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Onychomycosis: Prevalence and spectrum of fungal etiological agents among patients attending the dermatology clinic at All African Tuberculosis and Leprosy Rehabilitation Training Center

BY

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This is to certify that the thesis prepared by Sinkinesh Wolde Behaksira, entitled: Onychomycosis: Prevalence and spectrum of fungal etiological agents among patients attending the dermatology clinic at All African Tuberculosis and Leprosy Rehabilitation Training Center and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAU	Addis Ababa university
AIDS	Acquired Immune Deficiency Syndrome
ALERT	All African TB and Leprosy Rehabilitation Training Center
CAF	Chloramphenicol
HIV	Human Immune Deficiency Virus
LPCB	Lacto-Phenol Cotton Blue
KOH	Potassium Hydro-Oxide
LPCB	Lacto-Phenol Cotton Blue
NDM	Non-Dermatophytesmoulds
PH	Power of Hydrogen Ion
SDA	Sabrouad Dextrose Agar
SFI	Superficial Infection
SPSS	Statistical Packages for Social Sciences

Abstract

Background: Onychomycosis is among the most frequent forms of human infections; being estimated to affect more than 20-25% worldwide, and their incidence is constantly increasing particularly in developing countries. Although onychomycosis is considered to be a trivial disease the psychological effects of onychomycosis is highly considerable and because of its high morbidity it is a costly disease in terms of loss of working days and treatment. Anyone can get a fungal nail infection. Some people may be more likely than others to get a fungal nail infection, including older adults and people who have nail injury or nail surgery, Diabetes and a weakened immune system and blood circulation problems.

Objective: - To determine the prevalence of onychomycosis and profile fungal etiologic agents collected from clinical suspected samples in All African TB and Leprosy Rehabilitation Training Center.

Methods: -a cross sectional study design from January to May, 2018 was conducted. A total of 303 study participants were collected by convenient sampling techniques. By using standard structured questionnaire socio-demographic data was collected and fungal pathogen from scraped nail sample was isolate and identified by using KOH microscopic examination and cultivation on sabouroud and chromo agar. Data was analyzed using SPSS version 23 software and P- value < 0.05 was considered as statically significant.

Results:-The overall prevalence is of onychomycosis 185/303(61.3 %) in our study site. Non Dermatophytes was the most prevalent (34.5%), followed by dermatophytes (28.1%), yeast (25.9%) and mixed (10%). fungal pathogen *Aspergillus* was the most common species (spp) isolated among the non dermatophytes, whereas *t.tonsurans* and *t.mentagrophyte spp* were among the dermatophytes and from yeast genes *Candida albican* were isolated.

Conclusion: The prevalence and profile of fungal onychomycosis infections seems to be related to age, life style, and the presence of co-morbidities (P= 0.03). The present study indicated that non dermatophytes were the main agents causing onychomycosis. In the meantime performing direct examination and fungal culture was important to diagnose and treat the patients. Predisposing factors have a contribution to increase the incidence of onychomycosis.

Key words Onychomycosis Prevalence Risk factors

1. Introduction

1.1 Background

Onychomycosis is a denomination used to describe fungal infection of one or more of the nail units and can be caused by dermatophytes, yeasts or non-dermatophytic molds [1]. It accounts for up to 50% of all nail diseases and 30% of all mycotic infections of nail [2-4]. Till late 1990s onychomycosis was a poorly debated topic of medical science, which has been highlighted only in the last decade. In developing countries, higher priorities in socioeconomic concerns and health issues for other diseases have resulted in low awareness of onychomycosis by physicians and general public alike. Although onychomycosis is hardly life threatening, its high incidence and prevalence and the associated morbidity makes it an important public health problem. Onychomycosis shows a worldwide prevalence rate of 2% to 50% and varies with age, time and geographic location [5].

