

Socio-economic, geo-ecological characteristics and the growing number of exposed populations are the key factors of variations on rate and occurrence of fungal disease globally. The increase in the number of immunocompromised patients, some of whom are highly susceptible to fungal infections, is the major driver of mycoses infections in both developed and developing countries worldwide (Brown *et al.,* 2012).

Timely diagnosis of mycoses is critical to effective treatment. Common approaches for laboratory diagnosis of mycoses include direct microscopic examination of mycoses and this should be the fast point-of-care (Backx *et al.,* 2014).

Antifungal drugs, including griseofulvin,terbinafine, clotrimazole and intraconazole, are to be more and widely available. Such measures and with continued national efforts in education and training in managing of mycoses will have the possibility to improve patient outcomes significantly (Rodrigues & Nosanchuk, 2020)

In Kenya the extent of mycoses in patients with skin conditions has not been established. It is thought that mycoses are more common in patients with skin problems but definitive studies have not been done in Alupe, KEMRI western Kenya. Reports indicate that nearly 20%-25% of people globally are suffering from mycoses (Chikoi *et al.,* 2018).

A study conducted in Britain estimated that more than one million eyes go blind every year because of mycoses. Almost one billion people have skin mycoses, which makes this disease common on the world than dental and headaches caries (Rodrigues & Nosanchuk, 2020). Similar studies have not been done in Alupe, KEMRI clinic Western Kenya.

Tinea infections of the skin and scalp represent a relatively common problem especially in the tropical and subtropical regions of the world where the warm and humid climates provide a favorable environment for the causative organisms (Gupta *et al*., 1999).

Opportunistic fungal infections are significant and progressively predominant cause of disease in some patient. These fungal infections can occur both in immune-compromised and immune-competent people. Since the number of patients who are immunocompromised is increasingly rising, it is important for clinicians to consider mycoses disease in the diagnosis of these patients with skin conditions. The diagnosis of mycoses is fairly straight forward; however, most patients do not benefit from it because the diagnosis is not routinely done. *Pityriasis versicolor* is an important cause of superficial fungal infection prevalent in the tropics and subtropics (Burkhart, 2006; Kelly, 2012). The present study is to identify the causative agents of mycoses associated with the skin on patients attending Alupe, KEMRI clinic.

1.2 Statement of the Problem

Mycoses are common in patients with skin diseases , especially Tinea and *Pityriasis versicolor*. Most of the mycoses infections are opportunistic and may need to be taken into consideration particularly in the immuno-suppressed individuals. Mycoses associated with the skin infection continue to cause morbidity and mortality in patients. Mycoses can be grouped into four types according to the tissue levels originally inhabited that is cutaneous, subcutaneous, opportunistic and Superficial mycoses. An early specific diagnosis and subsequent treatment to combat these infections are also equally relevant to management of common fungal infections.

1.3 Justification of the Study

Mycoses infections have neither been the focus of intensive study nor of active control programs in the sub-Saharan Africa, including Kenya. This neglect is likely because fungal infections in healthy individuals tend to be relatively benign. There is a glaring lack of information on the epidemiology of mycoses in Kenya. The limited scientific information affects patient management, diagnosis and control programs. The frequency of mycoses infection in the skin is not known. Also another aspect that is not known is, which mycoses are common causative agents of the skin infections in patients attending Alupe KEMRI clinic, yet this knowledge is essential for patient management. Despite the availability of antifungal treatment, mycoses remain a common condition in the country. This study was conducted in Alupe, KEMRI Clinic

in western Kenya. The data from this study will help in the diagnosis and clinical management of common mycoses associated with the skin in patients.

1.4 Research Questions

1. What are common mycoses associated with the skin on patients attending Alupe, KEMRI clinic?
2. What are the contributing factors for mycoses on patients attending Alupe, KEMRI clinic.
3. What are the susceptibility patterns of mycoses pathogens to antifungal drugs?

1.5 General Objective

To determine the most common mycoses associated with the skin on patients attending Alupe, KEMRI clinic.

1.5.1 Specific Objectives

1. To isolate, characterize and determine the spectrum of agents of mycoses associated with the skin in Alupe, KEMRI clinic.
2. To identify contributing factors for mycoses on patients attending Alupe, KEMRI clinic.
3. To determine antifungal drug susceptibility patterns of the isolated mycoses pathogens in Alupe, KEMRI clinic.

CHAPTER TWO

LITERATURE REVIEW

2.1 Mycoses

Mycoses are fungi infection of animals or human beings caused by any fungus that invades tissues and can be grouped into classes based on their intrusiveness. Mycoses that cause superficial infections of the, hair, nails and epidermis are known as cutaneous mycoses. Mycoses that infiltrate the epidermis and the dermis to infect deeper tissues are called subcutaneous mycoses while mycoses that spread throughout the body are known as systemic mycoses (Gupta, 1994; Kelly, 2012).

Mycoses are common and multiplicity of ecological and physiological conditions that contribute to the progress of fungal diseases and are ubiquitous in nature. Mycoses of the skin was the 4th greatest common skin disease in 2010 affecting 984 million persons (Hay *et al.,* 2010). Mycoses are caused by a diversity of fungi, and with a variety of medical signs. Despite their worrying impact on human health, mycoses on the skin have been constantly neglected over the years (Rodrigues & Nosanchuk, 2020). These neglected tropical diseases have specific characteristics. First of all, they affect the poorest people without access to safe hygiene, and basic health services. Moreover, they are generally chronic and gradually develop, becoming increasingly worse if left undiagnosed and untreated. The injury these diseases cause can be permanent. At the same time mycoses can cause serious pain and disability in life, with long-term concerns for families and patients of the affected people. Individuals with mycoses are mostly stigmatized and socially rejected, which can distress their mental health (Heath *et al*., 2012; Molyneux, 2013).

Mycoses have weighty effects on human health. Approximately one billion people have skin mycoses. Fungal spores contribute to significant reactive airway diseases in over 10 million individuals. In total it is estimated that over 300 million people of all ages suffer from serious skin mycoses each year globally. Notably, over 1.5 million of these individuals are likely to die from mycoses disease (Bongomin *et al*., 2017). Some fungi are pathogenic to humans which cause fungal infections and can be

categorized according to the genera *Trichophyton, Microsporum* or *Epidemophyton* and by primary habitation which comprise geophillic, anthropophilic and zoophilic (de Hoog *et al*., 2017; Tedersoo *et al.,* 2014). Mycoses can be grouped into four types according to the tissue levels originally inhabited that is cutaneous, subcutaneous, opportunistic and Superficial mycoses

2.2 Cutaneous mycoses

Cutaneous mycoses are pathogenic fungal infections that cause the keratinized layers of the skin and its adjuncts hair and nail. It does not disturb the existing tissues. These infections are mostly caused by yeasts and dermatophytes that affects the skin, hair or nails and are common globally and their occurrence is continuously growing (Budak *et al*., 2013). These mycoses are restricted to the outermost layers of the hair and skin (Mahmoudabadi, 2005). In countries where people lack a dequate immune response as a result of immunosuppressive drugs and exposure to radiation or other serious infections cutaneous mycoses becomes common infections (Guto *et al.,* 2016; Jeng *et al.,* 2010).

The prevalence of cutaneous mycoses remains unknown (Faini *et al.,* 2015) . Worldwide, over 300 million people of all ages suffer from severe cutaneous mycoses every year, accounting for 1.4 million fatalities. This number is related to the yearly deaths from malaria and tuberculosis, which account for approximately 600 000 and 1.5 million deaths per year respectively (Brown *et al.,* 2012). The genus *Trichophyton* gives rise to most of the tinea dermatophytoses, including tinea capitis, tinea pedis, and tinea unguium (onychomycosis) (Goldstein *et al*., 2000; Guto *et al.,* 2016).

In addition to the recognized pathogens, nondermatophyte molds are now becoming main in cutaneous mycoses and a major cause of morbidity worldwide (Dias *et al*., 2013). They are very common fungal infections affecting the skin that occur to people, animals and especially poultry all over the world. Most of them cause fungi called Dermatophytes, which are about 3-4 genera. They include a large group of Species that share several common traits and cause Annular rash in the skin, hair and nails, do not invade the living tissue in these infections, located in the host cells some physiological changes due to the presence of fungus and its biological metabolic

consequences Skin infections are one of the most common infections in the world (Dias, *et al*., 2013).

Cutaneous mycoses represent a significant basis of morbidity for people globally, especially in the tropics. Fungal infections affect the hair, skin or the nails, and tend to blossom in the warm and humid tropical countries (Charles, 2009; Shrum, *et al*., 1994).

These fungi are onygenalean anamorphic and anamorphs species belonging to the group *Trichophyton, Epidermophyton and Microsporum*. The significant characteristic of the cutaneous mycoses is their restraint to the lifeless keratinized tissues, except for rare cases where the patient is immunosuppressed (Ogawa *et al.,* 1998).

An example of cutaneous mycoses is "ringworm" or 'tinea', an infection of the skin by a dermatophyte (Gupta *et al*., 2004; Oke *et al.,* 2014; Peters, 1995).

2.3 Opportunistic mycoses

Cases of opportunistic mycoses are anticipated to increase each year due to most fungal infections are not cured well and progresses into chronic illiness .Globally, the mortality rate and infection due to opportunistic mycoses such as *Aspergillus, Candida, Rhizopus* and *Trichophyton* amongst patients with weak immunity have been growing (Yoon *et al*., 2014).

Opportunistic mycoses are mycoses of the body which occur almost exclusively in weakened patients whose normal defence mechanisms are compromised. Opportunistic mycoses are significant and progressively predominant cause of disease in some patient. These fungal infections can occur both in immune-compromised and immune-competent people. Since the number of patients who are immunocompromised is increasingly rising, it is important for clinicians to consider mycoses disease in the diagnosis of these patients with skin conditions (Batzlaff & Limper, 2017).

Many fungal infections are life-threatening in nature but are neglected (Rodrigues & Nosanchuk, 2020). Some of which are opportunistic and affect immune-suppressed patients globally (Garcia-Solache & Casadevall, 2010).

Mycoses most commonly isolated from skin of immunocompromised patients are commensal, endogenous or saprophytic mostly from the environs. The most common species are *Mucor* species, *Rhizopus* species, *Aspergillus* species, *Cladosporium* species and *Candida* species (Pfaller & Diekema, 2004). The rate of opportunistic mycoses has increased significantly for the past two decades .This rise in infections is associated with morbidity and mortality and is directly associated to increasing patient populations at risk for the upsurge of severe mycoses, which comprises patients with AIDS and advanced age (Pfaller & Diekema, 2004; Walsh *et al.*, 2004).Yeast infection remains one of the most important cause of opportunistic mycoses globally (Nucci *et al*., 2010).

The incidence of opportunistic mycoses has grown due to the expanding population of immunocompromised patients, including elderly, cancer and HIV/AIDS patients. Even though some effective treatment options are available, opportunistic mycoses are associated with high morbidity and mortality rates (Nucci *et al*., 2010; Chung *et al.,* 2000).

Opportunistic mycoses fail to persuade infection in most immunocompromised individuals but can do so in those with weakened host resistances. Mortality is high because most mycoses are problematic to make a diagnosis, particularly in their initial and curable phases There are five types of medically significant fungi; *Rhizopus*, *Aspergillus*, *Cryptococcus*, *Mucor* and *Candida.* (Szalka & Prinz 1991).

2.4 Subcutaneous mycoses

Subcutaneous mycoses are a group of fungal diseases formed by a various cluster of fungi that infect the skin,subcutaneous tissue, and in some cases the underlying organs and tissues. The contributing agents are usually found in the leaves,organic material and soil and are led by traumatic damage of the skin. The mycoses usually remain

restricted and gradually extent to the nearby tissue; signs are usually absent or least. Subcutaneous mycoses usually produce typical lesions, but they may also closely look alike and be confused with other diseases. The appearance of lesions can be altered beyond recognition by earlier treatment, for instance with topical steroids (Warnock, 2012).

These are chronic, restricted mycoses of the skin and subcutaneous skin following the disturbing establishment of the aetiologic cause. The causative mycoses are soil saprophytes of area epidemiology whose capability to adjust to the skin environs and provoke illness is very variable . They are widely found in tropical and subtropical regions of the world (Bhat *et al.,* 2016; Bonifaz *et al.*, 2010).

Subcutaneous mycoses are a group of fungal infections of dermis and subcutaneous tissue which consist *Penicllium* species. Subcutaneous mycoses are widely found in tropical and subtropical regions of the world. They are hardly observed in Europe. Most cases in Europe are witnessed in returning travelers, archaeologists, immigrants and aid workers (Bonifaz *et al.*, 2010)

Subcutaneous mycoses are much less common than cutaneous mycoses, these are categorized by various group of infections that frequently result from straight penetration of the fungus into the dermis and subcutaneous tissue through traumatic harm. The fungus spreads by local deep tissue invasion from the inoculation spot. The mycoses generally remains confined and then gradually spreads to next tissue and finally to the lymphatics. Subcutaneous mycoses can occur in immunocompromised individuals and healthy people, these infections can spread widely (Koga *et al.,* 2003).

