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Prevalence of Dermatophytic Infections among Students of Nigerian Higher Institution Using Occlusive Leather Footwear

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Authors' contributions

This work was carried out in collaboration between all authors. Author MU designed the study, wrote the protocol and wrote the first draft of the manuscript. Author MBM managed the literature searches and analyses of the study. Author BA performed the hemacytometry analysis. Author IBM managed the experimental process. Authors AAA and IYT identified the species of fungi. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Prevalence of dermatophytic infections amongst students of Nigerian higher institutions using occlusive leather footwear was carried out. The aim was to evaluate the prevalence of dermatophytic infection associated with occlusive leather footwear worn by students of Nigerian Institute of Leather and Science Technology (NILEST), Zaria, with view to isolate, enumerate,

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compare the fungal loads between students who use occlusive footwear alongside with socks and those who do not, and microscopically identify the etiological agents associated with leather footwear.

Place and Duration of Study: The study was conducted at the Department of Science Laboratory Technology, Nigerian Institute of Leather and Science Technology, Zaria, Kaduna state, Nigeria for the period of February, 2014 to July, 2014.

Methodology: Forty swab samples were collected from the feet surface of students that wear occlusive leather footwear at Nigerian Institute of Leather and Science Technology (NILEST), Zaria, Kaduna State, Nigeria; twenty swab samples from those who use occlusive footwear with socks and also twenty swab samples from those that do not use occlusive footwear with socks. The swab samples were inoculated on solidified Sabouraud's Dextrose Agar (SDA) and incubated for 4 weeks and examined at 2 to 3 days intervals for fungal growth at temperatures of 28℃ and 37℃. After incubation, the colonies developed on the medium plates were counted by hemacytometry and recorded. Potassium hydroxide microscopic observation was used to identify the isolated moulds.

Results: The total prevalence of dermatophytic infections in the study area was found to be 85%, and the samples collected from the students using occlusive footwear without socks were found to harbor more fungal loads (72.5%) compared to those collected from students that use occlusive leather footwear with socks (27.5%). Thus, using socks reduces the level of dermatophytic fungal load for occlusive footwear. The dermatophytes isolated include Epidermophyton, Trichophyton and Microsporum species as well as yeast species.

Conclusion: Both students that wear occlusive leather footwear with socks and without socks harboured dermatophytic fungi, and the overall prevalence of dermatophytic infections in the study area was determined as 34 (85%). But, the infection is more rampant in the students that wear occlusive leather footwear without socks to dry the excessive sweat in their feet resulting from overstay in footwear or rigorous athletic activities.

Keywords: Athlete's foot; dermatophytosis; footwear; leather; prevalence; occlusive.

ABBREVIATIONS

NILEST : Nigerian Institute of Leather and Science Technology
SDA : Sabouraud's Dextrose Agar
KOH : Potassium Hydroxide

1. INTRODUCTION

Fungal infection of the feet is a serious disorder, which is associated with using non-aerogenic leather footwear, especially during rigorous athletic activities. Human mycoses are infections of skins or underlying tissues caused by fungi. These can be superficial, in the case of athletes or progressive deep-seated infections such as invasive aspergillosis [1-3]. The disseminated infections may lead to high levels of morbidity and mortality if not accurately diagnosed and treated [4].

Dermatophytes are susceptible to common disinfectants, particularly those containing aerosol, iodine, or chlorine. Some other factors such as enlightenment, customs, and traditions of people, hygiene levels, and environmental sanitary conditions may influence the prevalence of dermatophytosis [5].

Leather footwear having leather sole is most hygienic. It is sufficiently strong, air permeable, and flexible. Footwear for winter use in cold climates is usually insulated with wool, natural or stimulated fur, or any one of the various synthetic materials. In summer, and during athletic activities, footwear such as sandals with sufficient air exchange along with cotton socks should be used to avoid feet lesions. Leather footwear that did not conform to the aforementioned specifications tends to predispose one to fungal infections and feet lesions [6].

Footwear has been implicated as extrinsic cause of foot ulcers in people with diabetes and high-risk foot condition [7]. The pathways usually involve injury when a foot impaired sensation and /or deformity experience minor trauma or elevated plantar pressure, resulting in tissue damage [8]. In a descriptive and case control study, a footwear-related pivotal event resulted in amputation in 21 to 36 percent of the patients

when ulcer etiologies were identified as fungi [9,10].