Several factors have been implicated in the increase of the disease such as reduced peripheral circulation, diabetes, nail trauma, difficulty to maintain proper nail hygiene, chronic smoking, communal bathing etc. Infected nails serve as a chronic reservoir of infection, which can cause mycotic infection of skin [6]. Not only it can cause an aesthetic problem but also it will affect the physical, emotional, social and occupational well-being of the patients. Clinically onychomycosis is sub-classified into various forms such as distal lateral subungual-onychomycosis, proximal sub ungula onychomycosis, and white superficial Onychomycosis and total dystrophic onychomycosis [7]

Therefore, it is imperative to have a clinical suspicion for this condition and to identify the cause to treat it. Ethiopia being a developing nation located in the tropic with wet humid climate appears to fall in to the category of regions with high prevalence of onychomycosis. Furthermore, Ethiopia as one of the developing countries, socioeconomic constraints and other common prevalence health issues have led to a low awareness of onychomycosis by physicians

And general population and hence conducting further studies to know the actual magnitude of onychomycosis as well as the spectrum of its etiological agents among the general population is of the highest priority.

1.2 Statement of the Problem

Onychomycosis, fungal infection of the nail, represents approximately 30% [8] of all fungal nail infections and at least 50% of all nail disorders [9]. Its incidence is constantly increasing particularly in developing nations. Although onychomycosis is considered to be a trivial disease the psychological effects of ringworm is highly considerable because of its high morbidity and it is a costly disease in terms of loss of working days and treatment. Onychomycosis is predominantly caused by dermatophytes; however, non dermatophytic fungi are becoming increasingly implicated in causing onychomycosis.[8]

The distribution of onychomycosis and its causative agents vary with a humid geographical region are influenced by a wide range of factors. The prevalence of onychomycosis and the predominant etiologic agents are poorly known in Ethiopia. Therefore, determining the prevalence of onychomycosis and its etiologic agents in Ethiopia is timely and an active field of study.

1.3 Significance of the Study

- Knowledge of the prevalence of onychomycosis, provides relevant information on the extent of the disease epidemic , helps to identify infection control mechanisms and selection of appropriate antibiotics for empiric treatment
- Fungal pathogens isolate in this study can be used for further study such as drug susceptibility study
- Assessment of the prevalence of fungal nail disease is important to determine the size of the therapeutic problem and to better understand the etiology and changes over time
- The results obtained in this study may be used as a baseline data for epidemiological studies of onychomycosis in the country.

2. Literature review

2.1. Literature review general

Fungal nails may appear brittle broken and lifted or separated from the nail bed. Changes in the nail appearance are the first sign and symptom of onychomycosis[7]In Europe and USA on 2013 Sigurgeirsson B., and BaraneR. Evaluated the prevalence of onychomycosis based on published relevant studies and the finding shown that eleven population-based and 21 hospital-based studies were identified. The mean prevalence in Europe and North America was 4.3% [95%] Confidence Interval in the population-based studies, but it was 8.9% (95%) for the hospital-based studies. Both population-based and hospital-based studies showed that onychomycosis is more common in toenails and is seen more frequently in males. The main causative agent was a dermatophyte in 65.0% (95% CI: 51.9-78.1) of the cases. *Trichophytonrubrum* was the single most common fungus and was cultured on average in 44.9% of the cases. Moulds were found on average in 13.3% and yeasts in 21.1%. In the same study Onychomycosis is a common disorder, but it may not be as common as cited in the literature, because hospital-based studies might overestimate the prevalence of onychomycosis. It is more frequent in males, and toenails are more commonly affected. Dermatophytes, particularly *T.rubrum*, are the main causative agents of onychomycosis [14]

A systematic review of the literature and calculated pooled prevalence estimates of onychomycosis in at risk patient populations conducted byGupta K. in 2014. The prevalence of dermatophytes toenail onychomycosis was as follows: general population 3.22% (3.07, 3.38), children 0.14% (0.11, 0.18), the elderly 10.28% (8.63, 12.18), diabetic patients 8.75% (7.48, 10.21), psoriatic patients 10.22% (8.61, 12.09), HIV positive patients 10.40% (8.02, 13.38), dialysis patients 11.93% (7.11, 19.35) and renal transplant patients 5.17% (1.77, 14.14). Dialysis patients had the highest prevalence of onychomycosis caused by dermatophytes, elderly individuals had the highest prevalence of onychomycosis caused by yeasts (6.07%; 95% CI= 3.58, 10.11) and psoriatic patients had the highest prevalence of onychomycosis caused by non dermatophyte moulds (2.49%; 95% CI= 1.74, 3.55). An increased prevalence of onychomycosis in certain patient populations may be attributed to impaired immunity reduced peripheral circulation and alterations to the nail plate which render these patients more susceptible to infection. [24]

