EVALUATION OF FUNGAL MICROBIOTA OF TOE WEBS OF GODFREY OKOYE UNIVERSITY PRIMARY SCHOOL CHILDREN.

BY

HASSAN, UMMIE.

GOU/U22/MCB/526.

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GODFREY OKOYE UNIVERSITY, ENUGU NIGERIA.

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MICROBIOLOGY
DEPARTMENT OF BIOLOGICAL SCIENCES
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GODFREY OKOYE UNIVERSITY, ENUGU NIGERIA

A PROJECT RESEARCH WORK SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN MICROBIOLOGY

SUPERVISOR: EMERITA. PROF. C.A OYEKA.

JULY, 2026

APPROVAL

This research work titled “Evaluation of Fungal Microbiota of Toe Webs of Godfrey Okoye University Primary School Children..” has been assessed and approved by the committees of the Department of Biological Sciences and the Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University, Enugu, Nigeria.

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CERTIFICATION

This is to certify that this project report title: “Evaluation of Fungal Microbiota of Toe Webs of Godfrey Okoye University Primary School Children” was carried out by Hassan, Ummie. with Registration Number GOU/U22/MCB/526 under supervision in the department of Biological Sciences, Godfrey Okoye University Enugu Nigeria.

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DEDICATION

This work is dedicated to God Almighty, who has been my ultimate source of strength and guidance. Through every challenge and triumph, His divine hand has sustained me, illuminating the path and making this achievement possible.

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ABSTRACT

Fungal infections of the toe webs are common among school children and may result in discomfort, itching, inflammation, and other superficial infections if not properly managed. This study is aimed to evaluate the Fungal microbiota present in toe webs of Godfrey Okoye Primary School pupils, Enugu State. A total of twenty (20) toe web samples were collected from pupils aged 7–12 years using sterile swab sticks under aseptic conditions. The samples were cultured on Potatoe Dextrose Agar and incubated at room temperature. Identification of fungal isolates was based on colony morphology and microscopic examination using Lactophenol Cotton Blue stain. Data obtained were analyzed using frequency counts and percentages. The fungal species identified were Aspergillus niger 3(20.0%), Aspergillus clavatus 2(13.3%), Candida albicans 2(13.3%), Cunninghamella bertholletae 2(13.3%), Penicillium verrucosum 3(20.0%), Talaromyces marneffei 2(13.3%) and Trichophyton rubrum1(6.8%). Aspergillus niger and Penicillium verrucosum had the highest frequency of occurrence, while Trichophyton rubrum had the lowest occurrence. The presence of these fungal organisms suggests that the toe web region provides a favourable environment for fungal colonization due to moisture, sweating, and poor foot hygiene practices. The study concludes that primary school pupils may harbour various fungal organisms in their toe web spaces and emphasizes the importance of proper personal hygiene, foot care, and health education in preventing fungal infections among school children.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The skin is the largest organ of the body and provides an essential physical and immunological barrier to environmental insults and pathogenic microorganisms. In addition to its protective role, skin is a complex micro-ecological environment for a variety of microorganisms, such as bacteria, fungi, viruses and archaea, known as the skin microbiome. The microbial communities live in dynamic interactions with the host and play important roles in the maintenance of skin homeostasis, immune regulation and protection against pathogenic invasion (Byrd et al., 2018). To date, the molecular microbiological and metagenomic sequencing of the skin microbiome has provided a completely new perspective on the diversity and complexity of microbial communities in humans, demonstrating that microbial communities differ significantly depending on the location and conditions of the skin, such as moisture, temperature, pH and sebum production (Chen et al., 2018).

Fungi are an important, but not extensively studied, part of the microbial community living on the skin. Cutaneous microbial ecosystems are complex and include a group of fungi collectively known as the mycobiome, which is associated with the human body and has been shown to play important roles in health and disease. The traditional culture-based approach under-estimates the diversity of fungi found on skin, but recent development of high throughput sequencing, technologies have shown that there are many fungal taxa present on skin, many of them commensals under normal physiological conditions (Nilsson et al., 2019). Fungi constantly communicate with bacteria and skin immune systems, affecting microbial balance, skin barrier function and vulnerability to infections.

The mycobiome composition of body surface is not consistent. Each part of the body has its own unique microbial environment, which varies according to moisture, temperature, nutrient status and how exposed the body is to the environment. In general, moist skin sites tend to contain more fungal species than dry ones because the moisture provides a more favourable environment for the growth and persistence of fungi. Researchers who combined culture-dependent and metagenomic methods have shown that the skin is spatially heterogeneous with some body sites as reservoir for commensal and opportunistic fungal species (Saheb et al., 2022). Thus, the amount and variety of fungi present at a specific site of the skin are mainly dependent on the type of site.

One of the most unique ecological niches of the human skin microbiome is the feet. The feet are oftentimes covered for long periods of time in footwear which creates a higher temperature, greater perspiration, and less ventilation. This microenvironment is made moist, which leads to microbial colonization and survival. The interdigital spaces or toe webs are especially favourable for fungal proliferation since there is little airflow and moisture can accumulate in the feet (Leung et al., 2023). Therefore, toe webs have been shown to be reservoirs of the fungal organisms and often associated with superficial fungal colonization and infection. The fungus flora of the toe-web spaces is a complex mixture of microorganisms such as dermatophytes, yeasts and non-dermatophyte moulds. Some of these organisms live in the host without causing disease, but others may be opportunistic pathogens, which could cause disease under favorable conditions. The fungal species that are most commonly associated with the interdigital spaces of the feet are dermatophytes including Trichophyton rubrum, Trichophyton mentagrophytes and Epidermophyton floccosum and are also considered to be important causative

organisms of superficial fungal infection. Pathogenic bacteria, host and environmental factors such as excessive moisture, impaired skin barrier function, hygiene issues and alterations of immune response all contribute to the transition from asymptomatic colonization to active infection (Leung et al., 2023). Therefore, knowledge of the composition of fungal flora from the toe-web spaces is not only significant for understanding the normal microbial flora of the web spaces but also for detection of potential habitats for pathogenic fungi.

Fungi-host interactions are mostly mediated by skin immune system interactions. Recent studies have shown that fungi actively interact and play a role in immune regulation and maintenance of the host's cutaneous homeostasis. Normal flora fungi may induce immune responses that prevent colonization by pathogenic microorganisms, and disruption of the normal flora may lead to inflammation and susceptibility to infection. Host-microbe interactions have been studied and reveal that the skin immune system constantly surveys the microbe communities, and responds to their fluctuations in abundance and composition, thereby maintaining the balance of the cutaneous ecosystem (Nakatsuji et al., 2021; Lunjani et al., 2021). These findings highlight the need to investigate the microbiome of fungi as not only a disease-causing organism, but also as a part of healthy skin physiology.

The skin-associated fungal communities are known to vary among children, a population that is of particular interest in investigations. The skin has many physiological and immunological changes that affect the colonization of the skin in children. The bacterial and fungal flora is very different at various developmental stages, and significant changes take place in bacterial and fungal flora as children approach puberty. Research has demonstrated that the skin microbial profile of healthy children varies from that of adults, which is attributed to the differences in

skin chemistry and immunity and environmental exposure (Park et al., 2022). Children may be more prone to picking up or passing on fungal organisms as they are still learning how to do personal hygiene and are often in situations that expose them to environmental microorganisms.

Fungi also proliferate and spread between children in the school setting. Exposure to a variety of microorganisms may occur through daily interaction with various activities in the school classroom, recreational sports, and playground interaction and sharing of facilities. In many locations, school children often walk in the absence of footwear or footwear is worn for long periods without proper foot hygiene, which can all contribute to the persistence of fungus in the toe-web spaces. In addition, schools, where people are in close proximity with each other, can also lead to the spread of microorganisms through indirect contact with contaminated surfaces. All of these contribute to the importance of primary school children for the study of the pattern of fungal colonization of superficial fungal infections.

Diversity and composition of the microbial communities associated with human skin can also be influenced by environmental factors. Fungal survival, growth and transmission is greatly affected by climatic conditions (humidity and temperature). It has been shown that the microbial skin community of people living in warm and humid environments differs from that of people in temperate environments (Callewaert et al., 2020). High temperatures and moisture in tropical climates creates ideal conditions for fungi to grow and can raise the risk for moisture-prone areas on the skin being colonized by fungi, like the toe-web spaces. Additionally, exposure to environmental pollutants, socioeconomic factors, sanitation and lifestyle behaviours could also shape the composition of the skin microbial communities (Leung et al., 2023).

Nigeria is located in the tropics and has climates that could be conducive to the proliferation of fungi during most of the year. High temperatures, seasonal humidity and different sanitation and hygiene habits can favour fungal colonization of the skin. However, much less is known about the fungal flora of the toe-web spaces of healthy primary school children despite these ecological features. A significant number of studies are published in Nigeria on fungal infections as a clinical disease, while very few studies are available on the overall fungal communities that grow on the skin surface without a clinical disease. This restriction has contributed to the lack of knowledge regarding the ecology of fungal organisms among school-age and the possible role that school-age children can play in the reservoir of future infections. In view of this, the present study aims at exploring the fungal flora of toe-web spaces of primary school children at Godfrey Okoye University Primary School, Enugu State, Nigeria.

1.2 Statement of the Problem

School children Fungal infection is one of the issues of major concern to the general population, particularly in tropical and sub-tropical regions. The importance of this problem is self-evident, but the fungal flora of toe webs of school children in Nigeria is not researched thoroughly. This gap will be bridged in this research that will provide information about the fungal flora of toe webs in this population.