Dermatophytic infections are fungal infections associated with wearing covered footwear with poor aeration, especially in athletes who wear cover footwear that create optimal condition for fungal growth, which eventually leads to fungal infection known as athlete's foot. *Tinea pedis* or athlete's foot contrary to the name does not affect just athletes but affects men more than women. It affects the webs between the toes first, before spreading to the sole of the foot in a ""moccasin"" pattern [11].

Dermatophytes are the primary causative agent of dermatophytoses, a disease that affects billions of individuals Worldwide. *Trichophyton rubrum* is the most common species of the superficial fungal infection of the foot webs known as athlete's foot. Although. *T. rubrum* is a recognized pathogen for humans, little is known about how its transcriptional pattern is related to development of fungus and establishment of disease. Dermatophytes are fungi that require keratin for growth. These fungi can cause superficial infections of the skin, hair and nails. Dermatophytes are spread by direct contact from other people (anthropophilic organisms), animals (zoophilic organisms), and soil (geophilic organisms), as well as indirectly from formites. The organisms may remain viable in the environment for over six months, thus accounting for widespread infections. Transmission occurs most often from person to person [12], for example by shedding of infected skin cells and by direct body contact, especially by sharing covered footwear [13].

Dermatophytes, consisting of organisms in the *Trichophyton, Epidermophyton* and *Microsporum* genera, are the primary etiological pathogens of various dermatophytoses, such as *Tinea capitis*, *Tinea corporis*, *Tinea manus*, *Tinea unguium* and *Tinea pedis* [14]. These infections are widespread and increasing in prevalence on a global scale. Indeed, in some geographic regions, dermatophyte infection is now considered a major public health concern. Unlike other fungi, dermatophytes can cause infections in healthy, immune-competent individuals. Estimates suggest that 30 to 70% of adults are asymptomatic carriers of these fungi [15]. *Trichophyton rubrum* is the most common superficial fungal species that can be readily diagnosed based on the history, physical examination and potassium hydroxide (KOH) microscopy. Diagnosis occasionally requires Wood's lamp examination and fungi culture or histologic examination. Topical therapy with azoles is used for most dermatophyte infections [16]. Dermatophyte infections are common in developed and developing countries alike. However, the species involve and the resulting clinical entities vary both geographically and with the time [17]. Dermatophytes (name based on the Greek for "skin plants") are a common label for a group of three types of fungus that commonly causes skin diseases in animals and humans [18].

Dermatophytic infection (*Tinea pedis*) is an infection of the foot, characterized by fissures, scales and maceration in the toe web, or scaling of the soles and lateral surfaces of the feet. It is more common in those who wear occlusive shoes [19]. In majority of cases, vesicles, erythema, pustules and bullae may also be present. Anthropophilic dermatophytes are the major cause of *Tinea pedis*. Most common agents are *T. rubrum*, *T. mentagrophytes var* interdigitale, *E. floccosum*, *T. raubitschekii* and *T. violaceum.* It has been estimated that *Tinea pedis* is so common that one in five adults is affected [20].

The incidence of *Tinea pedis* has increased worldwide with an estimated prevalence of 10% in the developed world [21], and expectedly higher rate in most developing countries.

Tinea infections are among the most common dermatologic conditions throughout the world. To avoid a misdiagnosis, identification of dermatophyte infections requires both a fungal culture on Sabouraud's agar media and a light microscopic mycological examination from skin scrapings. Preventative measures of *Tinea* infections include practicing good personal hygiene, keeping the skin dry and cool at all times and avoiding sharing towels, clothing, footwear or hair accessories with infected individuals [13].

In Nigeria, there are varying reports of dermatophytoses in different cities and communities [22]. But, similar research was never been conducted in Zaria, Kaduna state, Nigeria. Therefore, in the present study we aim to evaluate the rate of incidences of dermatophytic infection associated with occlusive leather footwear of Nigerian Institute of Leather and Science Technology (NILEST) students, Zaria with view to isolate, enumerate and

microscopically identify the etiological agents associated with occlusive leather footwear. Also, the fungal load in students who use occlusive footwear alongside with socks was compared with that of students who do not use footwear with socks.

2. MATERIALS AND METHODS

2.1 Sample Collection

Following reception of ethical approval from the institute's authorities, the consent of the volunteered students that agreed to participate in the study was sought. Participant's consent form was filled during the collection of sample to obtain information on demographic data such as age and sex. A total of forty swab samples were collected from the feet surface of completely asymptomatic students of Nigerian Institute of Leather and Science Technology (NILEST), Zaria, who wear occlusive leather footwear, using a random sampling technique. The samples were collected by scrapping the feet surfaces at advancing edge of the lesion (if any), and skin flakes greater than $5mm^2$ around the webs of the toes and soles of both feet using sterile blunt scalpel following disinfection of the site of collection with 70% isopropyl ethanol. The scrapings were collected on a piece of sterile brown paper. All samples were collected aseptically by a single examiner. The collected samples were transported to the laboratory within 2 hours for microscopic and cultural analysis. Sabouraud's dextrose agar (SDA) was used for culturing the swabs as adopted by Griffin [23] and Gupta [24]. The mode of wearing footwear with or without socks was recorded for each of the samples collected.