A prospective cross-sectional study was carried out in Barcelona city In the period of 2003–2004, among 1,305 children To evaluate the prevalence of tineacapitis, tineapedis, and tineanguium (9% immigrant population) between the ages 3 and 15 in 17 schools in Barcelona. A systematic examination of the feet (including nails and scalp).Dermatophytes0.15% in toe nails. The predominant etiologic agents in toe nail were isolated in 2.9% of the samples with a prevalence of 2.5% in feet, 0.23% in scalp, and were *Trichophyton mentagrophytes* in 45.7% of the cases and *Trichophytonrubrum* in 31.4%. In the nails, *t. rubrum* and *Trichophyton tonsurans* were isolated, while *t.mentagrophytes* (2 cases) and *Trichophytonviolaceum* (1 case) were identified in scalp samples. Forty-five per cent of dermatophytes were isolated from healthy feet,. The results of the study demonstrate a low prevalence of tineacapitis and tineanguium in school children of Barcelona. But on the other hand, high prevalence of dermatophytes in feet was found. It highlights the high prevalence of healthy carriers of dermatophytes in feet.[23]

A study for determine epidemiology of foot diseases, including tineapedis and onychomycosis in Hong Kong. Two groups were included: the institutional group clinical evaluation and mycological investigations by dermatologists; and the private group clinical evaluation only by the private physicians. Patients who had a regular visit to the clinics were randomly invited to examination of their feet. The prevalence rate of foot disease, fungal infections, toe nail onychomycosis were respectively 50.7%, 26.9%, 20.4% and 16.6%. More male and elderly patients were affected except that the sex prevalence in toe nail onychomycosis was not shown to be significant. Vascular disease, diabetes mellitus and obesity were the three most prevalent predisposing factors in foot fungal nail disease. Dermatophytes, in particular *Trichophyton rubrum*, were shown to be the most common pathogen in nail infections and [26]

Study conducted byMayser P, Freund V, Budihardja D Toenail Diabetes mellitus may be associated with serious sequelae, such as renal disease, retinopathy, and diabetic foot. A recent large prospective study has shown that onychomycosis is among the most significant predictors of foot ulcer. As the severity of onychomycosis may be associated with the length of time the individual has had the infection, early intervention is advisable owing to the progressive nature of the fungal infection. If left untreated, toenails can become thick, causing pressure and irritation, and thus act as a trigger for more severe complications. (26)

In Egypt Prevalence of non dermatophyte molds in patients with abnormal nails involving 32 patients was carried out by El Batawi [10]. The prevalence of non dermatophyte molds was greater the commonest isolates accounting 47%. deramtophytes were isolated in only five patients (15.6%) of which *M. canis* and *t. violaceum* being the most common dermatophytes. Yeasts were isolated in only three patients (9.4%). than dermatophytes. Non dermatophyte molds were isolated from 19 cases (59.4 %) *Aspergillus* species.

A prospective study to evaluate the epidemiology of foot mycoses of three hundred and ninety-two patients was undertaken in Tunisians in order to determine the fungal etiological agents and to identify possible risk factors. During one year (2013-2014). Complete mycological diagnosis was carried out on all patients. A total of 485 samples were collected; tineapedis and tineanguium were confirmed in 88.2% of cases. Dermatophytes were isolated in 70.5% and the most frequent pathogen was *t.richophytonrubrum* (98.1%), followed by yeasts (17.7%) commonly *Candida parapsilosis*. Non-dermatophyte molds (NDMs) were observed in 8.02% cases and *Fusarium* sp. was the frequent genus (29.1%). The main predisposing factors of fungal foot infections were practicing ritual washing (56.6%) and frequentation of communal showers (50.5%). This is a recent survey of foot mycoses in Tunisiz. [25]

The prevalence, epidemiology and risk factors for onychomycosis Kuvandik et al[11]. One hundred and nine hemodialysis patients were enrolled. Onychomycosis was diagnosed in 26.6% of hemodialysis patients. Diabetes mellitus was present in 68.9% of patients with onychomycosis. Toenail scraping cultures were reported to be positive in 19.7% of patients with dystrophic nail changes. Logistic regression analysis revealed that the presence of diabetes mellitus and the mean duration of hemodialysis were the significant predictors associated with the development of onychomycosis.