1.3 Aims and Objectives

The aim of this study is to evaluate the fungal microbiota present in the toe webs of Godfrey Okoye Primary School pupils.

1.4 Specific Objectives are to:

1. To collect toe web samples from Godfrey Okoye Primary School pupils.
2. To isolate fungal organisms present in the toe web samples.

3. To identify the fungal isolates using cultural and microscopic methods.

4. To determine the frequency of occurrence of the fungal isolates.

5. To determine the distribution of fungal isolates according to age and sex.

CHAPTER TWO

LITERATURE REVIEW

2.1 Skin Microbiota and Mycobiome

Human skin microbiota is the association of microorganisms, such as bacteria, fungi, viruses, and archaea, that live on the surface of the skin. This ecosystem is very diverse and site-specific depending on local physiological and environmental conditions. The skin is a dynamic habitat that is colonized by microbes which interact with one another and with the host immune system and help to maintain barrier integrity and defence against pathogenic invasion. Byrd et al. (2018) noted that the skin microbiome does not simply exist as a collection of microbes, but rather as an active, biological system that plays a complex, host–microbe interaction role in maintaining the health of the skin.

The microbial flora of the skin is different on various body surfaces. Previous studies by Chen et al. (2018) showed that biogeographical parameters like moisture, sebum production and external environment affect the skin microbial communities. Sebaceous sites, for instance, will promote the growth of lipophilic organisms, whereas moist areas will promote growth of moisture-dependent microbes. This spatial variation is consistent with the idea of the skin being a microbial surface comprised of many ecological niches.

Bacterial communities have received the majority of the attention in skin microbiology studies, but fungal organisms have an important role in the skin ecosystem and have been underreported. The fungal community of the skin (the mycobiome) contains a wide range of taxa such as yeasts (Malassezia spp.), filamentous fungi and dermatophytes. Depending on host and environment, these organisms can be commensals, environmental transients or opportunistic

pathogens. Nilsson et al. (2019) highlighted that advances in high-throughput sequencing technologies have revealed a much greater diversity of fungal species on human skin than previously detected using traditional culture-based methods, which significantly underestimated fungal diversity.

Malassezia is the most prevalent fungal genus on normal human skin, which is well-suited to the lipid-rich environment and is distributed at several sites on the body. Other fungal taxa, however, may be found depending on environmental exposure, hygiene practices and anatomical location. Kashaf et al. (2022) also showed that the combination of culture-based methods and metagenomic sequencing offers a more holistic way of understanding the multi-kingdom microbial communities colonizing the skin, with the introduction of functional interactions between bacteria and fungi that can influence microbial stability and health of the skin.

Notably, the skin mycobiome is not an independent ecosystem but a tightly coupled ecosystem with the bacteria and immune system. These interactions help to maintain microbial competition, immune regulation, and ecological balance. Disturbance of these interactions may lead to dysbiosis, which has been linked to inflammatory skin diseases and susceptibility to infection. Therefore, environmental factors as well as microbial competition and host immune regulation are important factors in determining the ability of fungi to persevere on the skin.

2.2 Fungal Ecology of Human Skin

Fungi are a part of the human skin microbiota, but they tend to be less abundant and diverse than bacteria. Nevertheless they are important in the maintenance of the ecological equilibrium of the skin and in some conditions, in the development of disease. The fungal community associated with skin has also been identified as an essential component of the skin ecosystem that continuously interacts with immune

responses of the host and bacterial community, known as the skin mycobiome (Nguyen & Kalan, 2022; Chandra et al., 2021).

The skin mycobiome is highly stable with limited number of fungal genera, with Malassezia often being the most abundant genus, regardless of the skin site in healthy skin (Roux et al., 2022; Chandra et al., 2021). These fungi are lipid-dependent yeasts that grow on the surface of skin in sebaceous and moist places, using the lipids found on the skin for growth. Malassezia species have been identified as commensals and pathogens associated with a range of skin diseases, proving to be harmless residents and opportunistic organisms depending on the host and environmental conditions.

In addition to Malassezia species, there are other groups of fungi present on the skin, such as Candida species, dermatophytes (Trichophyton spp.), and environmental moulds. Depending on the ecological conditions of the skin surface, these organisms can be transient or permanent colonizers of the skin surface. Many of these fungi are present at low levels in healthy individuals and do not cause disease; however, disruption of the skin barrier, changes in humidity and immune suppression can lead to a change in abundance such that these fungi can multiply and cause superficial or invasive disease (Nguyen & Kalan, 2022).

Microenvironmental factors have a significant effect on the presence and persistence of fungal flora on the skin. Fungal colonization tends to be more prevalent in moist areas than in dry areas, and on places with increased warmth and reduced airflows. There is a higher fungal colonization where the skin is moist, warmer and with less airflow, especially in terms of the distribution of fungi on the human body, which is unique to the site. This notion of different microenvironments in the skin, including different types of skin sites (sebaceous, moist and dry), is the

reason why some fungal taxa are concentrated in specific parts of the body (Chen et al., 2018). The difference in these ecological factors is important in determining the structure of fungal communities, and is crucial to infection susceptibility by fungal overgrowth.

The fungal organisms on the skin are not present alone, but can interact with bacterial populations and host immune responses in a dynamic fashion. It appears that fungal–bacterial interactions play a role in colonization resistance, microbial competition and in maintaining the homeostasis of the skin. Disturbing these interactions can cause dysbiosis, which has been linked to a number of dermatological issues such as dermatitis and superficial fungal infections (Nguyen & Kalan, 2022; Ianiri et al., 2022). In addition, the immune system is also central to controlling fungal populations by recognising the presence of fungal associated molecular patterns and responding accordingly to prevent overgrowth.

It is important to note that the skin mycobiome is still not as well-studied as bacterial communities, although it is now known more than in past decades. The majority of fungal species found on the skin remain to be characterized in terms of functionality, pathogenicity and interaction with other microorganisms. This lack of knowledge is especially significant in clinical and public health settings, where fungal skin infections are still prevalent, particularly in settings where fungi thrive.

2.3 Toe Web Spaces as a Niche for Fungal Colonization

One of the most unique ecological niches of human skin is the toe-web spaces or interdigital spaces of the feet. The interdigital areas are often exposed to hyper-hygienic conditions that are wet, warm, moist, and have poor ventilation, which is a very favorable environment for microbial colonization and persistence. The toe-web spaces are ideal for the growth of the fungus, both commensal and pathogenic.

Therefore, the interdigital area has been the focus of many studies in dermatology and microbiology as one of the most important foci for fungal organisms related to superficial skin infections (Leung et al., 2023).

Footwear habits and perspiration, among other physiological conditions, have a significant impact on the ecological conditions of the toe-web spaces. Worn shoes and socks for an extended period of time decreases the amount of air flow around the feet, and sweaty skin is trapped between the folds of the toes. This moisture also allows stratum corneum to become more soft which makes it more favourable for the fungus to adhere, grow and survive. The main risk factors for fungal colonization of the toe-web spaces and subsequent development of tinea pedis are excessive sweating, prolonged occlusion, and insufficient drying of the feet, according to (Leung et al.,2023).

Other studies have shown that the fungal flora present in the toe-web spaces is distinct from that of other sites such as the axillary, rectal, and nasal cavities. Previous molecular studies by (Maseda et al.,2023) found that the fungal diversity of foot-associated skin sites is one of the highest on the human body. The scientists noted that the diversity of fungi in the feet was more variable than in the sebaceous areas, which represents the effects of different environmental factors in different parts of the body. These results support the concept that the toe-web spaces create a specialized ecological space in which a variety of fungal communities can develop. Many fungal organisms have been isolated from between the toes of the feet. The most common dermatophytes reported are those of the genera Trichophyton, Epidermophyton, and Microsporum. Among these, Trichophyton rubrum is found to be the most common dermatophyte found in the toe-web of people suffering from tinea pedis all over the world (Leung et al., 2023). Fungi that are routinely isolated

from interdigital areas include Candida spp, Malassezia spp and some non-dermatophyte moulds. Many of these organisms can be present in an asymptomatic colonizing state, but shift to a pathogenic state with environmental or host changes. Physicochemical properties of the toe-web environment affect microbial community structure, in addition to the species composition of fungi. (Akaza et al.. 2023) assessed the link between skin surface microbial communities and skin pH, and found that changing skin surface conditions can have a major impact on bacteria and fungi. The interdigital spaces are often exposed to moisture retention and changes in skin barrier function, which can also affect local pH and help perpetuate and establish fungal persistence and colonization. These results emphasize the need to take into consideration microenvironmental factors when studying fungal flora of the feet.

The toe-web spaces are also important areas of microbial interaction. In the presence of fungal organisms, there are also bacterial communities that compete for nutrients and resources and can either inhibit or promote fungal growth. Research on the microbial ecology of skin has revealed that interactions among fungi and bacteria may have an impact on the colonization dynamics and on the risk of infection development (Chen et al., 2018). Therefore, it is important to consider the fungal flora found in toe-web spaces as part of the skin's microbiome and not individual microorganisms.