Subcutaneous mycoses, though rare, they are commonly reported from northeast India. Their variety differs with geographic area. Examples of subcutaneous mycoses include, *Cladosporium* species, *Curvularia* species and *Rhizopus* species (Verma *et al*., 2018). *Rhizopus* species are common and they grow at the place of transcutaneous trauma. Infection gradually progresses as the etiologic agent lives and adjusts to the adversarial host skin environs. These fungal is sometimes found in small outbreaks, including in skin lesions and nosocomial infections after accidents (Bhat *et al.,* 2016; Queiroz-Telles *et al.,* 2003).

Another example of subcutaneous mycoses that is associated with the skin is *Cladosporium* species which cause infection in animals and humans. It is regarded as the basic development of sclerotic body, dark-coloured filamentous hyphae as well as yeast-like cells in the attacked skin. Two cases of subcutaneous mycosis in immunocompromised male patients aged 58 and 55 years attending dermatology outpatient department in India, were reported. The second case was diagnosed as chromoblastomycosis which was caused by Cladosporium species (Nath *et al*., 2015).

2.5 Superficial mycoses

Superficial mycoses are main cause of morbidity globally (Dias *et al*., 2013). Superficial mycoses are restricted to the stratum of the hair and skin. With superficial mycoses, fungi hardly attack the dermis. In particularly rare cases, internal tissues can also be affected. An example of such a fungal infection is *Tinea versicolor*, mycoses that normally affects the skin of young people. It generally affects the back, legs ,upper arms and chest. Tinea versicolor is instigated by a fungus that exists in the skin of some adult people. Usually does not affect the face. This fungus causes spots on the skin that are either hypopigmented lesions or brighter than the skin (Charles 2009; https://en.wikipedia.org/wiki/Mycosis#cite_note-9; Kallini *et al*., 2014).

These mycoses survives in two ways, one of them making noticeable spots. Aspects that causes the mycoses to be more visible, consist of immune or hormone anomalies as well as high humidity (Heffernan 2008; Kallini *et al*., 2014).

Superficial myscoses is mostly in the tropics, where humidity,damp and heat provide favourable environment for growth of mycoses (Charles, 2009; Odom, 1994). Direct contact is adequate to spread the infection from a unclean surface to person and can be transmitted to another. Dermatophytoses are the chief cause of mycoses in male, however pityriasis versicolor and candidiasis are examples of main superficial mycoses (Gupta *et al*., 2002; Kallini *et al*., 2014).

There are two clusters of superficial mycoses; non-inflammatory and inflammatory infections (Charles 2009; King-man, 2010).

1). Inflammatory superficial mycoses

These mycoses are caused by a cluster of fungi that normally are parasitic and live at the expense of keratin of nails, skin and hair. Examples of mycoses in this category are the following; *microsporum, epidermophyton* and *trichophyton*. Its more frequent in higher tropical and subtropical regions with humid and hot environment (Degreef, 2008). These mycoses can be be categorized as follows;

a) **Zoophilic**: these mycoses are found in animals and are spread to people through indirect contact or direct, causing inflammation and occasionally suppurative skin.
b) **Anthropophylic:** these mycoses are found in people and can be epidemic, with small inflammation. They are transmitted from individual to individual or through objects.
c) **Geophilic:** these mycoses are found in soil and infect humans sporadically through direct contact, causing inflammation.

2). Non-inflammatory superficial mycoses

These type of mycoses can cause minor inflammation and it is described by Eichstedt in 1846, that it is a frequent chronic infection, caused by lipophilic mycoses of the *Malassezia* species, which is mostly found on the skin and scalp minus clinical signs. like an opportunist, these can be an provoking element in many circumstances of seborrheic dermatitis. The three types commonly related to this disease are: *Malassezia sympodialis, Malassezia furfur* and *Malassezia globose* (Zaitz *et al*., 2000).

These non-inflammatory mycoses produces dicarboxylic acids such as azelaic acid, which inhibits tyrosine kinase, causing hypopigmentation of the part involved in people with black skin, however hyperchromic lesions can also be seen (Crespo & Delgado, 2002). The mycoses have characteristics of round maculas or oval-shaped in seborrheic areas. Conditions such as poor hygeine malnutrition,

immunosuppression, humidity, sweating and heat can aggravate the alteration of saprophytic yeasts into invasive or a pathogenic form (Crespo & Delgado, 2002).

2.6 Epidemiological Characterization of Mycoses

Lately, mycoses have been distressing and causing serious problems in immunocompromised patients and aging people globally. Healthy people mostly have a resilient immunity against mycoses . Though, people who have a weak immune system especially children, those with HIV/AIDS, aging, and those have been taking immunosuppressants for extended times are commonly susceptible to mycoses (Yoon *et al*., 2014).

According to an investigation based on United States death certificate data from 1980 to 1997 prevalence of deaths due to mycoses increased 3.4 times from 1,577 to 6,577 and mortality increased from 0.7 to 2.4 per 100,000 persons (Rees *et al*., 1998; Falahati *et al*., 2003). Though, considering that many cases fail to be correctly identified with mycoses amongst terminally sick, the result is probably miscalculated, and more are anticipated to have died due to mycoses. Mycoses infection should be treated cautiously (Rees *et al*., 1998; Yoon *et al*., 2014).

Mycoses of the skin, hair, and nails are a common public health problem worldwide and have been found to be affecting 984 million people in the year 2010 (Hay *et al*., 2014). However, a population-based survey reported that they are rarely managed (Dos *et al.,* 2010; Oke *et al.,* 2014). The prevalence of mycoses is likely to reach 25% of the world's population, and its occurrence continues to raise (Ameen, 2010). Mycoses accounts about 7% of the Kenyan population with periodic tinea capitis accounting for 82% of the infections (Guto *et al.,* 2016). This increase may be a result of usage of antibiotics and immunosuppressive drugs (Jain *et al*; 2010; Kannan *et al.,* 2006).

Mycoses of the skin and nails have been found in the last few decades to affect 20-25% of the world’s population, making them one of the most frequent forms of infection (Bongomin *et al.,* 2017; Havlickova *et al.,* 2008). They represent a major public health problem in school-age children especially in low and middle-income countries (LMICs) like Kenya and Nigeria. The predisposing factors to acquiring the

infection are hygiene, overcrowding, and low socioeconomic factors (Bongomin *et al.,* 2017; Verma *et al.,* 2018). Prevalence of mycoses in Nigeria as reported ranges from 3.4% to 55% (Nweze & Okafor, 2005; Oke *et al.,* 2014).

There are approximately 40 different species of dermatophytes, of which the most common species that cause disease in human are *Trichophyton rubrum, Trichophyton tonsurans, Trichophyton interdigitale, Trichophyton mentagrophytes, Microsporum canis,* and *Epidermophyton floccosum.* On, a worldwide scale, *Trichophyton. rubrum* and *Trichophyton . mentagrophytes* together account for 80% to 90% of all dermatophytosis (Ameen, 2010; Martin & Elewski, 2000).

Although dermatophytosis occurs worldwide, individual dermatophyte species may vary in their geographic distribution and virulence. Poverty and overcrowded living conditions are important social determinants (Hainer, 2003; Maraki & Mavromanolaki, 2016). Factors contributing to the high frequency and chronic occurrences of dermatophytosis in developing countries may also include poor living conditions, children interaction patterns, and poor health seeking behavior (Andrews *et al.,* 2009; Maraki & Mavromanolaki, 2016).

Kenya is a developing East African country with a high rate of tuberculosis (TB) and a moderate HIV infection burden. These two diseases predispose patients to the development of opportunistic fungal infections. Sub-Saharan Africa being the epicenter of contagious diseases is likely to have many cases of mycoses that affects the eye and skin, (Bii *et al.,* 2006; Nweze & Okafor, 2010). Evidence shows that mycoses are often hidden killers causing a substantial morbidity and mortality in susceptible individuals (Dogra & Uprety, 2016). However, their impact is not widely acknowledged or appreciated as compared to other diseases. A great disparity of access to fungal diagnostic and treatment services in resource-constrained countries is apparent particularly in sub-Saharan Africa (Guto *et al*., 2016). Moreover, despite efficient diagnostic tests and safe effective drugs, research on mycoses in comparison to other pathogens is somewhat neglected (Guto *et al.,* 2016; Kozel & Wickes, 2014). Mycoses are emerging beyond their usually described limits for reasons that are not

well understood but land use forms and the practice of agricultural farming is now known to be among the cause of mycoses (Brandt & Park, 2013).

Studies of epidemiology and characterization of mycoses have shown that the medical community is important to acquire an understanding of the, epidemiology and pathogenesis of these mycoses which initially was not common pathogens.These will enable them to be more familiar with the alternatives for prevention and treatment (Walsh *et al*., 2004).

2.7 Diagnosis of Mycoses

Diagnosis and treatment of mycoses brings together worldwide known mycologist specialists to direct researchers,clinicians and patients in the use of existing information in the area of medical mycology to handle those who suffer from mycoses. Regular, diagnostic plans and examinations, comprising of directed culturing and basic techniques are essential and needs to be considered and combined to make the proper diagnosis of mycoses. To avoid a misdiagnosis and identification of mycoses it requires both light microscopic examination of skin scrapings and mycologic culture on Sabouraud's dextrose agar media (Gupta *et al*., 2003; Kozel & Wickes, 2014; Roberts *et al*., 1984).

Mycoses often resemble other illnesses. Visiting clinicians could help with earlier diagnosis. When a fungal infection is suspected,prompt diagnosis of infection is important to effective treatment. There are many challenges to diagnosis such as decreasing number of medical mycologists. In Africa there are about 200 Africa mycological association members. Mycologist experience problems in developing world because of opportunistic diseases that are affecting patients and needs to be isolated and identified (Gryzenhout *et al*., 2012). Time to get laboratory result is another challenge since fungal culture which is the primary test used to diagnose a fungal infection is a slow growing in which some fungi takes many week to grow and be identified. The approaches to diagnosis include dierect microscopic examination of mycological samples and culture (Kozel & Wickes, 2014; Schott's, 2007).

The diagnosis of mycoses infections remains a big challenge worldwide. Early diagnosis of mycoses is key because of the associated mortality and morbidity; Also it has been reported in the previous study that, diagnosis is challenging due to the nonspecific signs. (Haydour *et al*., 2019). Enhanced standards of care of mycoses depend on the development of new laboratory diagnostic, procedures and the development of new antifungal drugs (Sanguinetti *et al*., 2019).

Mycoses have been reported by other studies that they are of growing rate and significance in immunocompetent and immunocompromised patients. Appropriate diagnosis depends on proper use of laboratory testing in patients. Quick, correct diagnosis of mycoses relies on suitable application of laboratory testing (Hage *et al*., 2019).

Increasing numbers of immunocompromised patients have led to a growing number of people at risk of mycoses disease. Many has been realized in the laboratory diagnosis of these mycoses, such as culture and microscopy. Also it has been reported that there is availability of standardized susceptibility testing procedures that provide better helpful interventions (O'Shaughnessy *et al*., 2003). However, in a period of economic declines in health operations, impending challenges will include technically streamlined structures, the improvement of cost-effective, systems which provide early detection and identification of common and emerging mycoses. However, it will take more time to establish the clinical importance of new approaches in different set ups (O'Shaughnessy *et al*., 2003).

The present dilemma is to develop rapid and precise non– culture-based approaches, which will lead to timely diagnosis and timely introduction of proper antifungal treatment. Studies have shown that clinical laboratory in partnership with the clinician plays a fundamental part in the diagnosis of mycoses (O'Shaughnessy *et al*., 2003). Noble communication among the laboratory and clinician is key. It is key that the laboratory has procedures concerning the number of sample needed for the tests requested, and concerning correct sample transport, so that best results are achieved. Similary, it is prudent that the clinician informs the laboratory staff that there are clinical mycoses (O'Shaughnessy *et al.*, 2003).

2.8 Treatment of fungal infections

Mycoses treatments are limited due to host toxicity and fungi gaining resistance. Most forms of mycoses in patients respond well to many topical antifungal agents, such as azoles and terbinafine . If the disease is incessant, then ketoconazole, fluconazole, and itraconazole are effective (Ogutu *et al.*, 2010).

Mycoses of the nails, skin and hair are a major cause of morbidity globally. Selecting the correct treatment is not always easy due to the possibility of side effects and drug interactions (Dias *et al*., 2013).