The Prevalence of Fungi in Finger nail Onychomycosis has been studied by Gelotar et al [12]. Of a total of 45 study subjects 37.78% study subjects were identified to positive by culture, among which 17.78% were KOH positive and 20.00% were KOH negative. 62.22% were culture negative.

The predominant pathogens were yeasts accounting for (64.71%), followed by dermatophytes (17.65%). A mixed infection was identified in 11.76%. *Scytaalidiumhyalinum* was identified in 5.88% of the cases. Fungi commonly presented in the middle age, between 31-40 years of age, due to trauma at the work site and in women, due to their wet work.

Epidemiological, clinical and cultural study of onychomycosis conducted on 64 patients with onychomycosis revealed that distal sub-ungual onychomycosis (DSO) was the commonest type onychomycosis accounting for 47(78.35%) and this was followed by candido-nychomycosis 10(16.6%), proximal subungual onychomycosis 2 (3.34%) and superficial white onychomycosis 1(1.71%). The most common predominant organism isolated is *T.rubrum* in 22(35%) followed by *T.mentagrophytes* 6(10%) and the non dermatophytes fungi were isolated in 3 cases (5%) [13].

A study on the prevalence onychomycosis and its etiology in a Tertiary Care Hospital, South India on 2016 showed that the out of 95 study subject 38 (40%) were positive for fungal infections of dermatophytes were isolated in 20 (52.6%) cases, while 18 (47.4%) had non-dermatophytes as fungal agents. *t. mentagrophyte* swas the most common species) isolated among the dermatophytes, whereas *Aspergillus* spp and *Fusarium* spp were among the non-dermatophytes [16].

An investigation on Onychomycosis in Eastern India demonstrated that out of 249 study subject enrolled in the study 126(50.6% were found to be positive for onychomycosis. Of these distal and lateral subungual onychomycosis was the commonest clinical pattern (56.6%) followed by proximal subungual onychomycosis (15.7%), total dystrophic onychomycosis (12.1%), white superficial onychomycosis (10.8%) and chronic paronychia (4.8%). Dermatophytes were most common (55.9%) cause of onychomycosis. Amongst them *Trichophyton rubrum* was the most common isolate (65.9%). *Candida albicans* was important (79.2%) amongst the yeast isolates. Non dermatophytic moulds were involved in 15.5% of cases [17].

Clinico-Mycological Pattern of Onychomycosis in Kashmir north India involving 150 study subjects suspected of onychomycosis studied by culture microscopic technique among the study subjects 66.6% fungal pathogens were detected and/or isolated by direct microscopy and culture. Males were infected more than females. The commonest age group infected was 21-30 years. Finger nails were affected more frequently than toe nails and distolateral subungual - onychomycosis was the most common clinical type seen in 66% patients. The etiological agents were dermatophytes (62.68%), NDM (29.85%), yeasts (7.46%). Among dermatophytes *T.rubrum* was the commonest etiological agent [18].

The study made in Cameroon 2012 Kondjo N.et.al there was two series of patients observed at a hospital dermatological service of Yaoundé and in a volunteer service of Douala were evaluated.

All the patients, regardless of the reason for the consultation, were examined by an expert dermatologist to discover signs of onychomycosis. Patients with suspected nail lesions underwent mycological examination according to the standard techniques.