The toe-web spaces are especially important from a public health point of view as they can serve as reservoirs of infection with fungal organisms. Pathogenic fungal species can be present in body without causing clinical manifestation and may become part of the body's disease burden and contribute to fungal dissemination and transmission in the environment. This is particularly important in communal

areas like schools, where communal facilities, proximity and poor hygiene may help spread fungal organisms. Therefore, it is important to be able to identify fungal colonies found in the toe-web spaces of apparently healthy individuals, to understand the pattern of fungal carriage and disease transmission in communities. The ecological importance of the toe-web spaces may also be more relevant in children as behaviour factors like playing outside, wearing school shoes for long periods of time and lack of knowledge on foot hygiene can make them exposed to fungal organisms. Although these factors could be significant, there are relatively few studies which have specifically investigated the fungal flora from the toe web spaces of healthy primary school children, especially in tropical developing nations.

2.4 Skin Fungal Flora in Children

Children's and adults' skin microbiomes have distinct composition and functional characteristics. These differences are attributed to the physiological growth, immune system maturation, hormonal changes and differences in environmental exposures. Early-life skin has different structural and biochemical properties, is more hydrated, sebum production is lower, and the skin has fewer natural moisturizing factors that give rise to an ecological niche for microbial colonization. These developmental attributes are also responsible for the establishment and development of the bacterial and fungal communities that inhabit the skin (Zhou et al., 2025; Maseda et al., 2023).

Initial investigations in paediatric skin microbiota revealed that microbiota communities are established at an early age and gradually matured with time. (Zhou et al., 2025) demonstrated that the skin microbiomes of infants and young children are extremely broad and distinct, influenced by their exposure to the environment and their mothers. Likewise,(Maseda et al.,2023) reported that there were

significantly different skin and nasal microbiota between children and adults, with children having distinct microbial profile across different body sites. These findings set the groundwork for the notion that the microbial ecology of the skin changes throughout childhood and is sensitive to developmental and environmental influences.

More recent longitudinal studies have also provided greater detail on the development of skin microbiota with growth and pubertal development. (Park et al.,2022) explored the bacterial/fungal community in healthy children during pubertal development, and showed that the composition of microorganisms changed greatly during puberty. The study concluded that fungal communities, especially those with a high relative abundance of Malassezia species, are more abundant with the rise in sebaceous gland activity during puberty. This is linked to hormonal changes that influence the lipids in the skin and hence the environment is more conducive to lipophilic fungi.

Microbiological transmission and colonization have been noted as factors influencing the skin microbiome of children, not only during puberty, but also during early-life. Skin is initially colonized at birth by maternal, environmental and other microorganisms and then gradually changed throughout infancy and childhood. Using a genome-based analysis of early-life skin microbiota,(Hwang et al.,2021) found that infant skin is populated with a variety of bacterial and fungal species, and that both maternal transmission and environmental exposure contribute to the composition of microbiota. This study also revealed that fungal species are already present from the early life and their functional role and ecological stability are gradually changing with time.

The fungal community is less stable and more variable in children than in adults.

These differences are due to variation in skin physiology, immune system maturation, and hygiene habits and environmental exposure. Dwyer and Scharschmidt (2022) highlighted the importance of early-life interactions between host and microbes in educating and priming the immune system, especially in the skin where exposure to microbes educates immune tolerance and defence responses. Any disturbance of these interactions could affect the susceptibility of a host to colonization by opportunistic fungi and to infection.

Comparisons of fungal community between different ages have revealed distinctive fungal community patterns in children compared with adults across all studies. (Kotakeyama et al.,2021) have reported that fungal diversity is greater in children and decreases into more specialized and less abundant fungi in adulthood, with lipophilic fungi, like Malassezia, dominating the mature skin environments. Likewise, (Maseda et al. ,2023) found that children had more diverse environments of fungi, suggesting that they are more exposed to fungi and have more unstable skin microenvironment than adults.

It is especially crucial to consider the transition stage from childhood to adolescence in understanding fungal ecology on the skin. An understanding of fungal ecology on the skin is especially important during the transition from childhood to adolescence. At puberty, the production of sebum by the sebaceous glands changes the environment of the surface of the skin, which causes the growth of Malassezia and other lipid-dependent fungi. (Park et al.,2022) confirmed that these hormonal and physiological shifts were closely related to changes in fungal community structure, leading to a gradual decrease in the diversity of fungi and an increase in the abundance of certain fungal taxa among older age groups.

Importantly, children are more likely to be exposed to the environmental sources of

the fungal organisms because of behavioural and lifestyle factors. Fungal acquisition and transmission is enhanced in school settings through outdoor activities, walking on surfaces without shoes, communal facilities, and close interpersonal interactions. The exposures are dynamic factors contributing to fungal colonization in paediatric populations and may affect site-specific colonization patterns, especially in moisture-prone areas (toe-web spaces).

While the skin microbiome in children is being explored, fungal communities are not as well studied as the bacterial communities. The vast majority of the available information is from high income countries and limited information is available from tropical countries where environmental conditions are favourable for fungal growth. While research like that of (Park et al., 2022) and (Masedaet al., 2023) provides baseline knowledge, the need for context-specific research in different geographical and climatic conditions should also be emphasized.

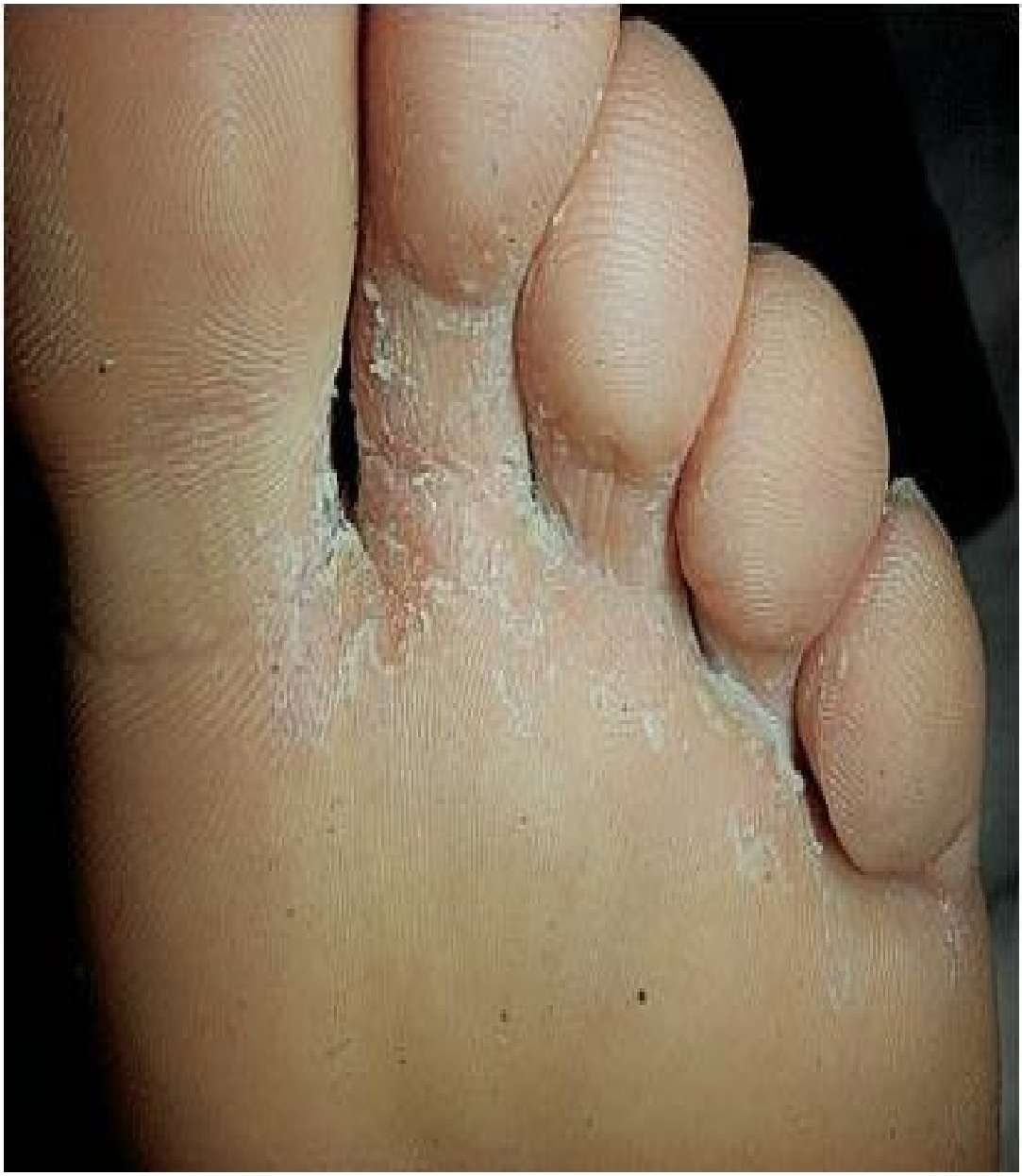

Fig 2.1: Fungal infections affecting toe webs.

(Adeyemi et al., 2019)

Source: Journal of Clinical Medicine England

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2.5 Environmental and Climatic Factors Influencing Skin Fungi

Fungal communities associated with the skin are shaped by fundamental environmental conditions and are characterized by their composition, diversity and functional behaviour. The skin microbiota is a system in direct contact with the external environmental factors; it is therefore very sensitive to variations in the environment's temperature, humidity, air quality and ecological conditions. Moisture and the environment's stability are crucial factors for fungal organisms, influencing both growth and survival (Callewaert, Ravard Helffer, & Lebaron, 2020).