The choice of an anti-fungal agent dependent on the site involved the extent of clinical infection, the age and general health of the patient and concomitant drug therapy (Kozel & Wickes, 2014). Mycoses may be managed either topically or systemically. Topical formulations are effective against localized infections but have limitations. They do not penetrate hair follicles, thick keratin, or the nail plate effectively. Patients may find them inconvenient which may affect compliance (Gupta *et al*., 1999).

The main treatments for mycoses are based on drugs with a practical approach to the most commonly used oral, systemic and topical drugs. However some mycoses can be sufficiently treated by topical antifungals (Gupta *et al*., 2003). The promising antifungal therapeutic drugs are; Itraconazole, Clotrimazole, Terbinafine and Griseofulvin. These are chemotherapeutic substances that basically act indirectly or directly on mycoses hence are of use in treatment of mycoses (Dias *et al*., 2013). The following drugs are indicated for the management of mycoses;

1. Itraconazole

Itraconazole is classified as azoles drug with three nitrogen atoms in the azole ring **(**Charles, 2009; Pasqualotto *et al*., 2010). Azoles are able to inhibit the demethylation of sterol's carbon-14 in fungal cell wall and finally inhibit the typical ergosterol biosynthesis by modifying its biochemical structure, and leading to inhibition of fungal progress and duplication. This drug can also used for systemic mycoses

treatments. All forms of azole share the similar mechanism of action and antifungal spectrum (Dias *et al*., 2013).

2. Clotrimazole

Clotrimazole is also classified as azoles drug but in the category of imidazole and it was the first imidazole derivative. It is available in form of spray, lotion, cream and pessaries. The absorption rate of clotrimazole is not more than 0.5% after application to the skin. It is used to treat dermatophytosis ; m*icrosporum* species , *epidermophyton* species and t*richophyton* species. It cures mycoses in 60 to 100% of skin patients (Dias *et al*., 2013)

3. Terbinafine

Terbinafine is classified as allylamines. This drug acts by hindering the squalene epoxidase

enzyme in mycoses cell membranes, leading to shortage in ergosterol and accumulation of

intracellular squalene (Pasqualotto *et al.,* 2010). Terbinafine is metabolized by cytochrome P450 system's isoenzymes, especially CYP2D6, that demonstrates its little possible for drug interactions. It is fungicidal against dermatophytes, also can be fungistatic or fungicidal against yeasts and can be used topically for *tinea versicolor* (Pasqualotto *et al*., 2010).

4. Griseofulvin

It has special act against dermatophytes and performs by interfering with DNA synthesis. This griseofulvin is fungistatic derived from the breakdown of *Penicillium griseofulvum* and is better absorbed in the body system.It is used for the treatment of dermatophytosis and is a drug of choice for the treatment of *Trichophyton* species (Zhang *et al*., 2007).

2.9 Prevention of Mycoses

Some of the main approaches of preventing mycoses are ; good practice of personal cleanliness, avoiding contact with the pathogen and avoiding touching animals with bare hands since they are reservoirs of mycoses (Gupta *et al*., 2003).

This can also be achieved by not sharing hats, combs, brushes, or other personal items, especially when mycoses are present. Wearing flip-flops or shoes in locker rooms, public showers, and around swimming pools can help to minimize contact with mycoses (Gupta *et al*., 2003; http://www.healthcommunities.com/fungal-infections/prevention.shtml 2000). The other approach is managing the environment to avoid enhancing fungus growth. Reduce moisture and humidity on the skin by drying it thoroughly and by changing sweaty clothes and socks. Cleaning or discarding infected objects and garments also helps prevent recurrences (Guto *et al*., 2016; http://www.healthcommunities.com/fungal-infections/prevention.shtml 2000).

Studies on prevention of mycoses have shown that health workers have an important part to play in early identification of epidemics of mycoses. This can be achieved by assessment of home environs and hospital for sources of infections, training of patients on preventive methods on patient environs and way of life. These includes sanitization for patients, health workers , minimizing of exposure to animals, soil, stagnant water, plants, dusty environs and air purification in hospitals (Smith & Kagan, 2005).

Mycoses live harmlessly in the environs; however, some species can cause illness in people. Health workers are likely to see patients with mycoses infections since they are in a position to offer guidance on methods of prevention in the spread of infection in the home and treatment (Gould, 2011).

Broader control of mycoses exposures in the public can also be enhanced by awareness, through learning concerning high risk activities and practices. Mycoses infections continue to be underestimated and serious cause of sickness and death. More should be done to prevent the significances of these diseases, though environmental exposure to these agents cannot be completely avoided in the public. Continuous community health efforts toward, describing, and tracing the mycoses infections can

aid to focus research on significance settings and priority of these mycoses (Brandt & Park, 2013; Lee *et al*., 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

The research was cross-sectional study which was conducted in Alupe, KEMRI clinic in western Kenya (Fig 3.1). The eligible patients were counsented before taking samples.

Figure 3.1: Study area: Busia County and Study site: Kemri Alupe

(https://www.nationsonline.org/oneworld/map/kenya_map2.htm) Source: UN Cartographic Section.

Busia lies at 1222m above sea level. The climate is humid with substantial rainfall. Even in the driest month there is a lot of rain. The average temperature in Busia is 22.0 °C | 71.6 °F with annual rainfall of 1691 mm | 66.6 inch. It borders Kakamega County to the east, Bungoma County to the north, Lake Victoria and Siaya County to the south and Busia District, Uganda to the west. It has a total population of 893,681 (2019 census) (https://www.google.com/search?q=busia+county+population&rlz=1C1GCEU_enUG827UG828&oq=busia+county+population&aqs=chrome..69i57j0l7.21556j0j4&sourceid=chrome&ie= UTF-8).

3.2 Study Site and population (*nationsonline.org*)

Alupe KEMRI clinic is eight kilometers North of Busia town (Fig 3. 1). The climate in the region is humid and damp. Cross border business within the neighboring country is rampant with significant risk factor for mycoses infecions. The study was sequential and 371 participants of all ages and both sexes were selected for this study.

3.3 Inclusion Criteria

Patients of any age, who presented with superficial mycoses, consented to participate in the study.

3.4 Exclusion Criteria

Patients who were not willing to consent and had no mycoses.

3.5 Sample size deternination

The minimum sample size was determined according to Fischer *et al.,* (1998).

The formula is

$$n = \frac{Z_{1}\text{-}\alpha/_{2}^{2}\ P\ (1\text{-}P)}{d^{2}}$$

Where;

n = minimum sample size sample size

$Z_{1}\text{-}\alpha/_{2}^{2}$= 1.96 (Standard normal deviate which corresponds to 95% confidence interval)

P= Estimated prevalence

d= The level of significance (5%)

Since there is no much information on mycoses infections in rural Kenya set-ups areas, a prevalence rate of 50% was used to calculate sample size. Assuming the prevalence at 95% confidence interval minimum estimated sample size was given as 384 in the formula below.

$$n = \frac{(1.96)^2 \times 0.5(1-0.5)}{(0.05)^2} = 384$$

According to the calculation, the estimated sample size of 384 could not be obtained as expected according to the sample size calculation with a prevalence of 50%. The samples obtained were 371 skin scarpings since some of the patients had been on self medication and had healed.

3.6 Patient recruitment

Patients presenting with symptoms of mycoses like skin scales, nail discoloration, skin lessions and ringworm who were attending the referral health facility were recruited. Consent was sought for those who were willing to participate (Appendix 1). This information included county, location, gender, age and clinical signs. Counseling and testing was conducted to determine the HIV status. Demographic data was collected through interview (Appendix II). Samples skin scraping, hair and nails were collected by qualified personnel.

3.6.1 Collection of Skin Scraping

Light scrapings were done to obtain skin scales, crusts, hair pieces and finger nails. This was taken from active edge of lesion using a blunt sterile scalpel blade (Karaman *et al;* 2017). The scrapings were collected in coded envelopes and taken to centre for infectious and parasitic disease control research Alupe and Mycology laboratory, center for Microbiology Research-KEMRI Nairobi for processing and analysis in which 91% of skin scrapings were detected by microscopy which provides the most rapid diagnosis (Karaman *et al;* 2017; Yee & Al Aboud 2020).

3.6.2 Transport and Handling of Specimen

The scrapings were collected in coded envelopes and kept at room temperature without refrigerating. Dermatophytes are inhibited at low temperatures and humidity will facilitate the growth of contaminants. The scrapings were taken to centre for infectious and parasitic disease control research Alupe and mycology laboratory, center for Microbiology Research-KEMRI Nairobi, for processing and analysis. Scrapings were transported to mycology Laboratory, center for Microbiology Research-KEMRI for confirmation and QC purposes.

3.6.3 Laboratory Procedures

3.6.3.1 Potassium Hydroxide Preparations

Mycological analysis of specimens were performed by mounting each specimen on a slide in drop of 20% potassium hydroxide (KOH) solution and observed under X40 microscope. Specimens showing hyphae or yeasts were considered positive (Karaman *et al.,* 2017).

3.6.3.2 Culture identification.

The skin scraping were seeded onto Sabouraud's dextrose agar (SDA) medium with chloramphenicol (Oxoid, U.K.), which acts as a broad spectrum antibiotic and incubated at 30^{o}C for two weeks. The colonial characteristics color of the specimen, texture, topography, and growth-rate, aerial and submerged type of hyphae were recorded. Slide culture were set in which fungi were inoculated in small blocks of nutrition deficient cornmeal agar medium covered with a coverslip and incubated. After incubation, the coverslip was removed from the agar block and placed on another coverslip to which lactophenol cotton blue (LPCB) was added and observed for microscopic characteristics morphology of micro conidia and macro conidia (Karaman *et al.*, 2017).

3.6.3.3 Identification of molds

Sabouraud Dextrose agar supplemented with 1% chloramphenicol was used for primary isolation and cultivation of molds. The media were prepared aseptically according to the manufacturer's guidelines and dispensed in 25 ml amounts into 90 mm-diameter sterile petri plates. This was done in a clean bench laminar cabinet which was disinfected by applying 70% alcohol. Each petri plate was labeled with the identification number given to corresponding samples after solidification of agar. Fungi were inoculated onto the center of the SDA plates by the use of inoculation sterile loop. Incubation was done at 30°C for two weeks and checked daily for any growth before sub-culturing positive ones.

Colonies with distinct morphological differences such as color, shape and size were picked using a wire loop sterilized by heating over a Bunsen burner and purified on Sabouraud dextrose agar plates supplemented with 1% chloramphenicol. The sub-cultures were incubated at 30°C for 2-5 days. Pure isolates were stocked at -20oC on glycerol media before identification. The isolated species were identified on the basis of the micro morphological appearance of colonies and macro morphological appearance of conidial and other appearances, according to the key described by Larone, (2002). Morphological features of molds were considered and main microscopic features such as colony color on agar, reverse and colony texture and colony diameter were helpful in identification (CLSI, 2012).

3.6.3.4 Identification of yeast

Features of yeasts were identified by observing their morphology. Biochemical tests were done following the identification of the cultures on agar media and microscopy. Purified yeast colonies from Sabouraud agar were sub-cultured onto CHROM agar for preliminary identification of yeasts and mixed cultures as described by (Sivakumar *et al*., 2009). CHROM agar has chromogenic,5- bromo 6-chloro 3-idolyl phosphate (Chromogenic substates) and 5- bromo 4-chloro 3indolyl N-acetyl-β-D-glucosaminide, which reacts with species specific enzymes to geve yeasts colonies which are different in color (Ghelardi *et al*., 2008). The preparation of media was done as per the manufacturer's instructionand then dispensed 25ml amount into 90mm

diameter sterile petri dishes. Then purified yeast colonies on Sabouraud agar were inoculated into CHROM agar using a sterile wire loop after which it was incubated at 30^0C for 48 hours.

3.6.3.5 Antifungal susceptibility testing of Dermatophytes isolates

The susceptibility testing of dermatophytes was performed as suggested by the Clinical Laboratory Standard Institutes using M38-A2 document, which is the reference method for Broth dilution of antifungal susceptibility testing of filamentous Fungi. In-vitro activities of four antifungal agents commonly used topically and orally were used. Itraconazole; It belongs to a class of drug triazoles, is used orally, intraveneous and suspension form and it is 3rd generation azoles that interfere with ergosterol synthesis. Clotrimazole; Belongs to a class of drug imidazole of second generation usually applied topically and orally and is well absorbed. It interfere with cytochrome P450 and it requires high dose. Terbinafine; Belongs to a class of drug Ally amines. It inhibits ergosterol synthesis by inhibiting the enzyme squalene epoxidase and is topically applied and used orally and Griseofulvin; It binds microtubule proteins and inhibits cell wall. Preparation for broth microdilution test containing the drug was dissolved in DMSO for which the highest desired test concentration was 16µg/mL, 1st weighed 4.8mg (assumed 100% potency) of the antifungal powder and dissolved it in 3.0mL DMSO. This provided a stock solution at 1600µg/mL. Then prepared further dilutions of this stock solution in DMSO. Diluted the solution in DMSO 1:50 in test medium and a further 2x twofold occurred when inoculated. Reducing the final solvent concentration to 1% DMSO at each concentration as well as in the growth control (drug-free medium (RPMI)) used in the tests as solvent control. All the tests were performed in sterile,flat-bottomed, 96-well micro plates. Aliquots of 100µL of the drug dilutions were inoculated into the wells with multichannel pipette. The susceptibility testing,100µL of the diluted inoculum suspensions was added to each well to bring the drug dilutions to the final test concentrations. Growth and sterility control wells were prepared. The micro plate contents were incubated at 28^0C, and were read. The minimum inhibitory concentrations (MIC), the MIC range, and the MIC at which 50% (MIC_{50}) and 90% (MIC_{90}) of the dermatophyte isolates were inhibited and was determined as per CLSI, guidelines (CLSL, 12).