2.2 Inoculation and Isolation of Dermatophytes from Samples

2.2.1 Direct microscopic examination

Direct microscopic examination of the scrapping placed on a microscope slide with one or two drop of 20% potassium hydroxide (KOH) and a cover slip was performed in accordance with Mackie and McCartney [25] to recover the type of mycelia, hyphal arrangement and the nature of spores. Wood's lamp was used for further characterization of the isolated fungal species. The sample was warmed for 5 minutes over a flame [26]. Each treated slide was then carefully examined under low (X10) and high (X40) power objective lens for the presence of hyphae and/or arthroconidia.

2.2.2 Fungal culture

Each scraping was cultured into Sabouraud's Dextrose chloramphenicol actidione agar [27]. A duplicate inoculation of the specimen was also cultured on Sabouraud's Dextrose cycloheximide agar to inhibit the growth of contaminants. The plates were incubated at 28℃ and 37℃ for up to 4 weeks and examined at 2 to 3 days intervals for fungal growth. Fungal isolates were sub-cultured onto plates of Sabouraud's agar. The isolates were examined visually and microscopically for morphology of fungi using lacto phenol cotton blue by slide culture technique. The dermatophytes species were identified by gross and microscopic morphology and by *in-vitro* colonial morphology, texture, color, and shape of the upper thallus and reverse pigmentations. The colonies developed were counted using hemacytometry by microscopic enumeration with a cell-counting hematocytometer (Neubauer chamber; Merck, S.A., Madrid, Spain). The isolates were identified by comparing their morphological and microscopic characteristics with standard reference organisms with those of known taxa, as described by Emmons [28] and Malmsten [29].

Identification of dermatophytes was done in accordance with Hartman and Rohde [30] and Cheesbrough [31]. The identification was based on colonies appearance, pigment production and the microscopic morphology of the spore produced.

3. RESULTS

Table 1 shows the prevalence of the isolated fungal species, which includes *Microsporum, Epidermophyton, Trichophyton* species and yeasts. The overall prevalence of dermatophytes in the study area was found to be 34 (85%).

Table 2 shows the prevalence of dermatopytes in relation to those students wearing leather occlusive shoes with or without socks. It was found that, those who do not wear leather footwear alongside socks, harbour more fungal loads (72.5%) compared to those who wear leather footwear with socks (27.5%).

Table 3 shows the general description of cultural and morphological characteristic of Fungi isolated from the feet of students who wear occlusive footwear in the study area. The isolated fungi are *Epidermophyton floccosum,*

Microsporum canis, Trichophyton mentagrophytes, Trichophyton rubrum and Trichophyton verrucosum.

Table 4 shows the prevalence of dermatophytosis in students that use occlusive leather footwear in relation to age. Students of age range 31 to 45 years were found to record highest prevalence of 42.5%.

Table 5 shows the prevalence of dermatophytosis in students that use occlusive leather footwear in relation to gender. The prevalence of dermatophytosis in the study area showed 52% and 35.5% for male and female students respectively.