Some of the most important environmental factors that can influence fungal colonization of the skin are humidity and temperature. Typically fungi grow well in warm and humid environments and this promotes germination, hyphal growth and persistence on keratinized surfaces. Research into interactions between the environment and skin has found that humidity increases fungal colonization on the skin, especially when the skin is occluded, like in the skin folds and interdigital spaces (Callewaert et al., 2020). These results apply to parts of the body that tend to retain moisture, such as the toe-web spaces, which are the result of sweating and decreased ventilation.

Fungus growth is favored especially by tropical and subtropical climates where the temperatures are high and the humidity is also high throughout the year. Superficial fungal colonization and infection are usually more likely to occur than in temperate areas. (Leung et al.,(2023) mentioned that dermatophyte infections such as tinea pedis are more common in warm and humid environments which is conducive for the survival and spread of fungi. This climatic advantage is responsible for the wide distribution of dermatophytes in tropical countries particularly where there are poor hygiene facilities available.

Environmental exposures to contaminated surfaces are an important factor in the acquisition of fungi, besides climatic factors. Fungal spores can be found in communal bathing facilities, footwear, locker rooms, soil and dust. People who come into contact with such environments are at risk for colonization, especially if the microenvironmental conditions on the skin are favorable. It has been shown that environment factors, such as exposure to external microbial reservoirs, impact the composition of skin fungal communities and can interact with the properties of the skin surface, such as pH, and affect the colonization of the skin surface by the microbial community (Akaza et al., 2023).

The effect of environmental conditions on fungal colonization is also influenced by skin surface characteristics (pH, hydration level and barrier integrity). Akaza et al.,2023) found that differences in bacterial and fungal community structure were related to differences in the pH of the skin. The pH of the skin may have a selective effect on the growth of certain fungal taxa. Additionally, in moist conditions, increased skin hydration promotes fungal binding further contributes to fungal growth, especially at sites where keratinized tissue is constantly exposed to friction and occlusion.

Environmental conditions also interact with behaviours and socio-economic factors that affect fungal colonization of skin. Humid environments can be exacerbated by inappropriate footwear (e.g. wearing closed shoes for extended periods), poor foot care (e.g. infrequent cleaning of the feet and poor drying after washing them), and the formation of microclimates that promote fungal growth. In many developing areas, including some of sub-Saharan Africa, these are exacerbated by environmental heat and humidity, giving rise to a greater risk of fungal persistence in susceptible anatomical locations, e.g. between the toes.

The relationship between the environment and the skin microbiome is a two-way street, in which the environment influences the makeup of the skin microbiome and the skin microbiome influences and affects its environment. Callewaert et al. 2020) pointed out that the skin microbiome is an environment–host interface that is constantly adapting to the environment and fluxes while balancing the internal ecosystem. Stressors in the environment may cause disruption of this equilibrium and sometimes the microbial community shifts toward opportunistic fungi.

The environment is especially favorable for fungal colonization of the skin in tropical countries like Nigeria. Fungi survive in a favorable environment all year, due to high ambient temperatures, seasonal rainfall regime and the consistent high ambient humidity. In combination with behavioural factors (footwear habits, hygiene practices), these conditions can greatly contribute to fungal colonization in moist regions of the body. Although these environmental factors are known, little site-specific information has been available for the distribution of the fungal flora in healthy populations in many tropical areas.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site and Population

The research was conducted in Godfrey Okoye University Nursery and Primary School in Enugu State, Nigeria. Sampling was carried out within the school compound. The study site was selected due to its proximity to the Microbiology Laboratory at Godfrey Okoye University, which makes it suitable for sample collection and processing. The result of the study will be beneficial to the GOUNI community. Toe web samples were collected from primary school pupils from basic 1- 6.

Data Collection Method; the pupil's data were collected using structured questionnaires, Assistance was provided to the pupils in filling the questionnaires. The questionnaires were designed to obtain information on relevant variables which include age, sex, class, parental occupation, and personal hygiene practices.

3.2 Preparation of Culture Media

The isolation of fungal pathogen was conducted using Potatoe Dextrose Agar (PDA). The medium was prepared by measuring accurately 15.6g of Potatoe Dextrose Agar powder which was weighed and dissolved in 400ml of distilled water according to the manufacturer's instruction. The mixture was carefully swirled to endure a complete dissolution, then sterilized in the autoclave for 15mins at 121degree Celsius. After cooling at about 45 degree Celsius, 15 mls of the medium was distributed into 20 petri dishes and allowed to solidify at room temperature.

3.3 Preparation of Normal Saline

The required standard of preparing normal saline solution was accomplished by adding 8.5g of NaCl into 1 liter of distilled water and boiled for 10 minutes, it was

allowed to cool. The solution will be used for fungal suspension for microscopy and culture.

3.4 Sample Collection

Sampling of the toe webs of pupils in Godfrey Okoye Nursery and Primary School was done using sterile swab sticks, the samples were collected from the interdigital part of the feet of the pupils. The procedure used in the collection of the sample was the swabbing of the toe webs of the pupils in a mild manner. The samples were then sent to the lab for processing.

3.5 Processing of Samples

Sample collection was done according to the method of Nwankwo and Edward (2025). Sterile swab sticks were used to collect the samples after cleaning the toe webs of the pupils with 70% alcohol. These were inoculated into respective petri dishes and properly labeled A1- A20 according to the volunteers. The inoculated plated were incubated at room temperature of 25 degree Celsius for 3- 7 days to ensure proper growth of the isolates. The isolates were viewed macroscopically and microscopically using X10 and X40 magnification respectively.

3.6 Preparation of Pure Culture

A total of 14.04g Potatoe Dextrose Agar powder was weighed and dissolved in 360mls of distilled water according to manufacturer's guide and autoclaved at 15mins at 121 degree Celsius. After cooling 5mls of the medium was transferred to 20 bijou bottles respectively. Each colony was subcultured into the labeled bijou bottles respectively and allowed to grow for 5- 7 days.

3.7 Slide Culture Method of Identification

The slide culture method was employed according to the method of Agu and Chidozie (2021) to identify the isolates, which could not be identified properly

through direct microscopy. A sterile scapel was used to cut about 10cm of already prepared PDA medium and was placed in a clean petri dish containing a filter paper, bent glass rod and clean glass slide. The agar block was placed on the clean glass slide, using a sterile wire loop, the fungal colonies obtained from the slant culture were carefully inoculated onto the corners of each agar block and covered with a coverslip. Two (2)ml of distilled water were added to the filter paper to provide a humid and wet environment for fungal growth. The setup was allowed to grow at room temperature in for 4- 5 days.

3.8 Procedure for Staining of Fungal Isolates for Identification

The slide culture was taken down by removing the coverslip and placing Lactophenol Cotton Blue (LCB) stain. The agar block was discarded. One drop of Lactophenol Cotton Blue stain was placed on the slide where the fungi grew then it was covered with a cover slip. The slides were observed using a microscope using X10 and X40 magnification.

CHAPTER FOUR

RESULTS

4.1 Colony Morphology and Appearance

The findings revealed that the toe webs region of the pupils harboured different fungal Species. A total of seven (7) fungal species were isolated from the samples examined and are shown in figures 1,2,3,4,5,6, and 7 respectively. The Cultural and Morphological characteristics of Fungal Isolates are also shown in Table 1.

Figure 1: was identified as Aspergillus clavatus which initially appeared white and later turned blue-green with a powdery or velvet-like texture. Under microscopic examination, it showed septate hyphae, conidiospores and chains of conidia. Figure 2: showed Aspergillus niger, appeared as black colony with pale yellow reverse, under the microscope possessed a septate hyphae, conidia and phialides. Figure 3: Candida albicans, the colonies showed typically cream to white appearance which became wrinkled as the cultured aged with smooth texture, also had a bread-like smell. The microscopic observation revealed budding yeasts cells and pseudohyhae. Figure 4: Cunninghamella bertholletae, displayed grayish to brownish colonies with a fluffy and cottony texture with a colorless reverse. The non-septate hyphae were broad and irregularly branched with small-celled spores. Figure 5: Penicillium verrucosum, had a dull green appearance and a powdery texture which also had a brownish under side, the microscopic feature showed or resembled a paintbrush

structure with a septate hyphae and hyaline. Figure 6: Talaromyces marneffei, it displayed a bluish- greenish like appearance and a velvet feeling, also with smooth and branched conidiophores. The hyphae were septate, the conidia were arranged in a round to oval form. Figure 7: Trichophyton rubrum: the initial surface color was cream but developed light pinkish coloration with time with a cottony texture, the underside appearance showed a deep red pigmentation. Microscopically, microconidia appeared in numerous quantities with septate hyphae.