3.6.3.6 Preparation of the Antifungal drug micro dilution plates

The modified protocols described by CLSI, 2008 referred to as (M38-A2) standard method for conidium forming filamentous fungi to test for antifungal drug susceptibility dermatophytes (Janssen- Cilag Beerse, Belgium) was used in this study. The Azoles and Griseofulvin antifungal stock solutions were prepared by dissolving 16µg of each antifungal in 10ml of 100% dimethyl sulphoxide (DMSO) (Sigma chemicals Co., St. Louis, Mo., U.S.A.) in separate tubes to get a concentration of 1600µg/ml. The stock solutions were kept frozen in 1ml aliquots at -70^{o}C. A working solution of each antifungal was prepared by diluting 100 µl of the stock solution in 900 µl of RPMI-1640 medium containing L-glutamine and 0.165M morpholine propane sulfonic acid (MOPS) without bicarbonate (GIBCO BRL, Life Technologies, Paisley, Scotland) to get a concentration of 32µg/ml for the azoles.

Candida parapsilosis ATCC 22019 (The American Type Culture Collection (ATCC) was used as quality control strain to test for the antifungal drugs. The reference strain was grown in 10ml brain heart infusion broth (Difco) at 35^{o}C overnight.

The suspension was diluted to two folds with brain heart infusion broth containing 20% glycerol (Sigma), and dispensed in screw-capped tubes, sealed and stored at -70^{o}C.

The reference strain was tested with every batch of antifungal susceptibility of the isolated dermatophytes species. Two fold serial dilutions of the antifungal agents was prepared with RPMI 1640 medium. The final concentration of the antifungal agents ranged from 32 to 0.063µg/ml for itraconazole, terbinafine, griseofulvin, and clotrimazole. Sterility control (negative control) and growth control (positive control) were included in each plate. For each antifungal susceptibility, antifungal control using *C. parapsilosis* ATCC 22019 reference strain was inoculated to test for the validity of the four antifungal agents according to the (CLIS), M38-A2,standard method (Jessup *et al.*, 2000).

3.6.3.7 Preparation of the Dermatophyte inoculum.

Dermatophyte isolates were re-seeded on oatmeal cereal agar and potatoes dextrose agar slants for 7 days at 28°C; which supported the conidial growth (Jessup *et al.*, 2000). Sterile normal saline (0.85%) was then added to the slant culture and gently swabbed with a cotton tip applicator to dislodge the conidia from the hyphal mat. This suspension was adjusted to 5 ml with sterile normal saline. The cell density was adjusted to give final inoculums concentration of 104 (colony forming unit) CFU/ml. Exactly 100µ1 of the organism suspension was transferred into sterile, flat-bottomed, 96-well micro plates except for the negative control wells. The plates were incubated aerobically 7-10 days at 28°C, except for *E. floccosum* and *M. canis* at 35°C.

3.6.3.8 Reading and interpretation of the panel.

The minimal inhibitory concentration (MIC) endpoint was determined according to CLSI M38-A2 standards. The concentration at which the organism was 80% inhibited compared to the growth control was considered the MIC for azole antifungals. The point at which no visual turbidity was observed was considered MIC for the griseofulvin and terbinafin antifungals. The quality control strain *Candida parapsilosis* MIC endpoint was determined as ≥80% inhibition of the positive growth control for azoles (CLSI M38-A).

The result from the laboratory was communicated back to the clinician for patient management where necessary. All infected patients were offered the appropriate treatment in the Kenya Medical Research Institute (KEMRI) referral Centre in Alupe. The HIV sero-positive patients were referred to Alupe District Hospital for care and treatment of opportunistic infections.

3.7 Data Management and Analysis

3.7.1 Data Entry and Cleaning

Microsoft Excel 2007 was used in data entry, cleaning and coding before analysis. Data was stored in Ms Excel®worksheet and in a hard covered book.

3.7.2 Data Analysis

Data was presented in form of tables and graphs. Data was analyzed using STATA version 12 SE. Categorical variables were summarized as frequencies and the corresponding percentages. Continuous variables were summarized using the median and the corresponding lower and upper quartiles as well as the minimum and the maximum values. Simple descriptive statistics were used to determine social demographic. Simple logistic regression was used to determine relationship between infections and ages.

3.8 Ethical Considerations

Approval was sought from SERU at KEMRI through the Centre of Infectious and Parasitic Disease Control Research SSC protocol number 2186 prior to the study. Consent was sought for those who were willing to participate. All procedures were carried as per Clinical Laboratory Standards Institute, (CLSI) and Kenya Medical Research Institute (KEMRI) Mycology Laboratory Bio-safety guidelines.

3.9 Limitations of the study

- Patients expected to get results immediately but mycoses culture takes time to grow and because of this patients get results late than expectated.
- Some mycoses never grew in the primary media due to prior exposure to antifungal drug while some were bacterial infections.

CHAPTER FOUR

RESULTS

4.1 Factors associated with mycoses in Alupe KEMRI clinic

Structured questionnaire was administered to get information on patients consented to participate in the study. 371 samples from equivalent number of patients were collected. 117 samples were collected from children under 18 years while 132 samples were collected from farmers The data on social-dermographic; Age, sex and occupation was collected as shown in Table 4.1, it was noted that patients on age category 5-14 81(21.8%) were the most affected with 85-94 1(0.3%) being the least affected respectively. In sex category,women were most affected 212(57.1%) while male were 159(42.9%) respectively. It was also noted that farmers were more affected 132(35.6%) while other recorded least affected in occupation 12(3.8%).

Table 4.1: Factors associated with the skin on patients attending Alupe KEMRI clinic

Characteristic	Category	Frequency
Age in years	0-4	21(5.7%)
	5-14	81(21.8%)
	15-24	56(15.1%
	25-34	66(17.8%)
	35-44	47(12.7%)
	45-54	39(10.5%)
	55-64	38(10.2%)
	65-74	15(4%)
	75-84	7(1.9%)
	85-94	1(0.3%)
Sex	Male	159(42.9%
	Female	212(57.1%)
Occupation	Business	68(18.3%)
	Farmer	132(35.6%)
	Employed	43(11.6%)
	Unemployed	114(30.7%)
	Other	14(3.8%)

4.1.1 Age group category in years

The structured questionnaire was used to capture the age of patients and the age ranged from 0 to 94 years with the mean age of 31.0± 20.0. In age categorization it was noted that between 0 and 4 years there were 5.7% (21), between 5 and 14 years 21.8% (81), between 15 and 24 years 15.1% (56), between 25 and 34 years 17.8% (66), 35 and 44 years 12.7% (47), 45 and 54 years 10.5% (39), 55-64 years 10.2% (38), 65 to 74 years 4.0% (15), 75 to 84 years 1.9% (7) and 85 to 94 years 0.27% (1). Age group between 5 to 14 years 21.8% (81), were the most affected group by mycoses. Table 4.2

Table 4.2: Mycoses according to age categories

Age group	Frequency	Percent
0-4	21	5.7
5-14	81	21.8
15-24	56	15.1
25-34	66	17.8
35-44	47	12.7
45-54	39	10.5
55-64	38	10.2
65-74	15	4
75-84	7	1.9
85-94	1	0.3
Total	**371**	**100**

4.1.2 Mycoses infections among the gender in Alupe KEMRI clinic

The total number of patients were 371 in which 42.86% (159) were male and 57.14% (212) were female.

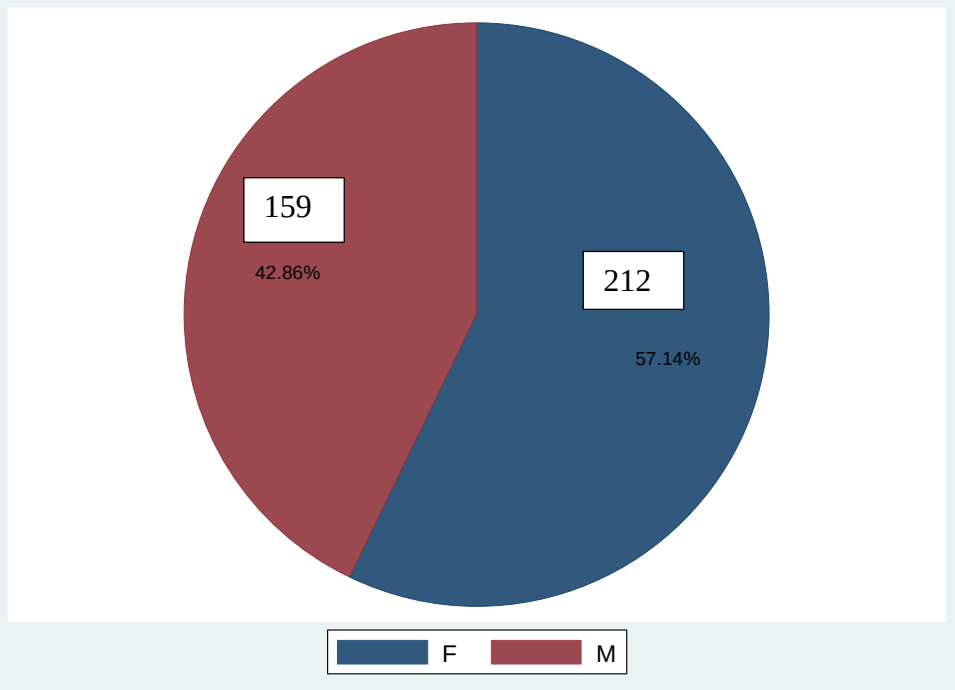


Figure 4.1: Relationship between gender and mycoses infections

In this study mycoses infections in relation to age and gender were as follows; 0 to 4 years [2.7% (10)] males and [3%(11)] females, 5 to 14 years 8.9% (33) males and 12.9% (48) females, 15 to 24 years there were 5.1% (19) males and 10% (37) females, 25 to 34 years 7% (26) males and 10.8% (40) females, 35 to 44 years there were 7.5% (28) males and 5.1% (19) females, 45 to 54 years 5.1% (19) males and 5.4% (20) females, 55 to 64 years there were 3.5% (13) males and 6.7% (25) females, 65 to 74 years 1.9% (7) males and 2.2% (8) females, 75 to 84 years there were 1.1% (4) males and 0.8% (3) female and 85 to 94 years 0.3% (1) female. Table 4.3

Table 4.3: Mycoses distribution by gender and age group in years

Sex	0-4	5-14	15-24	25-34	35-44	45-54	55-64	65-74	75-84	85-94
Females	11	48	37	40	19	20	25	8	3	1
Males	10	33	19	26	28	19	13	7	4	0
Total	**21**	**81**	**56**	**66**	**47**	**39**	**38**	**15**	**7**	**1**

The study shows that participants 54 years and below 83.6% (310) were more affected by mycoses compared to those who were above 55 years 16.4% (61) with a mean of 30.98 and a median age of 28.00. Figure 4.2.