4. DISCUSSION

The findings of the research study recorded the prevalence of *Microsporum species* (10%), *Epidermophyton floccosum* (15%), *Trichophyton species* (60%) and yeast as shown in Table 1, and the overall prevalence of dermatophytic infections in the study area was determined as 85% (Table 1). This showed that the Nigerian Institute of Leather and Science Technology (NILEST) is endemic of dermatophytosis. This study is first of its kind conducted in Zaria, Kaduna state, Nigeria. The high prevalence in the study area may be attributed largely because of the using occlusive leather footwear without socks, hides and skin contact by the students who engage in tanning activities in the institute, sharing footwear among students, excessive and rigorous athletic activities that result in profuse feet sweating, hot humid climate, and lack of personal engagement of the people to fight the infection, which provides a favorable environment for the organisms causing dermatophytosis. This number is higher than the figure recorded in other parts of Nigeria, that is, Kogi state (69.67%) [32], Southeastern Nigeria (51.80%) [33], Ebonyi (21.10%) [34], South-Eastern Nigeria (41.00%) [35], Kano (9.50%) [36], Plateau (82.73%) [37]. It was also higher than the figure recorded in Alexandria (7.4%) [38], and in Libya (31.10%) [39], but lower than the figure recorded in Mysore (94.00%) [40] and Assam (92.85%) [41]. The difference may be due to host's socioeconomic characteristics (age, gender, family size, individualistic and communal life style), over-crowding, geography, level of hygiene practice, nature of school infrastructure and amenities, locality, climate, affinity for contact sports, frequent contact with domesticated animals, participation in tanning processes without wearing protective boots, and nature of health care system that facilitates the transmission of dermatophytic infections of the area of study [42-44]. Another study reported that *Tinea pedis* is most commonly caused by anthropophilic species like *Trichophyton rubrum* (60%), *T. mentagrophytes* (20%), *Epidermophyton floccosum* (10%) and more rarely by *M. canis* and *T. tonsurans*. However, the true etiology in any given patient may be complicated by the presence of saprophyte fungi, yeast and /or bacteria. It has been observed that 9% of the cases of *Tinea pedis* are caused by infecting agents other than dermatophyte [45-47].

Table 1. Prevalence of dermatophytic infection in the study area

Fungal species isolated	Frequency	Prevalence (%)
Microsporum species	4	10%
Epidermophyton floccosum	6	15%
Trichophyton species	24	60%
*Yeasts	*6	*
Total prevalence of dermatophytes	34	85%

** Non-dermatophyte isolates*

Table 2. The hemacytometric distribution of fungal loads in relation to the samples collected from those who wear occlusive footwear with socks and those who do not

Mode of wearing footwear	Total number of samples	Dense fungal load >100 colonies	Sparse fungal load < 100 colonies
With socks	20	12 (60%)	8 (40%)
Without socks	20	17(85%)	3 (15%)
Total	40	29(72.5%)	11(27.5%)

Table 3. General description of cultural and morphological characteristic of fungi isolated from the feet of students who wear occlusive footwear in the study area

S/No	Cultural and morphological characteristics	Suspected organism
1	Colonies were flat, white to cream in color with powdery to suede-like surface and yellowish and Pinkish brown reverse pigment. Microscopically, it appears with numerous sub-spherical to pyriform microconidia. Multi-septate macroconidia are present (Appendix Plate 1).	*Trichophyton mentagrophytes*
2	Colonies were white to buff, fluffy and downy, with a yellow brown to wine red, reverse pigmentation. Microscopically, it appears with scanty to moderate numbers of slender clavate to pyriform microconidia. Macroconidia were absent.	*Trichophyton rubrum*
3	The colonies are olive green to yellow-mustard colour. The colonies are folded and lumpy, with orange to brown reverse pigmentation. Microscopically, it appears as blunt-clavate with smooth walls.	*Epidermophyton floccosum*
4	The colonies are white, sometimes yellow or gray with velvety appearance and heaped, smaller colonies, with white and sometimes yellow reverse pigmentation. Microscopically, it appears with rare macroconidia, long, thin and smooth wall and many chlamydospore chains.	*Trichophyton verrucosum*
5	The colonies are white and fluffy center with golden yellow border. It has closely spaced radial grooves, with yellow reverse pigmentation that become dull brown with age. Microscopically, it appears with few microconidia that form along hyphae, which is pyriform to round in shape (Appendix Plate 2).	*Microsporum canis*

Table 4. Prevalence of dermatophytosis in the students of NILEST that use occlusive leather footwear in relation to age

Age range (years)	No. of samples collected	Frequency of dermatophytosis (%)	Prevalence
0-15	0	0 (0%)	0%
16-30	13	11 (84.62%)	27.5%
31-45	19	17 (89.47%)	42.5%
46-60	8	6 (75%)	15%
>60	0	0 (0%)	0%
Total	40	34	85%

Table 5. Prevalence of dermatophytosis in the students of NILEST that use occlusive leather footwear in relation to sex

Sex	No. of samples collected	Frequency of dermatophytosis (%)	Prevalence
Males	26	21	52%
Females	14	13	32.5%
Total	40	34	85%

The students using occlusive leather shoes without socks were found to harbour denser fungal loads (72.5%) compared to those students who wear cover shoes with socks (27.5%). According to [15], *Trichophyton rubrum* is the most common cause of the superficial fungal disease of the feet, especially in individuals that wear footwear without socks.