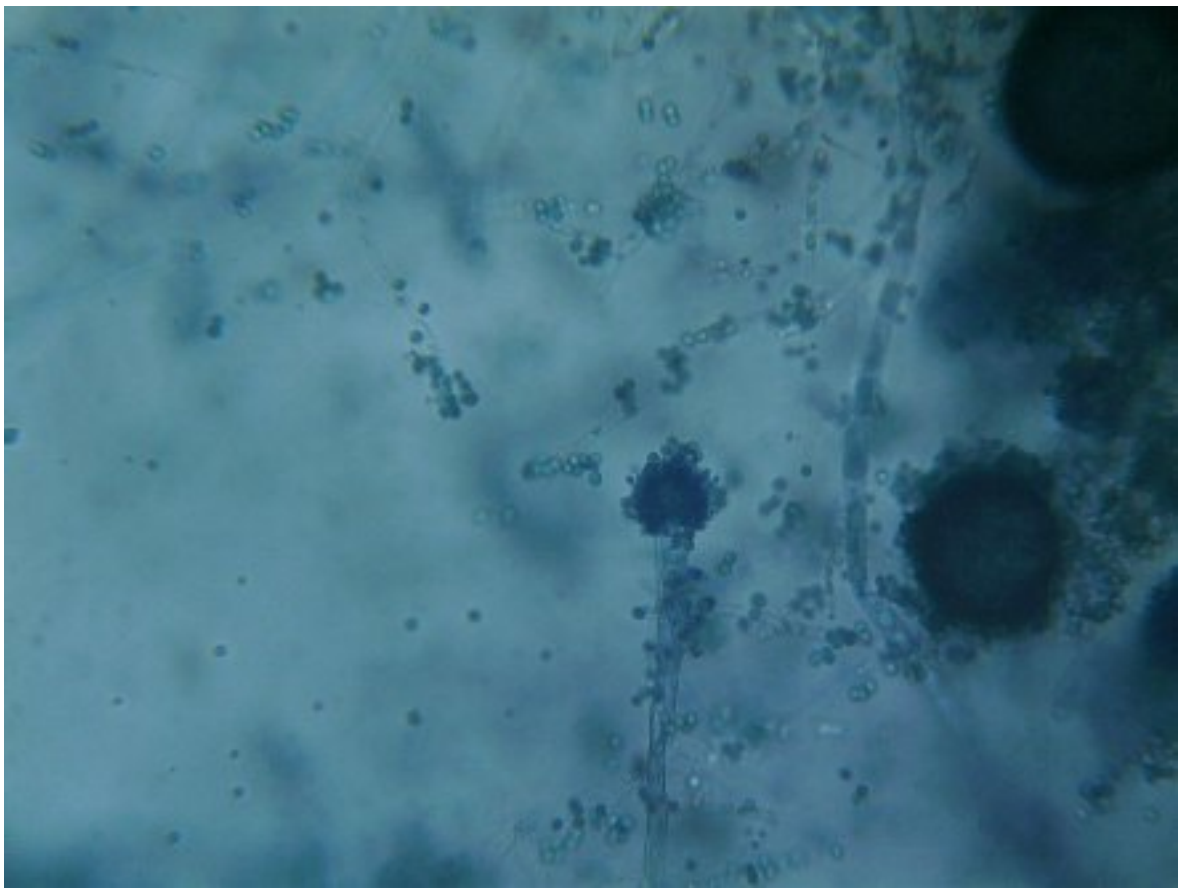

Figure 4.1: Aspergillus clavatus

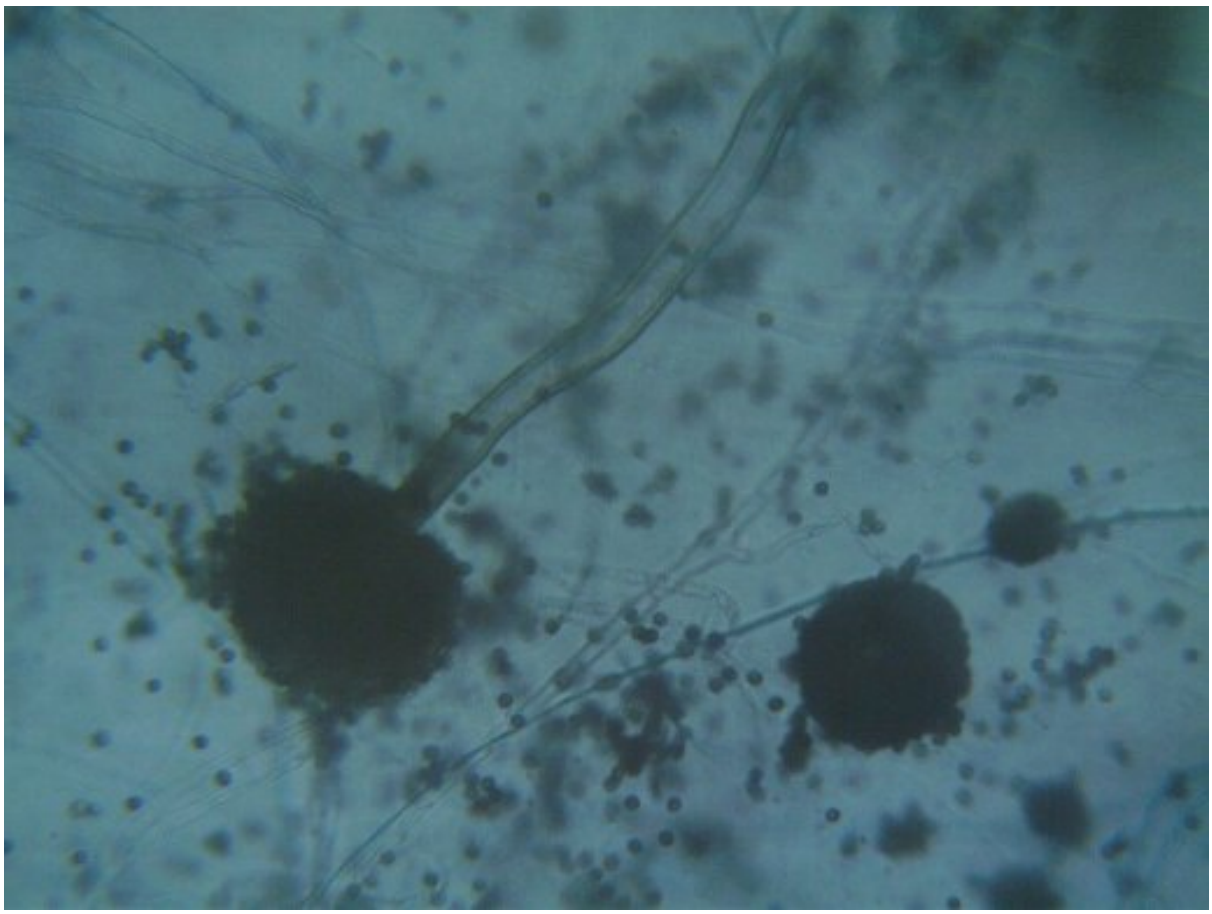

Figure 4.2: Aspergillus niger

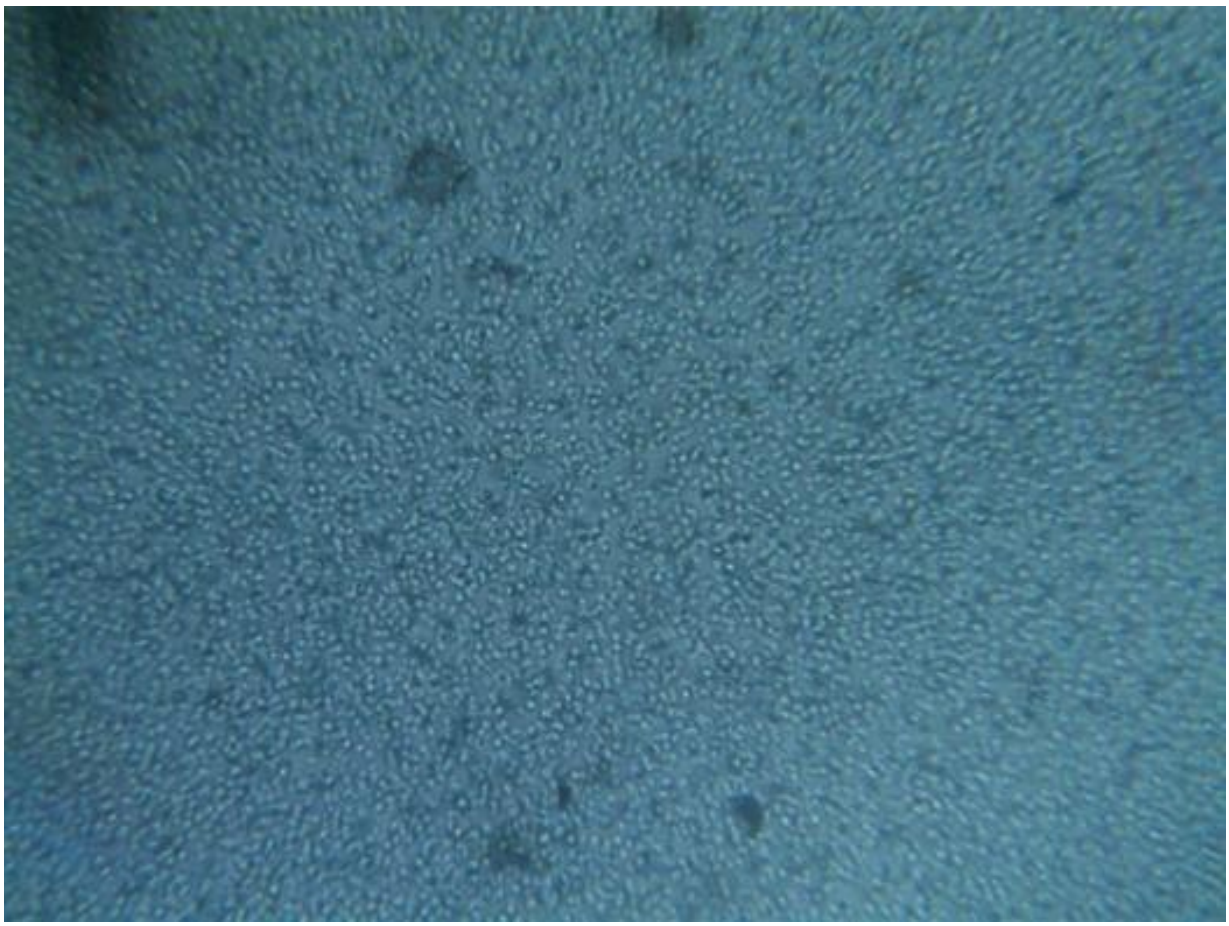

Figure 4.3: Candida albicans

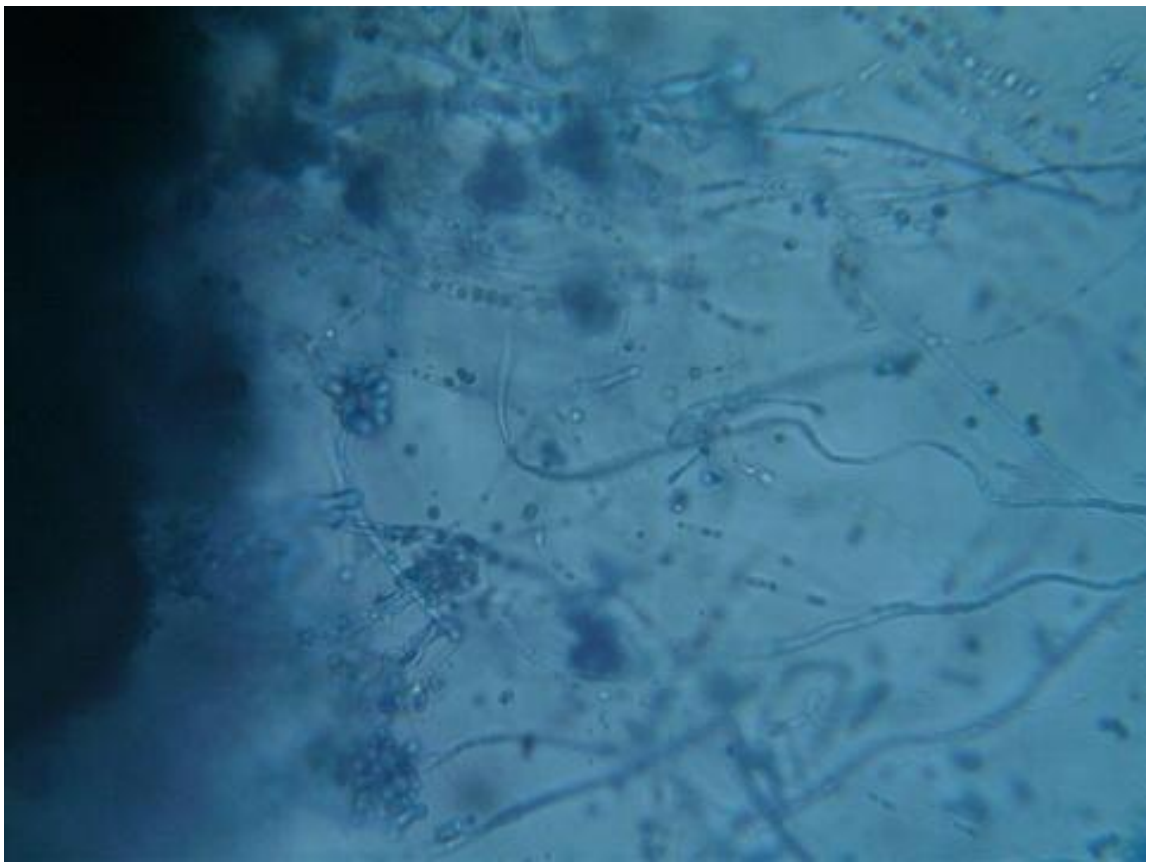

Figure 4.4: Cunninghamella bertholletae

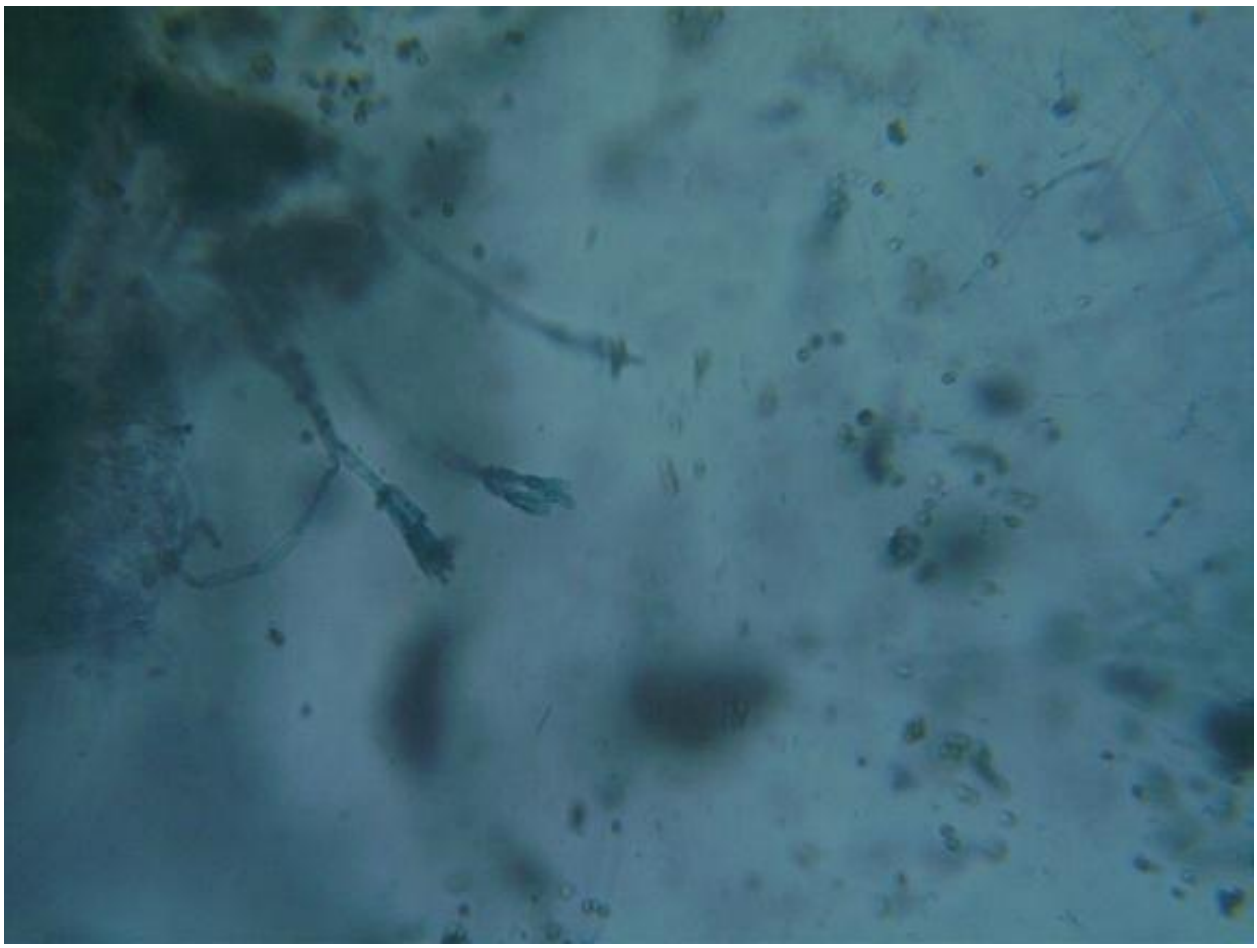

Figure 4.5: Penicillium verrucosum

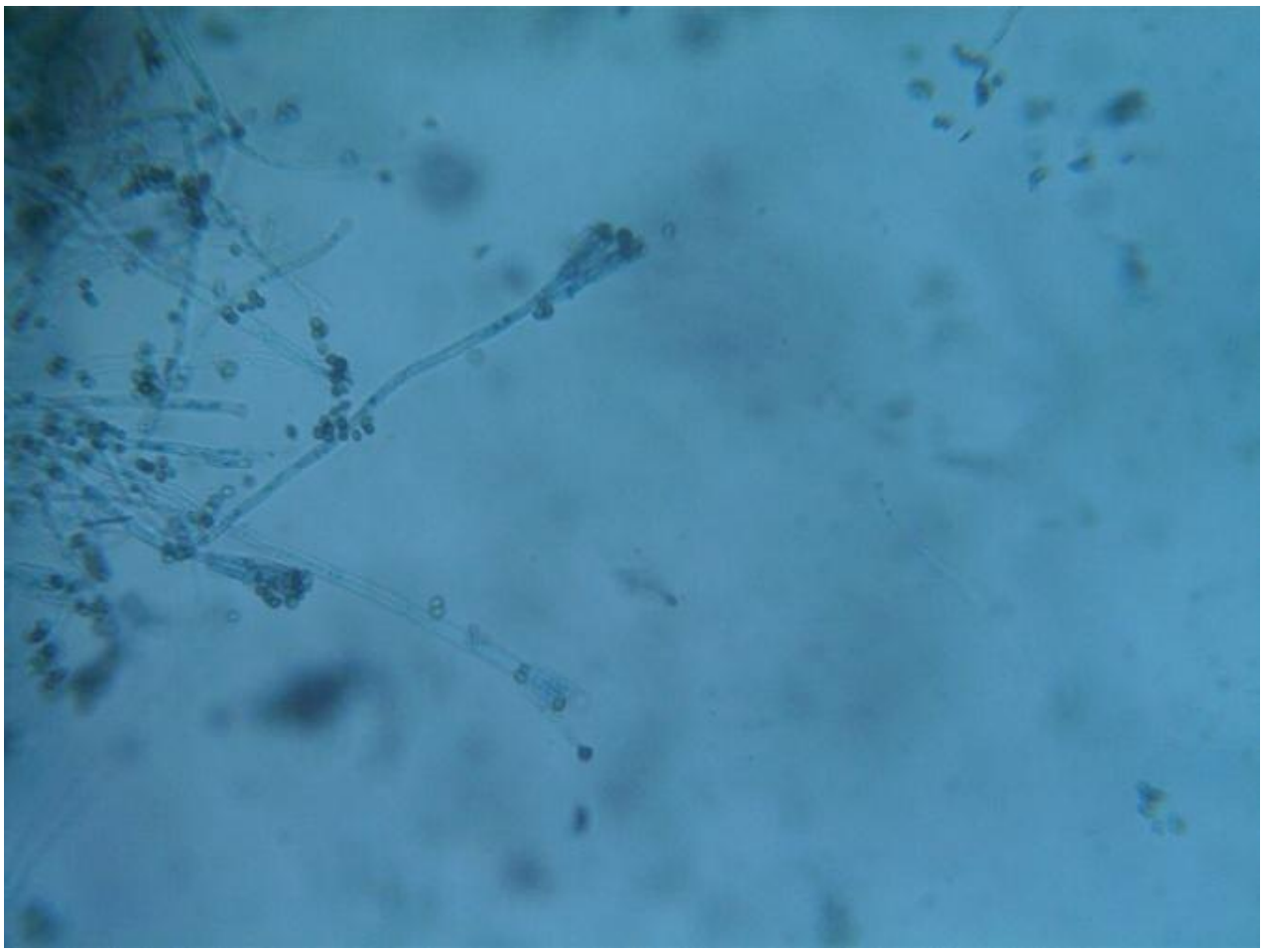

Figure 4.6: Talaromyces marneffei

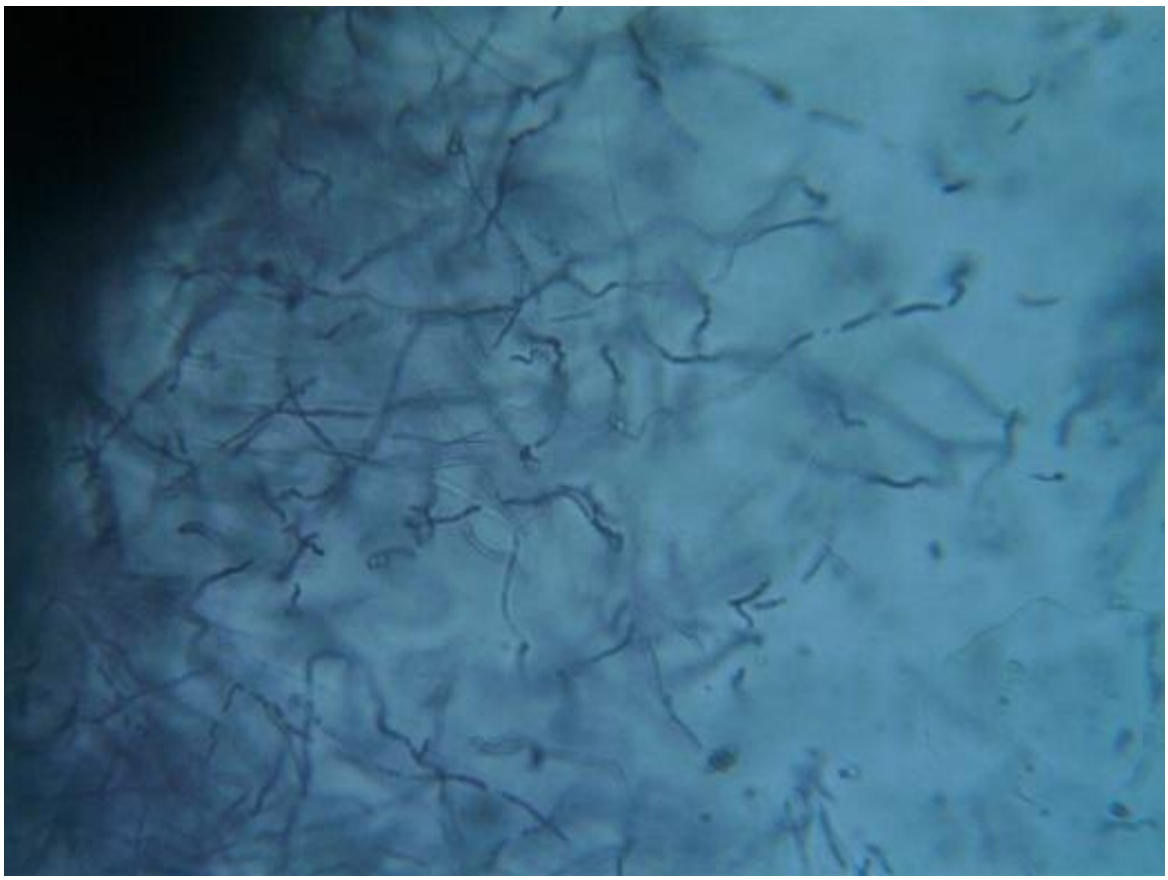

Figure 4.7: Trichophyton rubrum

Table 1: Cultural and Morphological Characteristics of Fungal Isolates

Isolates	Macroscopic characteristics	Microscopic characteristics
Aspergillus clavatus	Bluish-green or dull green coloration with whitish edges which are somewhat filamentous	Branched septate hyphae with smooth round conidia
Aspergillus niger	Black appearance with a powder texture as a result of the production of conidia	Septate hyphae, vesicle is covered with conidial structures and a long smooth conidiophore.
Candida albicans	The colonies appears creamy white with smooth and well defined edges often gives a bread-like smell	Budding yeasts are in an oval to round shape, the pseudopyphae gives an elongated chain appearance
Cunninghamella bertholletae	The colonies are highly elevated and has a cotton texture with a greyish to pale brown appearance	Produced sporangiospores which are small and round or oval, it possess an aseptate hyphae