Among a total of 590 sample (317 males and 273 females), aged 16-83 years, onychomycosis was mycologically confirmed in 52 cases (8.8%). The infection was most common in the fifth decade. Fingernails were affected in 12 cases, toenails in 30 cases, and both fingernails and toenails in 10 cases. Dermatophytes were isolated in 57.7% of cases, the most common species being *Trichophytonrubrum* (16cases) and *Trichophytonviolaceum* (8 cases). Non-dermatophytic mould, including *Aspergillus* spp, *Fusarium* spp., and *Neoscytalidiumdimidiatum*, was found in 10 cases. Onychomycosis was more common in patients from low social-economic classes [21]

Teklebirhan and Bitew [19] have conductedcrosssectionalstudy on the profile of dermatophyte and non dermatophyte fungi in patients suspected of dermatophytosis against 305 study subjects. The results of their study demonstrated that onychomycosis was the predominant clinical manifestation accounting 51.1% of the cases of which 119 (76.3%) were from females and 37 (23.7%) from males. *t.violaceum*. Seventy five percent of yeasts and 77.6% non dermatophyte molds were isolated from nails. They conclude that along with dermatophytes, non-dermatophyte fungi are also emerging as important causes of dermatophytosis.

A cross-sectional Study conducted in Ethiopia, Harari Regional State from April to June 2015 attempted and to determine the prevalence and etiological agents of dermatophyte infections of hair, skin, and nail among primary school children study was carried 428 primary school children. Skin scrapings, hair samples, and nail clippings were collected from children who showed dermatophytosis. Prevalence in 428 school children, 211 (49%) male and 217 (51%) female, 100 (23.4%) had culture confirmed dermatophytosis and tineacapitis took the overall prevalence of 18% (77/428). *T.violaceum* was isolated from4 3 samples, followed by *T. rubrum* in 24. The highest prevalence of dermatophytosis was seen in. As a result, the study found a high prevalence of dermatophytosis in the Harari's Regional State school children and tineacapitis was the predominant clinical finding which needs an intervention [21]

2.2. Conceptual frame work

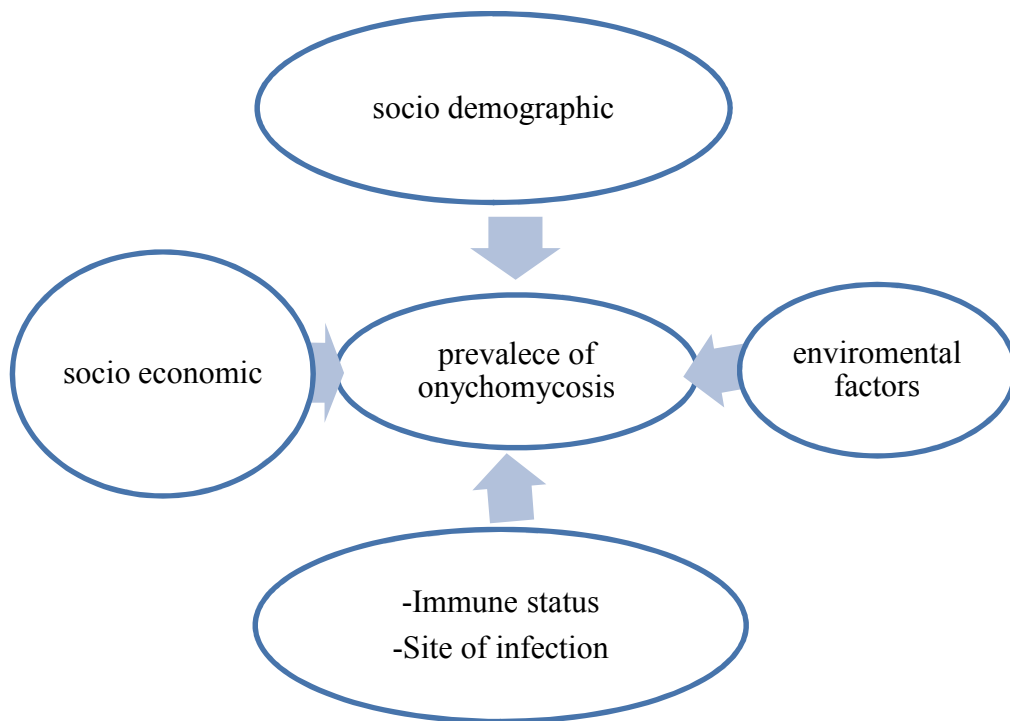


Figure 1: Conceptual framework (constructed by me) [1-12]

3. Objective

3.1 General objective

To determine the prevalence of onychomycosis and the spectrum of dermatophytes and non dermatophytes fungi and their association with socio-demographic characteristics among patients attending the dermatology clinic in ALERT Hospital Ethiopia, from January 2017-May 2018.