Therefore, the analysis of dermatophytic infections associated with occlusive leather footwear of NILEST students showed the presence of dermatophytic infections, and the overall prevalence of dermatophytic infections in those who wear occlusive shoes with socks and those who wear occlusive shoes without socks was found to be 85%. According to the world health organization [48], dermatophytes affect about 25% of the world population. It is estimated that from 30 to 70% of adults are asymptomatic hosts of these pathogens and that the incidence of the disease increases with age. The dermatophytes isolated include *Epidermophyton floccosum, Microsporum canis, Trichophyton verrucosum, Trichophyton mentagrophytes* and *Trichophyton rubrum* (Table 3). This agrees with the findings of [49], who reported that dermatophytes are known to grow best in warm and humid environments and subtropical regions and this probably explains why they are very common in Africa. For instance, some species of dermatophytes such as *Trichophyton mentagrophytes,* var. *interdigitale*, *Microsporum canis*, *Epidermophyton floccosum*, and *Trichophyton rubrum* are distributed all over the world. However, other species have partial geographical restriction. For examples, *Trichophyton schoenleinii* is found in Africa and Euroasia while *Trichophyton soudanense* is restricted within Africa. Therefore, proper use of occlusive footwear should be maintained to avoid the infection with dermatophytosis. Chadwick [50] reported that old shoes that may have become colonized with dermatophytes should be replaced. Clean, absorbent socks are recommended, preferably made from natural fibres, such as cotton. When in communal areas, the individual should avoid direct contact with the floor by wearing flip flops and never wear other people's shoes.

The students' demography was categorized on the basis of age groups of 0-15 years to 60 years and above in groups of 15 years interval, and it was found that the maximum prevalence (42.5%) was observed in the age group of 31 to 45 years (Table 4). This may be due to the presence of feet flakes and cracks that favour the growth of the fungi in the feet of middle-aged individuals. Carlo and Bowe [51] reported similar assumption, that nearly everyone in the population is exposed to the common fungi that cause *Tinea pedis*. Each person's immune system determines whether infection results from such exposure. As adults age, tiny cracks develop in the skin of their feet, increasing the susceptibility to tinea infections. Once acquired, a fungal infection can linger inactively for years and later become active when a person reaches the age of 60-70. Robbins and Elewski [52] reported that the prevalence of *Tinea pedis* increases with age. Most cases occur after puberty. Childhood tinea is rare.

The prevalence of feet dermatophytosis in the males and females was found to be 52% and 32.5% respectively (Table 5). This agrees with a previous study [53], who reported that the *tinea pedis* infection was pronounced in males, 78 (70.9%) as compared to females 32 (29.1%). Also, [52] reported that *tinea pedis* has no predilection for any racial or ethnic groups, but the disease more commonly affects males compared with females.

5. CONCLUSION

Both students that use occlusive leather footwear with either of the socks and without socks were found to harbour dermatophytic fungi; and the overall prevalence of dermatophytic infections in the study area was found to be 34 (85%). But, the infection is more rampant in the students that use occlusive leather footwear without socks to dry the excessive sweat in their feet resulting from overstay with footwear or rigorous athletic activities. *Tinea pedis* was found to be more prevalent in males compared to their female counterparts. The infection was found to be more common among adults of age range 31 to 40 years.

It is therefore recommended that, people with prior foot ulcers should need varieties in shoe types and styles. These individuals should be encouraged to use adequate aerogenic footwear and minimize the time spent in footwear. The feet should be cleaned and dried before putting on socks or footwear. Protective gears should be worn by those who engage in mingling with hides and skin during tanning processes, to protect one from contacting zoophilic dermatophytosis. Also, prompt diagnosis and proper treatment of dermatophytic infections with antifungals such as terbinafine, griseofulvin, azoles (ketoconazole, Itraconazole, oxiconazole, miconazole) and Whitfield ointment is very important to prevent complications of secondary bacterial infections.

Many factors may have been attributed to the continuous prevalence of dermatophytic infections in this area. These factors may include lack of information or ignorance of the exact

cause, and correct treatment approaches to the disease, poor environmental sanitation, animal contact, sharing footwear among students, and impoverished economy among others.

CONSENT

All authors declare that written informed consent was obtained from the patients and the authorities of the Nigerian Institute of Leather and Science Technology (NILEST), Zaria, Kaduna state, Nigeria for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Plate 1. Showing growth of *Trichophyton mentagrophyte* isolated from foot surface
The colonies are flat, white to cream in color with wrinkled, powdery to suede-like surface. It shows yellowish and Pinkish brown reverse pigmentation

Plate 2. Showing growth of *Microsporum canis* isolated from foot surface
The colonies appear as white, granular, with fluffy center and golden yellow border. It possesses yellow to brown reverse pigmentation

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