Penicillium verrucosum	Surface texture is powdery or granular the colonies show a dull green or greenish color	The hyhae is septate, conidial head appears like a brush-like structure, conidiophore is branched into mutalae and phialides

4.2 Frequency of Fungal Isolation from Site of Study

Frequency of occurrence of fungal isolates is shown in Table 2. Aspergillus niger and Penicillium verrucosum were the most predominant organisms with a frequency of 3(20.0%) which suggests that they are the most frequently occurring fungi in the toe webs. Aspergillus clavatus, Candida albicans, Cunninghamella bertholletae and Talaromyces marneffei each had a frequency of 2 (13.3%), indicating a moderate level of prevalence. Trichophyton rubrum was the least common organism in this investigation with a frequency of 1 (6.8%).

Table 2: Frequency of Occurrence of Isolated Fungi

S/N	Fungal Isolate	Frequency	Percentage
1.	Aspergillus clavatus	2	13.3%
2.	Aspergillus niger	3	20.0%
3.	Candida albicans	2	13.3%
4.	Cunninghamella bertholletae	2	12.3%
5.	Penicillium verrucosum	3	20.0%
6.	Talaromyces marneffei	2	13.3%
7.	Trichophyton rubrum	1	6.8%
Total		15	100%

4.3 Distribution of Fungal Isolates According to Age and Gender of Pupils