3.2 Specific objectives

- To determine the prevalence of onychomycosis in the study site.
- To determine the risk factor for onychomycosis.
- To compare prevalence of dermatophytes and non-dermatophytes implicate in causing onychomycosis.

3.3 Hypothesis

The prevalence of onychomycosis in the study site will be the same to the findings of study conducted by G/egziabher T and Bitew A, TikurAnbesa, Ethiopia.

4. Materials and Methods

4.1 The Study Setting and Period

The study was conducted from January to May 2018 at ALERT hospital, Addis Ababa, Ethiopia. ALERT is a medical facility on the edge of Addis Ababa, specializing in Hansen's disease, also known as "leprosy". It was originally the All Africa Leprosy Rehabilitation and Training Center (hence the acronym), but the official name is now expanded to include tuberculosis: All Africa Leprosy, Tuberculosis and Rehabilitation Training Centre.

ALERT's activities focus on its hospital, rehabilitation of leprosy patients, training programs for leprosy personnel from around the world, and leprosy control (administration of the Ethiopian Ministry of Health's regional leprosy control program). From the beginning, ALERT provided leprosy training for medical students from Addis Ababa University. Also at ALERT is the Armauer Hansen Research Institute, founded in 1970, specializing in leprosy research. There is currently 240 bed teaching hospital, which includes dermatology, ophthalmology, and surgery departments, also an orthopedic workshop, and a rehabilitation program.

ALERT is the continuation and expansion of the leprosy hospital originally built by Dr. Thomas Lambie in 1922, which was later named the Princess ZänäbäWärq Hospital. A memorandum to found ALERT was signed Dec. 11, 1965 by representatives of the Ministry of Health, Addis Ababa University, the International Society for the Rehabilitation of the Disabled, The Leprosy Mission, and Dr. Eugene Kellersberger of the American Leprosy Mission, who had had the vision for establishing such a multifaceted center and had been the main promoter of the project.

Currently ALERT laboratory is one of accredited laboratory among accredited laboratories in Ethiopia.

4.2 Study Design

A cross sectional descriptive study was conducted from January to May 2018.

4.3 Population

4.3.1 Source Population

The source population was study participant attended dermatology clinic at ALERT hospital during the study period.

4.3.2 Study Population

Study populations were those who were sent to mycology laboratory with clinically suspected cases of onychomycosis and people who fulfill the inclusion criteria were included.

4.4 Sample Size and Sampling Procedure

Convenient sampling technique was used, in which clinically suspected cases of onychomycosis (superficial mycoses) prescribed by physicians were included. According to a cross sectional study conducted at Tikur Anbessa Hospital Addis Ababa, Ethiopia the prevalence of dermatophytes and non-dermatophytes associated with onychomycosis 16.1% was [19], and 95% CI, and 5% margin of error and 10% for the non-response rate in determining my sample size for this study. Therefore the sample size is given as follows:

$$N = \frac{(Z\alpha/2)^2 * (p) * (1-p)}{d^2}$$

Where: n = the sample size

$(Z\alpha/2)^2 =$ at 95% confidence interval Z value ($\alpha = 0.05$) = 1.96

P = the proportion of occurrence of onychomycosis 16.1% (0.16)

d = margin of error at 5% (0.05)

$$N = \frac{(1.96)^2(0.161(1-0.161))}{(0.05)^2} = 205.6 = 206$$

10% non response rate = $\frac{10 \times 206}{100} = 20$, Sample size was therefore 206 + 20 = total sample size was **226**. Actually we have collected and analyzed 303 samples to increase the sensitivity of our study.