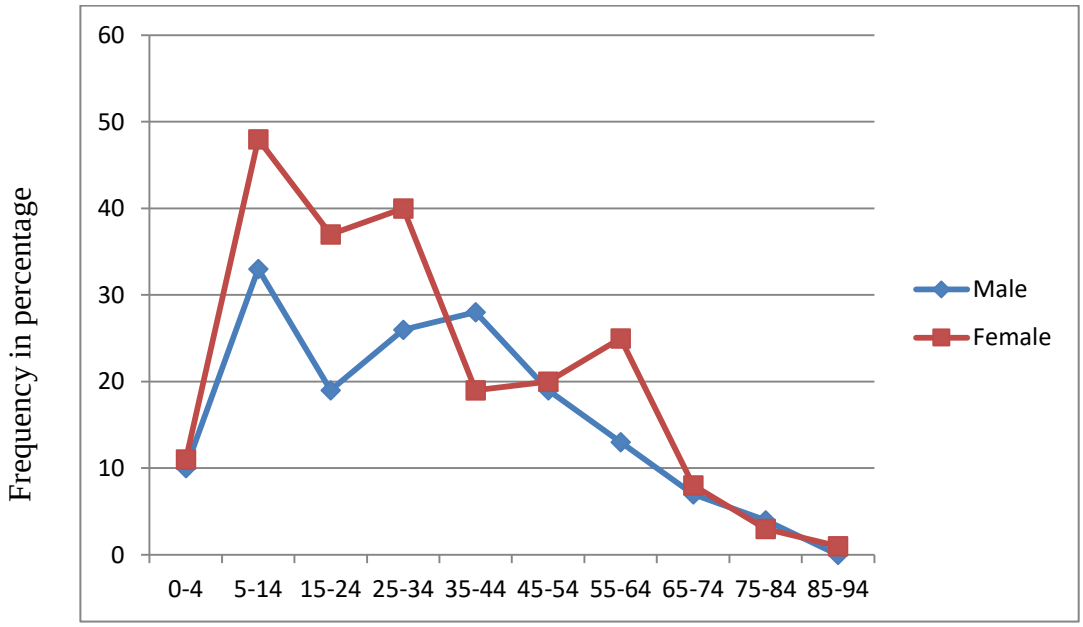


Figure 4.2: Age and gender distribution for mycoses

4.1.3 Mycoses among occupation in patients with skin mycoses attending Alupe KEMRI clinic

In this study 371 patients were recruited, their occupation were grouped into five categories; Business 18.30 % (68), farmers 35.6% (132), employed 11.6% (43), unemployed 30.7% (114), others 3.8% (14). It was observed in this study that the most affected occupation was farmer followed by unemployed while other was least affected by superficial mycoses. Figure 4.3

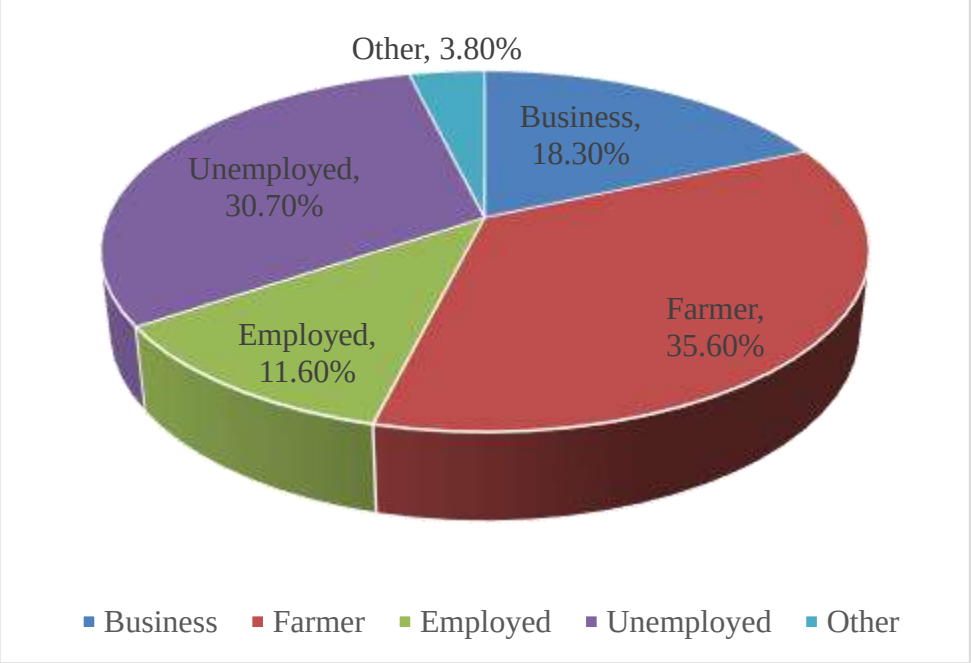


Figure 4.3: Mycoses among occupation in patients

4.2 Anatomical Sites of Mycoses Infection

In a population of 371 it was noted that 7.6% (28/371) had the mycoses infections on the face, 15.4% (57/371) had infections on hand, 26.4 % (98/371) had infections on the leg, and 12.9% (48/371) had infections on the neck 12.4% (46/371) had the infections on the neck, scalp, hand, 1.9% (7/371) had infections in the private part, 13.5% (50/371) had infections on the scalp, 8.9% (33/371) had infections on the trunks and 1.1% (4/371) had infections on the whole body. Figure 4.4

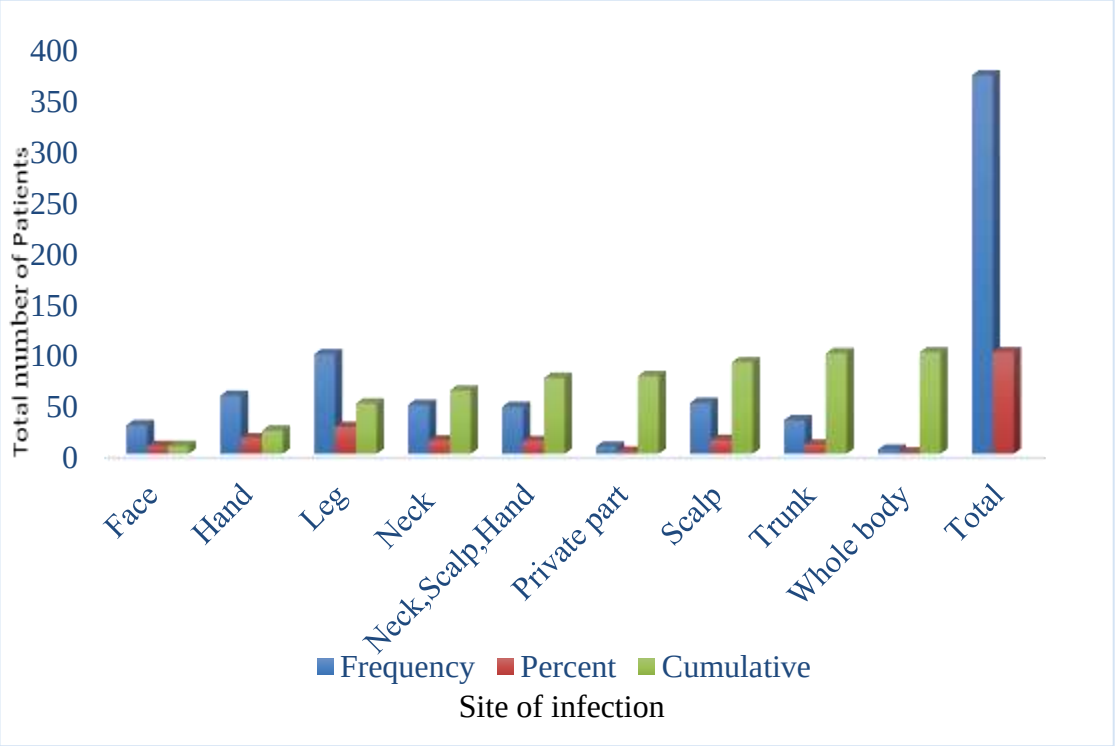


Figure 4.4: Site of mycoses infection

4.3 Mycoses isolates from patients in Alupe KEMRI clinic

Out of 371 study population it was observed that spectrum of agents of mycoses associated with the skin were: *Alternalia* species was 3.8% (14/371) cases, *Aspergilus* species 12.1% (45/371), *Cladosporium* species, 2.2% (8/371), *Penicillium* species was 4.3% (16/371), *Rhizopus* species 3.2% (12/371), *Trichophyton* species 27.8% (103/371), Yeast 2.7% (10/371) ,others 6.7% (25/371) and No growth obtained 24.8% (92/371). Table 4.4.

Table 4.4: Spectrum of agents of mycoses associated with the skin on patients in Alupe KEMRI clinic

Mycoses Species	Frequency	Percent
lternalia species	14	3.8
Aspergillus species	45	12.1
Cladosporium species	8	2.2
No growth obtained	92	24.8
Penicillium species	16	4.3
Rhizopus species	12	3.2
Trichophyton species	103	27.8
Yeast	10	2.7
Others	25	6.7

4.3.1 Aspergillus versicolor

The *Aspergillus versicolor* was identified by the use of Lactophenol cotton blue (LPCB) stain which is used for staining in microscopic identification at X400 magnifications for detection of hyphae and conidia which had the vesicle like a circle at the center in which filamentous were anchored to. Topography and reverse of *Aspergillus versicolor* on primary media (SDA). Plate 4:1

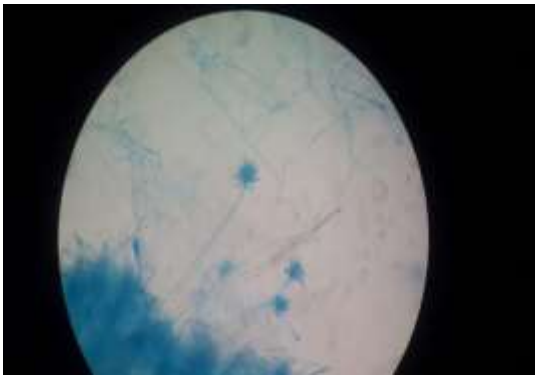


Plate 4.1: Microscopy of Aspergillus versicolor stained in LPCB at X400 magnifications

Plate 4.1 a) **Plate 4.1 b)**

Apergillus versicolor in SDA media **4.1**a) Topography Texture: is suede-like, with radial grooves **4.1** b) Reverse is a reddish-brown or reddish-purple

4.3.2 Rhizopus

Colonies of *Rhizopus* grew very fast and filled the Petri dish and matured within 5 days at 37°C. The texture was cotton-candy like. From the front, the color of the colony was white which turned grey to yellowish brown. The reverse was white to pale. Plate 4.2.

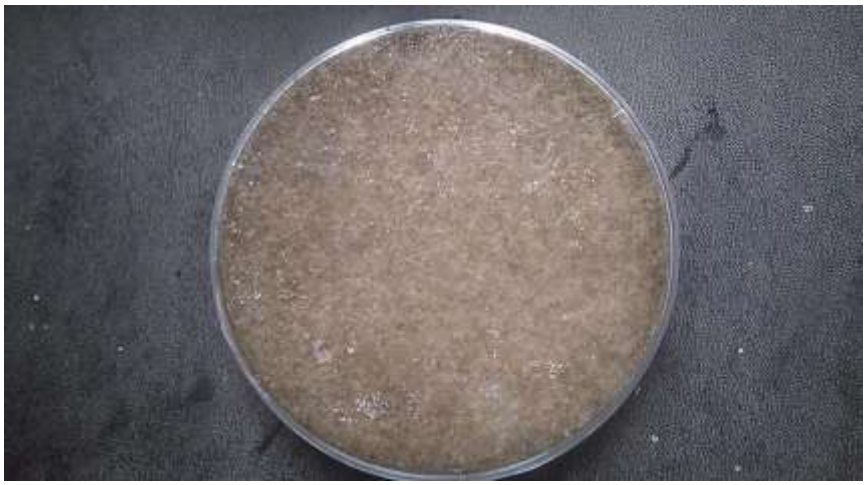


Plate 4.2a: Morphological appearance of Rhizopus

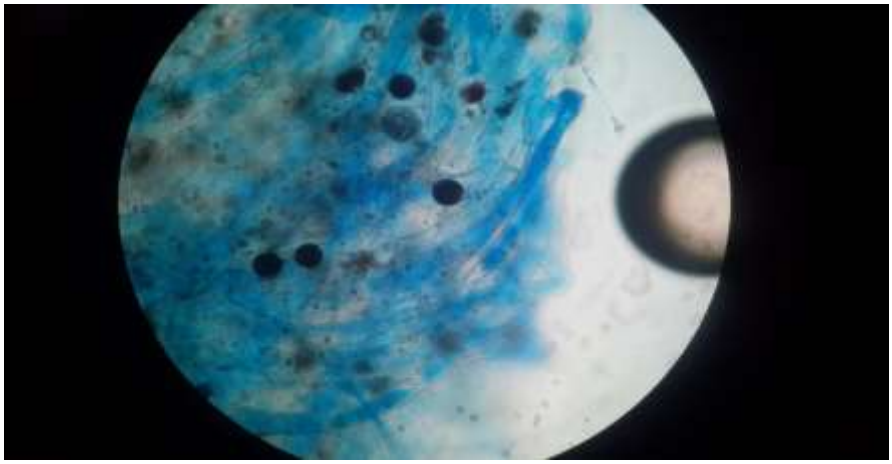


Plate 4.2b: Microscopic of *Rhizopus* stained in LPCB under X400 magnification

4.3.3 Trichophyton

The growth rate of colonies was slow. The texture was waxy, glabrous and cotton like. The front color was white to bright yellowish beige. Reverse was yellowish, pinkish, reddish-brown in color. Plate 4.3 (a-c).

Plate: 4.3a **Plate:4.3b** **Plate: 4.3c**

Plate 4.3a: Colony morphological reverse of Trichophyton species. Plate 4.3b The front color of Trichophyton species. Plate 4.3c Microscopic of Trichophyton species stained in LPCB at magnification of x400.