The distribution of fungal isolates according to the age and gender of the pupils sampled is shown on Table 3. The data shows that age 9-10 group had the highest number of fungal isolates. In this group males had 5 isolates (33.3%) and females had 2 isolates (13.33%). Age 7-8 group had 4 isolates (26.67%). The 4 of these isolates were found only in female pupils. No isolates (0.00%) were found in males in this youngest age group. The age 11-12 group had the lowest number of isolates, with 2 isolates (13.33%) in males and 2 isolates (13.33%) in females.

Table 3: Distribution of Isolates According to Age and Gender of Pupils

Fungal Isolates	Age 7 - 8		Age 9 - 10		Age 11 - 12		
	Male	Female	Male	Female	Male	Female	
Aspergillus niger	-	-	3	-	-	-	
Candida albicans	-	-	-	2	-	-	
Penicillium verrucosum	-	3	-	-	-	-	
Aspergillus clavatus	-	-	-	-	2	-	
Cunninghamella berthoelltae	-	-	-	-	-	2	
Talaromyces marneffei	-	-	2	-	-	-	
Trichopyton rubrum	-	1	-	-	-	-	
	0	4	5	2	2	2	Sub total
	(0.0%)	(26.67%)	(33.33%	(13.33%	(13.33%	(13.33%)	15
							Total

))) 100%

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The results obtained from this research showed the fungal flora presents in school pupils of Godfrey Okoye University Nursery and Primary Schools. The results showed that varieties of fungal organisms, including environmental and medically significant fungal species were present in the toe webs of the pupils. This supports the findings of (Rychlik, et al 2025) who reported that school children frequently contract fungal infections especially in tropical areas where warm, humid weather encourages fungal growth.

A total of seven fungal species were isolated, namely, Candida albicans,

Cunninghamella bertholletae, Penicillium verrucosum, Talaromyces marneffei and Trichophyton rubrum. The recovery of these organisms shows that the toe web region offers an ideal habitat for the colonization and growth of fungi Gupta and Foley, (2017) reported similar findings characterizing the toe web region as a damp, warm and conducive for the growth of fungi.

The frequency of the isolated fungi showed that Aspergillus niger and Penicillium verrucosum had the highest frequency of 3(20%), followed by Aspergillus clavatus, Candida albicans, Cunninghamella bertholletae and Talaromyces marneffei which had a frequency of 2(13.3%). Trichopyton rubrum was the least common organism with a frequency of 1(6.8%).

Trichopyton rubrum had the lowest frequency of occurrence while Aspergillus niger and Penicillium verrucosum had the greatest. The ubiquitous distribution of Aspergillus niger and Penicillium Spp in the soil, dust, and indoor habitats may be reason for their high incidence. This result is consistent with the findings of (Alter et al 2018), who found out that environmental fungi are often isolated from contaminated surfaces that school pupils routinely come in contact with.

The isolation of Trichopyton rubrum is particularly because it is one of the common causative agents of Tinea pedis (athlete's foot) although its occurrence was low in this study, its presence indicates possible fungal infection or asymptomatic carriage amongst pupils. Trichophyton rubrum species are among the major fungi causing toe web infections and superficial mycoses Gupta and Taylor (2025).

The presence of Candida albicans in the samples may be linked to sweating, excessive moisture, and inadequate drying of feet after washing. Gupta and Foley, (2017), Candida Spp prefer damp surroundings and can develop into opportunistic infections under the right circumstances.

The recovery of Cunninghamella bertholletae suggests environmental exposure to soil and matter. Similar findings associated with environmental fungi with contaminated surroundings and poor hygiene practices was reported by (Igbokwe et al 2019).

The fungal flora found in this research could be caused by a number of things, such as poor personal hygiene, wearing tight shoes for extended periods of time, sweeting and moist socks. (WHO, 2020) emphasized that poor foot hygiene and environmental exposure significantly contribute to fungal infections among school pupils.

Untreated fungal colonization can lead to symptomatic infection, itching, inflammation, unpleasant odor, and subsequent bacterial infection, the study's conclusions are significant for public health. Additionally, according to (Rychlik et al 2025) fungal infections may have a detrimental impact on a person's comfort, health, and quality of life.

5.2 Conclusion

Fungal organisms found in primary school pupils toe webs can cause superficial fungal infections and other associated health issues. Aspergillus niger, Candida albicans and Trichophyton rubrum were among the fungal species that were isolated from the toe webs samples of the pupils under investigation according to the study's findings.

Sweating, poor foot cleanliness, the toe webs region warmth and moisture, and extended wearing of closed hoes can all encourage the growth and colonization of fungi. Pupils with toe webs fungal infections may experience discomfort, inflammation, itching and irritation.

Thus, basic sanitary practices like frequent foot washing and drying, proper hygiene are crucial preventative strategies that helps lower the growth of fungal transmission in Primary school pupils.

5.3 Recommendations

Primary school pupils should be educated on good foot and personal hygiene habits, such as frequent cleaning and drying of feet, especially the spaces between the toes. In addition to preventing pupils from sharing personal belongings like towels and

shoes, parents and school administrators should make sure that pupils wear clean socks and well-ventilated shoes. To lessen the spread and incidence of fungal illness among school pupils, lastly, pupils exhibiting fungal infections should also be urged to seek medical assistance as soon as possible.

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APPENDIX I

The researcher assisting a primary school pupil in filling the questionnaire.

APPENDIX II

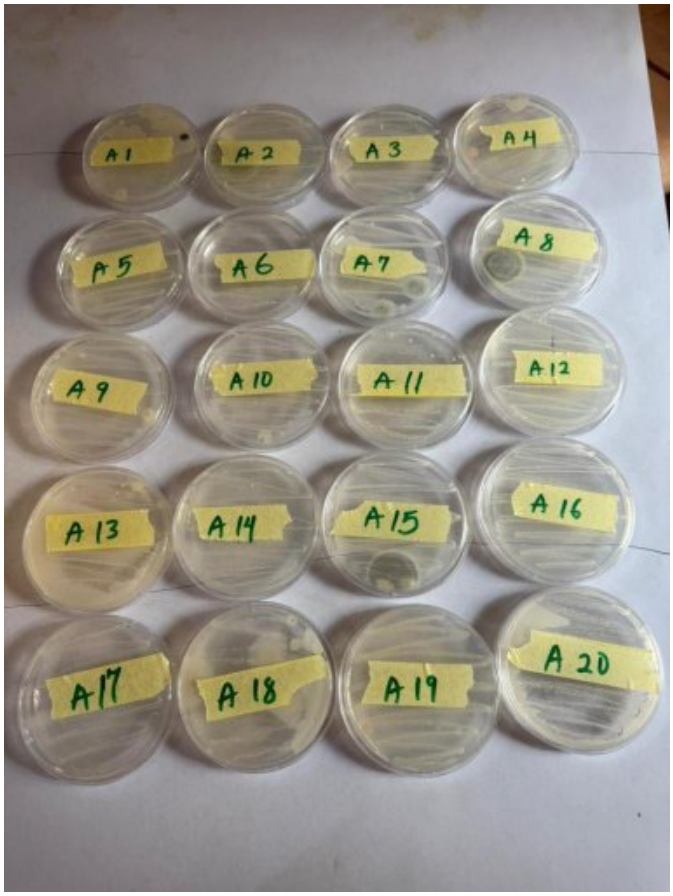
The researcher collecting toe web samples from a pupil.

APPENDIX III

Pictures showing Potato Dextrose Agar preparation.

APPENDIX IV

Picture showing the growth of the fungal isolates

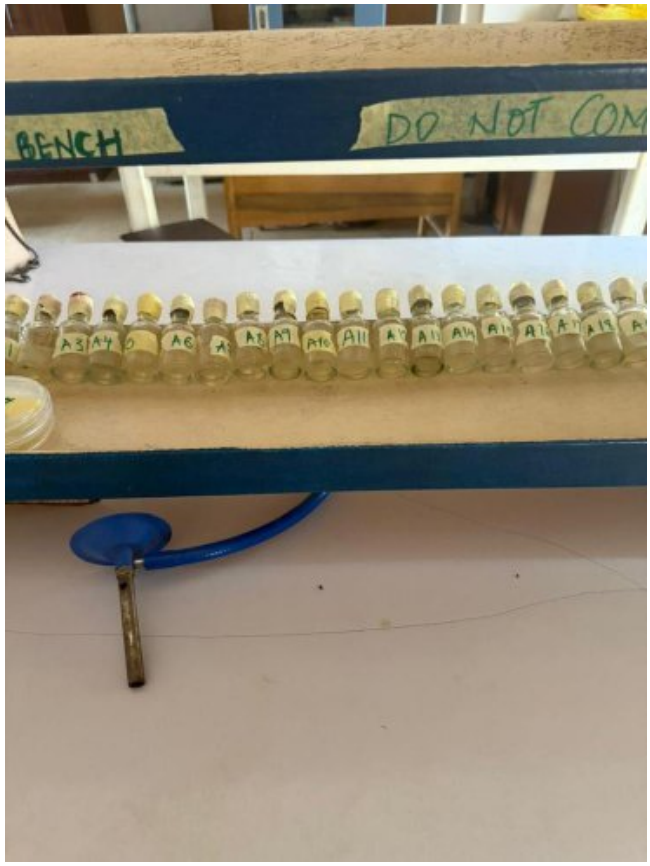


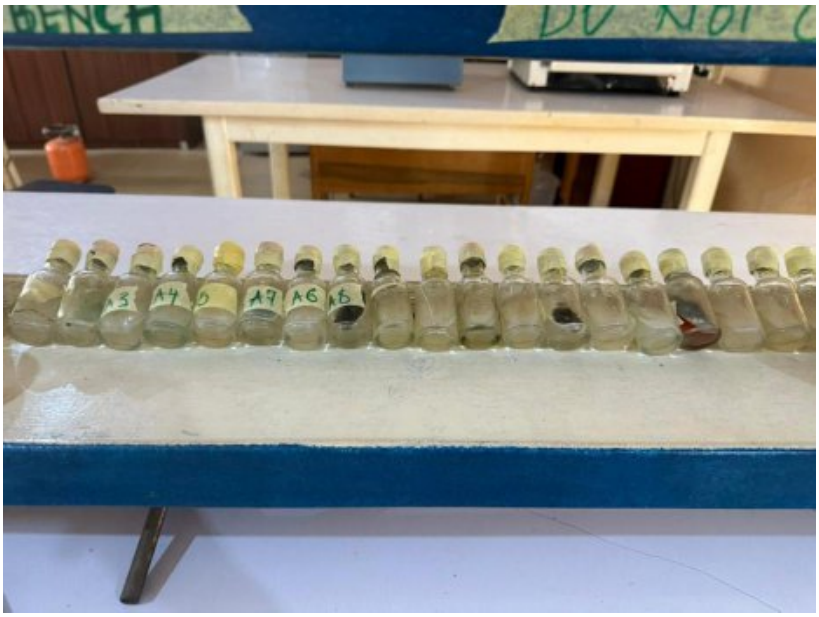
APPENDIX V

Picture showing the swab sticks used to collect the toe web samples.

APPENDIX VI

Picture showing the growth of the sub culture of the fungal isolates.





APPENDIX VII

Picture showing slide culture technique.