4.3.4 Alternaria

The growth rate of colonies was rapid. The colony was woolly, flat and covered by short grayish aerial hyphae. The surface was also pale gray to olive brown on surface with a light border. Reverse was brown to black in color. Plate 4.4 (a-c).

Plate: 4.4a **Plate: 4.4b** **Plate: 4.4c**

Plate 4.4a: Colony morphological reverse of alternaria species. Plate 4.4b The front color of alternaria species. Plate 4.4c Microscopic of alternaria species stained in LPCB prepartions at magnification of x400.

4.3.5 Cladosporium

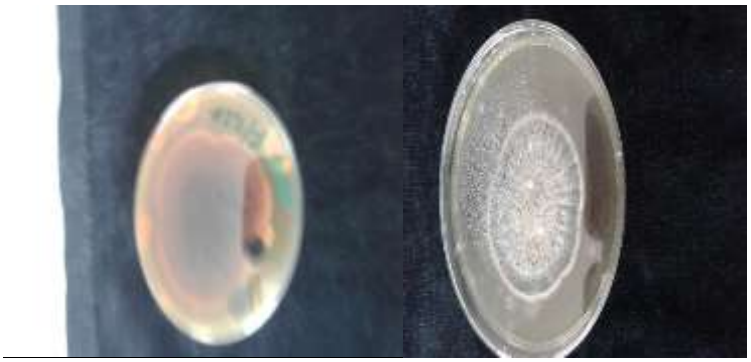
The growth rate of colonies was moderately rapid. The colony was black with grayish velvet, heaped and folded. Reverse was black in color. Plate 4.3.5 (a-c).

Plate: 4.5a **Plate: 4.5b** **Plate: 4.5c**

Plate 4.5a Colony morphological reverse of *Cladosporium species.* Plate 4.5b The surface color of *Cladosporium species*. Plate 4.5c Microscopic of *Cladosporium species* stained in LPCB prepartions at magnification of x400

4.3.6 Penicillium

The growth rate of colonies was rapid.The surface was white,powdery with a white border. Reverse was brown in color. Plate 4.6 (a-c).



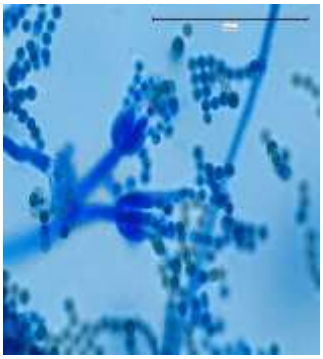


Plate: 4.6a **Plate: 4.6b** **Plate: 4.6c**

Plate 4.6a: Colony morphological reverse of *Penicillium species.* Plate 4.6b: The surface color of *Penicillium species*. Plate 4.6c: Microscopic of *Penicillium species* stained in LPCB prepartions at magnification of x400

4.3.7 Rhodotorula

Rhodotorula species are pigmented basidiomycetous yeasts. Colonies on saboroud dextrose agar appeared orange to red-orange because of the production of carotenoids. It presented big round blastospores in cornmeal agar with hyphae and pseudohyphae absent.

Plate 4.7: Smooth, glistening, round with mucoid red-orange colonies of *Rhodotorula species*.

4.4 Mycoses infections in relation to Anatomical sites

In this study mycoses were noted in the following sites; Out of 371 cases, face had 7.5% (28/371). Hand cases were 15.4% (57/371). Leg had 26.4% (98/371) cases. Neck cases were 13% (48/371). Neck, Scalp, Hand cases were 12.4% (46/371). Private part cases were 1.9% (7/371). Scalp cases were 13.5% (50/371). Trunk cases were 8.9% (33/371). Whole body cases were 1.0% (4/371). Table 4.5.

Table 4.5: Mycoses infections in relation to Anatomical sites

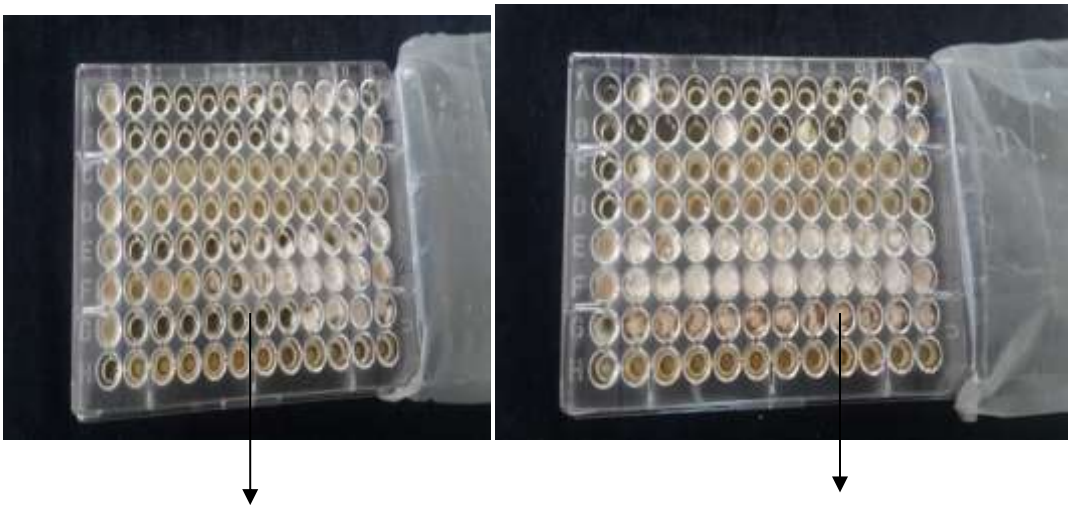
Site	Frequency	Percent
Face	28	7.7%
Hand	57	15.4%
Leg	98	26.4%
Neck	48	12.9%
Neck, Scalp, Hand	46	12.4
Private Part	7	1.9%
Scalp	50	13.5%
Trunk	33	8.9%
Whole Body	4	1.0%
Total	371	100%

4.5 Antifungal susceptibility testing of mycoses isolates

The susceptibility testing of pathogens was performed as described by the Clinical Laboratory Standard Institutes using M38-A2 document, which is the reference method for broth dilution of antifungal susceptibility testing of filamentous Fungi. The *in vitro* activities of the micro-dilutions of azole antifungal (itraconazole, clotrimazole, terbinafine and griseofulvin) were done. The minimum inhibitory concentrations (MIC), the MIC range, and the MIC at which 50% (MIC_{50}) and 90% (MIC_{90}) of the pathogens were inhibited are shown in Table 4.6. The inhibitory effect of antifungal drugs at the incubation endpoint of the NCCLS M27-A method was based on cell growth, which was measured in terms of culture turbidity (Figure 4.5). The MIC range in µg/ mL was recorded .Table 4.6.

(a) Minimal inhibitory concentration (MIC) endpoints of intraconazole drug

(b) Minimal inhibitory concentration (MIC) endpoints of clotrimazole drug



(c) Minimal inhibitory concentration (MIC) endpoints of terbinafinedrug

(d) Minimal inhibitory concentration (MIC) endpoints of griseofulvin drug

Figure 4.5: Minimal inhibitory concentration (MIC) endpoints of fungal drugs

Broth microdilution method M38-A2 approved protocol of CLSI (2008) for filamentous fungi was followed for determining the susceptibility of pathogens. The susceptibility testing was based on four drugs; Itraconazole,terbinafin,griseofulvin and clotrimazole.Table 4.6. These drugs are commonly used as the choice of anti-fungal

agent which dependent on age, overall health of the patient ,extent of clinical mycoses infection and site of infection.

Table 4.6: Antifungal susceptibility testing of mycoses isolates

Isolates	**Intraconazole µg /Ml**	**Terbinafine µg /mL**	**Griseofulvin µg /mL**	**Clotrimazole µg/mL**
Trichophyton mentagrophytes	0.25	0.5	16	2
Trichoderma	>32	>32	>32	>32
Cladosporium	0.25	<0.0625	>32	1
Fusarium sonai	0.25	0.5	>32	0.25
Aspergilus fumigatus	0.5	0.5	>32	0.5
Alternalia alternate	0.5	4	>32	0.125
Trichophyton verrucosum	1	<0.0625	>32	0.25
Trichophyton intergidale	0.25	0.125	>32	<0.0625
Trichophyton tonsurans	<0.0625	0.0625	<0.0625	<0.0625
Trichophyton concentrim	<0.0625	<0.0625	<0.0625	<0.0625
Trichophyton niger	2	0.5	>32	4
Rhizopus arrhizus	<0.0625	16	>32	<0.0625
Penicillium	0.25	0.125	>32	<0.0625
Aspergilus candidus	0.25	<0.0625	>32	<0.0625
Yeast	0.125	<0.0625	>32	0.25
Aspergilus versicolor	<0.0625	<0.0625	>32	0.25
Scylidium dimidiatum	1	0.5	>32	0.5

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The result of the current study showed that out of total number of skin samples examined, the incidence of *Trichophyton* species were the highest 27.8% (n=103) and *Cladosporium* species 2.2% (n=8) were the lowest respectively. This is because *Trichophyton* species are very common mycoses infections that are affecting the skin that occur to people, animals and poultry all over the world (Dias *et al.,* 2013). In the previous study it was reported that the genus *Trichophyton* gives rise to most of the tinea dermatophytoses, including tinea capitis, tinea pedis, and tinea unguium (onychomycosis) (Goldstein *et al.,* 2000). This study is in agreement with other studies done in Kenya and other places in which approximately 7% of the Kenyan population suffers from a significant mycoses infection, with recurring of *trichophyton* species accounting for 82% of the infections (Guto *et al.,* 2016 ; Nweze, 2010).

Cladosporium species was the lowest mycoses isolated from patients in Alupe KEMRI clinic. This is because it is rarely associated with animal and human opportunistic infections (Bensch *et al.,* 2012). According to a study carried out in the United states, it was reported that *Cladosporium* is generally associated with restricted superficial, subcutaneous or cutaneous mycoses (Sang *et al*., 2012). In previous study *Cladosporium* species have been reported that they are everywhere but hardly can cause mycoses infections (Sandoval-Denis *et al*., 2015).

Alternaria species was among the identified mycoses infection isolated from patients attending Alupe KEMRI clinic with 3.8%. The study is in agreement with the research done in India which reported that alternaria can be found on animal and human skin (Pastor & Guarro, 2008). Aprevious study on alternaria species in North India revealed that 3.3% of alternaria species was isolated from patients and the portal of entry was through the breakdown of the skin barrier (Pastor & Guarro, 2008). These mycoses has been reported to be opportunistic human infections and most present with clinical appearances and are subcutaneous and cutaneous infections (74.3%), according to the

study done by (Pastor & Guarro, 2008). Most patients present with red papules,erythema and the skin develop to ulceration and erosion.

It has also been reported by another study that, alternaria species is an opportunistic mould (Pfaller & Diekema, 2004). It causes onychomycosis which is a cutaneous infections that is commonly found in immuno-compromised patients. According to the study done in Spain, alternaria Species infected mostly immunocompromised hosts (Pastor & Guarro, 2008).

The common isolated mycoses from the skin of patients attending Alupe KEMRI clinic were aspergillus species with (12.1%). It is one of the common medically significant mycoses isolated and are opportunists. The mycoses involves a wide variety of clinical presentations, including, cutaneous ,keratitis , wound and otomycosis, infections (Gautier *et al*., 2016). The present study is in agreement with the previous one which reported that subcutaneous and superficial mycoses infections affect the keratinous and skin tissues and are among the most commonly occurring skin mycoses, affecting millions of individuals globally (Garber, 2001). Another study had revealed that aspergillus species is the most common species involved in human disease especially the skin (Gautier *et al*., 2016). The mycoses is most isolated from skin of immunocompromised patients (Pfaller & Diekema, 2004).

In this study *Penicillium* species represented (4.3%) of the total isolates of mycoses isolated from the patients attending Alupe KEMRI clinic. These mycoses may be due to zoonotic infectious diseases that can be naturally transmitted between animals and humans. Animals can be either mechanical or reservoir of mycoses of these zoonotic pathogens consequently the spread may be indirect or direct contact. *Penicillium* species have been identified as Zoonotic mycoses and can be indeed spread between animals and humans, and can cause major public health concern (Seyedmousavi *et al*., 2015). *Penicillium* species are among the mycoses infection of important emerging community health problem, particularly among patients infected with human immunodeficiency virus in the regions of endemicity in South East Asia, China and India (Vanittanakom *et at*., 2006). A previous study on health risks associated with

exposure to filamentous fungi has revealed that *Penicillium* species have been affecting different organs such as the eyes, ears, nails, and skin (Egbuta *et al*., 2017).

Rhizopus species was among isolates from patients and represented 3.2% of the total isolates. *Rhizopus* species is ubiquitous mycoses in nature, and a number of its species is used in food fermentation and the production of hydrolytic enzymes. The infection was probably acquired through direct traumatic inoculation through the skin. Previous investigation recorded that the infection can be gotten through direct traumatic inoculation through the skin or mucosa or inhalation (Rabie & Althaqafi., 2012). *Rhizopus* species affect patients who are immunocompromised due to underlying diseases like HIV/AIDS and diabetes (Kontogiorgi *et al*., 2007). *Rhizopus* species can be gotten through infection with spores usually happens when there is a break of the skin and underlying mucosa, such as spider bites, infected dressings, catheterization or contaminated wounds (Roden *et al.,* 2005).

According to this study,Yeast species contributed (2.7%) of isolates from mycoses on patients attending Alupe KEMRI clinic. The isolation most likely was due to warm and humid climate which is favourable for multiplication of the fungal. It has been reported by other studies that Y**e**ast species manifestation of the skin dwell well in skin wrinkles of human especially groin and armpits and is common in warm, moisty and hot places (Robert *et al*., 2015). The overgrowth of the mycoses *Candida* is the basis of yeast infections. Adults can also have yeast infections around lower abdomen the creases beneath the breasts ,in nail beds, and below other skin folds. Warm, moisty, tight clothing and poor sanitation may produce these conditions and the fungus will multiply quickly. According to the previos study the fungal was isolated from the area between fingers and toes. The fungus flourishes in, damp, moist and warm conditions.most of these Yeast species infections are superficial cutaneous and can clear up quickly with treatment. According to the previous study Yeast are emerging as opportunistic human pathogens and diagnosis remains puzzling and treatment are not optimal (Miceli *et al*., 2011).

The incidence of of mycoses has grown due to the expanding population of immunocompromised patients, including elderly, cancer and HIV/AIDS patients. Even though some effective treatment options are available, opportunistic mycoses are associated with high morbidity and mortality rates (Nucci *et al*., 2010).

A previous study established that worldwide, people of all ages suffer from severe mycoses infection every year (Brown *et al.,* 2012). This is in agreement with the current study where age category was a factor that contributed for mycoses infections (Caputo,1986). The prevalence of mycoses was significantly (p= > 0.0001) associated with age group of patients with higher infection among those aged 5-14 years with 81 (21.8%). Those aged between 85-94 had the lowest frequency 1(0.3%). This is probably because of overcrowding, unhygyne and vulnerability. The study is in unagreement with what was reported in South Korea in which by age group, mycoses infections among those aged 60-69 and 80-89 years were most prevalent (Lee *et al.,* 2014; Yoon *et al.*, 2014). The study which was done in northern Malawi had the highest prevalence rates of mycoses infections among subjects aged 15-24 years and rates were generally higher among males than among females (Oke *et al*., 2014; Ponnighaus *et al.,* 1996). A study done in India is disagreeing to my report in which it was reported that those in age group of 21-30 were more affected by mycoses compared to other age group (Kaur *et al*., 2008).

The prevalence of mycoses infection was noted to be higher in males 57.14% (212) and females 42.86% (159) respectively. In this study out of 159 males 88.7% (141) and 212 females 81.6% (173) had mycoses infection respectively. This would be females are more sensitive to their health , hygienic and cosmetician compared to males. The finding is thought to be attributed to the possibility that men tend to participate in sports and or outside activities more than women do. This has also been documented by other researchers that mycoses infections differ with sex (Nweze *et al.,* 2005; Rahbar *et al;* 2010). In a study done in South Korea it was noted that the prevalence of mycoses infections was greater among males (5.57%) than that among females (4.81%) (Yoon *et al.*, 2014).

The result of previous study had shown that socioeconomic status especially those in employment and business had been one of the core factors associated with the increase of infections among patients (Chikoi *et al.,* 2018). This is in agreement with this study in which contributing factor for mycoses infections was noted in occupation in which students 39.1% (145/371) were more affected while unemployed were least affected 3.2% (12/371).

Also what emanated from this study is that farmers 29.4% (109/371) recorded the highest number of mycoses. The farmers and students were mostly affected because of their nature of work especially in poor sanitary, hot and humid condition as well as overcrowding and living standards which is favorable environment for the fungi to grow. Researchers have documented similar report that mycoses are more common in students due to exposure to various agents that predispose them to mycoses infections (Ayanlowo *et al.*, 2014; Nweze *et al.,* 2014; Okafor *et al.,* 2011; Oninla *et al*., 2012; Oyeka *et al*., 2008; Sanuth *et al*., 2014). The favorable environment of hot and humid climate, poverty, poor sanitary conditions and overcrowding are well known factors that favor these fungi growth (Olutoyin *et al.,* 2017). Studies have shown that risk factors for superficial cutaneous fungal infection in certain occupations like farmers have a high prevalence compared to others as a result of direct contact with contaminated animals (Kaur *et al*., 2008; Yoon *et al.*, 2014).

Mycoses infection occurs in patients due to immunocompetence of the body system (Guto *et al.,* 2016). In this study I observed that in the HIV/AIDS status, out of 371 patients 55.5% (206/371) knew their HIV/AIDS status with 44.5% (165/371) did not know their status respectively. Of those who were negative were 183 of which 57.9% (106/183) were male and 42.1% (77/183) were female respectively. Unknown HIV/AIDS cases were 165 with male 57.6% (95/165) and female 42.4% (70 /165) respectively. Of the 23 HIV/AIDS positive cases 52.2% (12/23) were famales and 47.8% (11/23) were males respectively. Mycoses infections may be the early signs of HIV/AIDS associated immunosuppression. *Trichophyton* species was the most isolated fungi 27.8% (103/371) from this study with 43.5% (10/23) being isolated from HIV/AIDS positive. Silmilar study on mycoses has shown that HIV/AIDS infections is associated with mycoses infections and is more severe in this group

(Altman *et al*., 2015). More recent studies have shown that mycoses continue to cause morbidity and mortality in immunocompromised patients globally (Altman *et al*., 2015). Similar studies have shown that mycoses are important causes of serious and life threatening mycoses infections in the immunosuppressed (Badiee & Hashemizadeh, 2014; Balwierz, 2004 ; Kaur *et al.,* 2016).

In this study it was noted that most and least affected sites were legs 26.4% (98) and whole body 1.1% (4) respectively. Legs are more exposed to mycoses infections compared to other parts of the body. Research conducted in other places had similar findings (Olutoyin *et al* 2017). These reports are contradictory to the study done in Zambia in which most of the mycoses species isolated in their study were from the truck (general body infection). Also it does not agree with the research done by (Caputo,1986) which shows the most affected part is the scalp.

The study revealed that the distribution of mycoses differ between geographical region. Of the total participants, Busia County had the highest rate 69.54% due to poor sanitary, hot and humid condition in the area and nature of business within the border County which is favorable environment for mycoses. Some counties registered the lowest 0.27% . These findings are similar to what was reported by other studies in other countries that occurrence and aetiological agents differ from time to time with geographic zone and humidity (Silva-Rocha *et al* 2017). This study is in agreement with what was reported by other study that fungal species causing mycoses infections differs from one geographical region to another (Michaels & Del Rosso, 2012).

Antifungal susceptibility testing plays a significant part in managing and making decision especially on appropriate drug to be prescribed for treating a patient with specific kind of mycoses infections (Posteraro *et al.,*2014). The result of the present study found that mycoses organisms were more susceptible to itraconazole drug which belongs to a class of drug compared to other tested drugs; clotrimazole, terbinafine and griseofulvin. This could be due to interference of the drug with ergosterol systhtesis. This study is in agreement that itraconazole remains an important drug in the prevention and treatment of mycoses infection. Itraconazole has a broad-spectrum of activity and is available in both an intravenous and oral form (Lestner *et al*., 2013).

CHAPTER SIX

CONCLUSIONS

6.1 Conclusions

The findings of this study reported the prevalence of *Trichophyton* species was the most associated mycoses with the skin on patients attending Alupe KEMRI clinic with a prevalence of 27.8% while *Cladosporium* was the least isolated 2.2% respectively. It was also observed that out of 23 HIV/AIDS positive cases 43.5% had *Trichophyton* species infection.

According to this study, the age category was a factor that contributed for mycoses infections. The difference in age category between age groups was statistically significant ($p = < 0.0001$).

The study has also raised the importance to raise health awareness on mycoses infections both in health facilities and public places. It has revealed that to prevent mycoses infections it is essential to practice good hygiene and avoid overcrowding both in schools and public palces.

It is evident from this study that mycoses pathogens were susceptable to *intraconazole* drug and is an important agent in the prevention and treatment of mycoses infection.

6.2 Recommendations

I. There is need to raise health awareness on mycoses infections both in health facilities and public places.

II. To prevent mycoses infections it is essential to practice good hygiene and avoid overcrowding both in schools and public palces .

III. The clinicians need to know that itraconazole is a drug of choice for mycoses infections. However is important to routinely use mycological anaysis involving identification of fungal species and susceptibility testing for fungal treatment of patients.

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APPENDICES

Appendix I: Informed Consent Document (ICD)

Title: Mycoses Associated with the Skin on Patients Attending Alupe KEMRI Clinic.

Introduction

We are conducting a study to investigate the superficial fungal infections in western Kenya in order to look for solutions of reducing the disease burden in the area. In order to be sure that you are informed about being in this research, we are asking you to read (or will read to you) this consent form. The purpose of this consent form is to give you the information you will need to help you decide whether or not to participate in the study. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. Before you decide if you wish to be in this study, you need to know about any good or bad things that may arise if you decide to join. This form tells you about the study. This consent form may contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand.

Being in the study is your choice

When we have answered all your questions, you can decide if you want to participate in the study or not. This process is called ‘informed consent’. This consent form gives you information about the study and the risks will be explained to you. Once you understand the study, and if you agree to take part or your child under 17 years to take part you will be asked to sign your name or for your child under 17 years or make your mark on this form in the presence of a witness.

Before you learn about the study, it is important that you know the following:

Your participation in this study is entirely voluntary

You may decide not to answer questions, give any specimens or even withdraw from the study at any time

Purpose for the research

We are asking you to participate in this study to help us to assess the causative agents and factors associated with superficial fungal infections. We would also like to get information on the social demographic from you and we may request for your consent to collect skin scrapings from you in case you agree to participate in this component of the study.

Study groups

The study groups will comprise of children of both sex under the age of 17 years with superficial fungal infections, women and men who are parents or guardians of these children and adults women and men who will be above the age of 18 years with superficial fungal infections. All groups of people mentioned here are very important to this study

Procedures

If you agree to participate in this study by signing at the end of this form, you will participate in the following activities. You will be questioned about your personal life related to this study such as your region, county, village, date of birth and occupation. We shall also request you to provide us with details of length of the superficial fungal infections in your child / you.

We will take the scrapings specimen from the affected part to help us in investigation the cause of superficial fungal infections. If your child/ you will participate, we will request that you consent to take the scrapings sample from him/her for further investigations in the laboratory.

Precautions

Your child/you might feel a little discomfort if the affected area is cracked and cleaned with alcohol before specimen is removed, however, there are no other expected complications associated with this exercise. The team is well trained and experienced staff will guide you through this exercise and will take necessary precaution to ensure minimum discomfort.

Possible Risks/Discomfort

There are no invasive procedures that will be carried out on you or your child. You may feel uncomfortable during the interview due to the sensitive nature of some questions including loss of privacy. We will minimize risk and discomfort from the interview by using a trained staff to place you at ease during the interview. You may skip any question that you do not want to answer and may terminate the interview at any time without consequence. You will also be free to withdraw from the study any time you feel like.

Data security and Confidentiality

All the information gathered from by the research team will be in confidence for the sole purpose of this research only. Any records relating to your identity or child identity and test results will remain confidential. Your name or your child name will not be divulged in any report of the results, and you will receive copy of this consent form. No one will have access to the interviews except the investigators. The study team will provide you with examination results for the tests carried out. Strict data management procedures are intended to ensure confidentiality of the study subjects.

New findings

Results will be disseminated to the relevant health ministries in Kenya, local and international conferences, the district and other stakeholders in need of this information for the purposes of instituting interventional programs in the country. The findings of this project will be used to provide information to be used to provide

information to be used for improving management and help clinicians on diagnosis of common fungal infections.

Benefits

Results obtained will aid in making recommendations of employing new health approaches to reduce the burden of mycoses infections. The information from the study will help to provide data useful in clinical diagnosis and selection of drug of choice for antifungal treatment.

Costs to you

There is no cost to you for participating in the study.

If You Decide Not To Be In the Research

You are free to decide if you want to be in this research. Your decision will not affect the health care/service you would normally receive.

Leaving the Research

If you choose to be in the study, you can still decide not to complete the interview. If you leave the study, please tell the interviewer why you are leaving so that this information can be used to improve our work and provide more support if possible.

Problems and questions

If you ever have questions about this study, you should contact: Tom Mokaya, Study Principal Investigator, KEMRI, CIPDCR, P. O. Box 3-50400 Busia (K), (Mobile: +254 722 356 707); Email: mokayatom@yahoo.co.uk.

Your rights as a Participant

This research has been reviewed and approved by the Ethical Review Committee of Kenya Medical Research Institute (KEMRI), if you have any questions about your rights as a research participant you may contact the secretary of KEMRI Ethics Review

Committee, P.O. Box 54840-00200,Nairobi;Telephone numbers:020-2722541,0722205901,0733400003; Email address: erc@kemri.org or seru@kemri.org

Your statement of consent and signature

If you have read the informed consent, or had read and explained to you, and you understand the information and voluntarily agree to join this study, please carefully read the statements below and think about your choice before signing your name or making your mark below. No matter what you decide, it will not affect your rights in anyway:

The risks and benefits involved in this study have been read and explained to me.

I have been given the chance to ask any questions I may have and I am content with the answers to all of my questions.

I know that my records or records of my child will be kept confidential and that I may leave this study at any time

The name, phone number and address of whom to contact in case of an emergency has been told to me, and has also been given to me in writing.

I agree to take part or my child to take part in this study as a volunteer, and will be given a copy of this informed consent form to keep.

Participant`s Name (Printed) ……

Signature or Participant or Thumb Print …… Date…….

(For those who cannot sign)

If volunteers cannot read the form themselves, a witness must sign here or can sign for the child:

I was present throughout the entire informed consent process with the participant. All questions from the subject were answered and the participant has agreed to take part in the research.

Printed Name of Witness ……

Signature of Witness……Date ……

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

Printed Name of Person Who Obtained Consent (Study staff) ……

Signature of Person Who Obtained Consent: …… Date……

Note: You are not giving up any of your legal rights by signing this informed consent document.

Appendix II: Structured Questionnaire

Mycoses Associated with the Skin on Patients Atttending Alupe KEMRI Clinic

Study no…….Date of specimen collection…… (Dd-mm-yr)

Interviewer……..

1. Region…… County……..

Division……. Location……

Sub-Location……. Village……

2. Gender: Male ( ) Female ( )

3. Occupation…….

4. Age (years / date of birth) ( ) ( )

5. How is mycoses transmitted? (tick all that is applicable)

□Through touching body of infected persons

□Through touching contaminated objects

□Through coughing

□Though sharing clothes

□Through un-well cooked food

6. Do you think there is an effective medicine for superficial fungal infections?

□Yes

□No

7. Which medicines do you think are effective for treating superficial fungal infections?

□Injections

□Syrups

□Tablets

□Traditional medicine

□Cream/Ointment

□There is no effective drug for treatment of superficial fungal infections

8. Clinical signs: i. Has skin scales ( )

ii. Has nail discoloration ( )

iii. Skin lesions present ( )

Location (specify)……

iv. Acne present ( )

v. Annular plaque with raised edge ( )

vi. Ringworm of the scalp ( )

vii. Any 0ther (specify)……..

9. Diagnosis……..

Appendix III: Idhini ya Maelewano

Mada: Shida za Ugonjwa wa Ngozi Zinazo Ambatana na Virusi Vya Ukimwi Magharibi Mwa Kenya

Utangulizi

Tunafanya utafiti na kuchunguza shida zangozi katika eneo la Magharibi mwa Kenya ili kupata suluhisho mwafaka la kukabiliana na makali yake.Ili kuhakisha kwamba unaelewa kuhusika kwako katika utafiti huu,tunakuomba usome (au tukusomee)fomu hii ya idhini.Madhumini ya fomu ya idhini in kukupa maelezo yatakayo kusaodia kuamua kama utashiriki au la katika utafiti huu.Tafadhali soma fomu kwa makini.Unaweza kuuliza maswali kuhusu lengo la utafiti tunaokuuliza ufanye.Madhara yanayoweza kutokea na hata faida, haki yako kama anayejitolea na chochote kile kuhusu utafiti huu au fomu ambacho si wazi.Kabla ya kuamua ikiwa ungependa kuwa katika utafiti huu, unafaa kujua kuhusu mazuri au mabaya yanayoweza kutokea ikiwa unaamua kujiunnga.Tafadhali tuulize tukueleze chochote unachotaka kuelewa.

Kuwa Katika Utafiti ni Hiari Yako

Tukisha jibu maswali yako yote unaweza kuumua ikiwa utashiriki au la, huu mpangilio unaitwa ``Idhini ya maelewano`` Hii fomu inakupa maelezo kuhusu utafiti na madhara yanayoweza kupatikana.Ukishaelewa utafiti,na kukubali kushiriki,utaulizwa utie sahihi ya jina lako au utie alama yako kwa hii fomu ukiwa na shahidi.Ni vizuri uelewe yafwatayo;

-Kuhusika kwako katika utafiti huu ni kujitolea haswa.

-Unaweza kuamua kutojibu maswali,kutopeana sampuli au hata kujitoa kutoka kwa utafiti wakati wowote.

Madhumuni ya Utafiti

Tunakuomba ushiriki katika utafiti huu ili utusaidie kukagua visababishi na njia sinazotumika kwa kusambaza shida/ugonjwa wa ngozi.Tungetaka kupata maelezo kuhusu eneo lako na tungeomba idhini yako kuchukua sampuli ya ngozi kutoka kwako ikiwa utakubali kushiriki.

Vikundi vya Masomo/Utafiti

Vikundi hivi vitashirikisha watoto wa jinsia zote na chini ya miaka kumi na saba(17) walio na shida za ngozi (fangai), wanawake na waume ambao ni wazazi au walinzi wa hawa watoto na wanawake na wanaume walio zaidi ya miaka kumi na nane (18) walio na shida ya ngozi.Vikundi vyote vilivyo tajwa ni muhimu sana kwa utafiti huu.

Mpangilio

Ukikubali kushiriki kwa kutia sahihi mwishoni mwa fomu hii,utafanya yafwatayo;Utaulizwa kuhusu maisha yako ya kibinafsi ambayo yanauhusiano na utafiti huu kama vile Kaunti/jimbo,kijiji,tarehe ya kuzaliwa na kazi yako.Tutakuuliza pia utupe maelezo kuhusu kiwango cha ugonjwa kwa motto au kwako.Tutachukua sampuli ya eneo liloatirika la ngozi kutusiadia katika uchunguzi wa kutambua sababu/kiini cha ugonjwa.Ikiwa wewe au mtoto atashiriki,tunakuomba idhini ya kuchukua sampuli yake (mtoto) kwa uchunguzi zaidi katika maabara.

Tahadhari

Wewe au mtoto anaweza kuhisi maumivu kidogo ikiwa eneo lililoadhilika limechipuka wakati wa kupangusa na spiriti kabla ya kuchukua sampuli,ingawaje,hakuna matatizo mengine yanatarajiwa kuhusiana na shughuli hii.Wahusika ni watu walio hitimu na walio na uzoevu wa kutosha kutekeleza zoezi hili na watakwelekeza vilivyo kuhakikisha kuwa hakuna maumivu.

Hatari Zinazotarajiwa au Kutoridhika

Hakuna kudungwa au kuingiliwa wakati wa mpangilio huu il unaweza kuhisi vibaya wakati wa mahojiano unapoulizwa maswala yanayo fanya upoteze usiri wako.Tutajaribu kupunguza hatari hii kwa kutumia afisa aliyehitimu ili akuweke huru wakati wa mahajiano.Unaweza kuruka swali ambalo haungetaka kujibu na una uhuru wa kukatisha mahojiano wakati wowote bila madhara yoyote.Unaweza kujitoa kwa utafiti wakati wowote

Kuweka Siri kwa Habari Unayotoa.

Habari yote itakayo sanywa na kundi la watafiti itakuwa ya siri kwa nia moja ya utafiti pekee.Nakala yoyote ya utambulisho wako itawekwa kwa siri.Jina lako halitatolewa wakati wa kuandikwa kwa siri na utapokea nakala yah ii fomu ya idhini.Hakuna atakaye weza kupata ripoti ya mahojiano isipokuwa watafiti peke yao.

Kundi la utafiti litakupa matokeo ya majaribio yaliyofanywa.Utunzaji wa nakala zote ni kwa siri kubwa ili kuangalia siri ya mtu binafsi.

Matokeo Mapya

Matokeo yatasambazwa kwa vituo vya afya nchini,eneo la karibu na hata vikao vya ng`ambo,wilaya na washika dau wanaohitaji matokeo haya ili kuweka mikakati ya kukabiliana na shida hii au kuweka mipangilio mwafaka.Matokeo ya itafiti huu yatatumika kupeana maelezo yatakayo tumika kuboresha vita dhidi ya shida zangozi na pia kusaidia wahuguzi kutambua shida za ngozi kwa urahisi.

Faida

Matokeo yatakayo patikana yatasaidia kutoa mapendekezo kuhusu njia mpya za kiafya zinazoweza kupunguza mzigo wa shida za ngozi.Maelezo kutokana na utafiti yatasaidia kuwa kama ghala itakayo tumika katiaka kutambua/kufanya uamuzi kuhusu magonjwa au hata dawa bora zaidi kwa kutibu magonjwa ya ngozi ya fungi.

Gharama Kwako

Hakuna gharama kwa kushiriki katika zoezi hili.

Ikiwa Utaamua Kutoshiriki

Una uhuru wa kutoshiriki katika utafiti huu.Uamuzi huu hauta adhiri kushughulikiwa/kutibiwa kwako ambako ungepokea kwa kawaida.

Kukatisha Utafiti

Hata kama utashiriki katiaka utafiti, bado una uhuru wa kukatisha mahojiano.Na ikiwa utaamua kuachana na utafiti tafadhali mwambie mhoji wako sababu inayo kufanya uchukue mwelekeo huu ili maelezo haya yatusaidie kurekebisha au kuboresha jinsi tunavyo fanya kazi na kusaidia kazi hii

Shida/Maswali

Ukiwa na maswali kuhusu utafiti huu,wasiliana na Bw. Tom Mokaya, Mtafiti mkuu, KEMRI,CIPDCR S.L.P 3-50400 Busia (K).Nambari ya rununu +254 722 356 707.Barua pepe mokayatom@yahoo.co.uk

Haki Yako Kama Mhusika

Utafiti huu umeangaliwa na kukubaliwa na kamati ya Sheria Angalishi ya KEMRI (Sherika la Utafiti la Kenya); Ukiwa na swali lolote kuhusu haki zako kama mshiriki wa utafiti,unaweza kuwasiliana na mwandishi wa Kamati Angalizi S.L.P 54840-00200 Nairobi. Nambari ya simu; 020-2722541,0722205901,0733400003; Nambari ya electroniki erc@kemri.org

Sentensi Yako ya Idhini na Sahihi

Ikiwa umesoma idhini elekezi/ya maelewano,au umesoma na kuelezewa,na unaelewa maelezo na kwa hiari yako unakubali kujiunga na utafiti huu, tafadhali kwa makini soma maelezo/sentesi zifwatazo na ufikirie kuhusu uamuzi wako kabla ya kutia sahihi

jina lako au kutia alama chini hapa.Haijalishi uamuzi wako,haita athiri haki zako kw njia yoyote ile.

Athari na faida zinazohusiana na utafiti huu nimesoma na kuelezewa.

Nimepewa nafasi ya kuuliza maswali ambayo ningekuwa nayo na nimeridhika na majibu niliyopewa.

Najua nakala zangu zote ziko salama/siri na naweza kukatisha utafiti huu wakati wowote.

Jina, nambari ya simu na anwani ya anayehusika iwapo kuna dharura nimeshajuliswa,na nimepewa kwa nakala.

Nakubali kushiriki katiaka utafiti huu kama mhisani/anyejitolea,na nitapewa nakala ya fomu hii ya idhini ya maelewano/elekezi kuhifadhi.

Jina la Mhusika ……

Sahihi ya Mhusika au Alama ya Kidole Tarehe …….

<u>Ikiwa Wahisani/wanaojitolea Hawawezi Kujisomea fomu, Shahidi Lazima Atie Sahihi Hapa</u>

Nitakuwepo katiaka muda wote wa idhini elekezi/maelewano pamoja na mshiriki.Maswala yote kuhusu utafiti yalijibiwa na mshiriki amekubali kushiriki katika utafiti.

Jina la Shahidi……. ……..

Sahihi ya Shahidi Tarehe……..

Ninathibitisha kwamba jinsi na nia, faida zinazotarajiwa ,na pia hatari zinazoambatana na kushiriki kwenye utafiti huu zimeolezewa kwa mtu wa hapo juu.

Jina la mtu ambaye amechukua idhini (Afisa Mtafiti)…….

Sahihi ya mtu aliye chukua idhini Tarehe ……..

Tahadhari: Hautupilii mbali haki yako ya kisheria kwa kutia sahihi kwa fomu hii ya idhini.