4.5 Study variables

4.5.1 Dependent variables

- Prevalence of onychomycosis
- Types of etiologic agents for onychomycosis

4.5.2 Independent variables

- Socio-demographic characteristics such as: age, sex, and occupation
- Site of affected nail parts such as nail matrix bed and plate

4.6 Inclusion and exclusion Criteria

4.6.1 Inclusion and exclusion Criteria

All patients clinically suspected of onychomycosis were included at the study area and in the specified period of study. Abnormalities included: hyperkeratosis, nail thickening, longitudinal and transverse grooves, pitting, brittle nails, cracks, changes in color of nail plates such as black, yellow, and white discoloration and onychomycosis.

4.6.2 Exclusion Criteria

Study participant who do not want to participate in the indicated study and those under fungal treatment were excluded.

4.7 Data Collection Procedures

4.7.1 Data collection tool for socio-demographic factors

Standard questionnaire was used to collect detailed information on onychomycosis and history data of each study subject (age, sex, health status, and site of infection).

4.7.2 Laboratory methods

4.7.1.1 Sample collection transportation and identification

The specimens were collected for microbiological analysis from clinical abnormal nails were collected by scraping the distal portion of the nail and from affected parts suspected with onychomycosis and previously swabbed with 70% (v/v) ethanol was collected using sterile surgical blades by scraping and transferred in sterile glass Petri dish. Each of these Petridis was appropriately labeled with the patients name, age, sex, and date of collection.

4.7.1.2 Isolation, characterization and Identification of fungal pathogens

4.7.1.2.1 Direct microscopy (KOH)

Direct microscopy, by using freshly prepared 20% KOH to identify the presence or the absence of fungal filaments, budding yeast cells, the Pseudohyphae, the hyphae and the arthroconidia.

[AnnexVI]

4.7.1.2.2 Culture

All samples were inoculated into duplicate plates of Sabrouad Dextrose Agar (SDA medium supplemented with chloramphenicol and cycloheximide and SDA medium supplemented with chloramphenicol but not cycloheximide). Then all culture plates were incubated at room temperature for a period of 4-6 weeks to report result culture negative. All culture was examined from the colony texture, topography, pigmentation production and rate of growth every other day. A mold was identified microscopically while yeasts were identified following standard routine biochemical and assumptions procedures. All supplies (culture media and reagents) used in the present study was prepared according to manufacturer instruction.

4.7.1.2.3 Characterization, Identification and differentiation of fungal pathogen

All the culture growths were identified on the basis of the culture characteristics. Mold isolates were identified by examining macroscopic and microscopic characteristics of their colony. Texture, rate of growth, topography and pigmentation of the front and the reverse side of the culture were prepared for macroscopic identification. Identification of mold by microscopic, isolates was performed by placing pieces of culture colony from SDA and stained with Lacto phenol Cotton Blue (LCB) and compared with Atlas (practical guide and atlas for the diagnosis of fungal infection 2017).

4.7.1.2.4 Yeast identification

Candida albicans, *Candida glabrata*, *Candida krusei* and *Candida tropicalis* was differentiated after cultured on SDA took pieces of culture colony from SDA and inoculate on chromo agar Candida media observe the color change on media for identification. *Candida albican* (green), *Candida tropicalis* (metallic blue), *Candida krusei* (pink), Other *Candida* spices (white to move).

[VIII]

4.8 Data Management and Quality Assurance

Media was checked for sterility and growth supporting ability and standard protocols was followed. Data was checked for completeness before analysis.

4.9 Data Processing and Analyses

Results was compiled and entered in to SPSS version 23 Software and was analyzed to determine frequency and compare distribution of etiologic agents of onychomycosis by age, sex and anatomic site of infection involved. Categorical continuous variables Chi-square was calculated distribution and association of etiologic agent and socio demographic and P .value < 0.05 was considered statistically significant.

4.10 Dissemination of Results

The results were submitted and presented to the school of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University and were submitted to the study health institutions, Association of Medical Laboratory (AMLA). And will be sent to peerreviewed journals for publication.

4.11 Ethical Consideration

All ethical considerations and obligations were fully addressed and the study was conducted after the approval of the Internal Review Board (IRB) of the department of Medical Laboratory Sciences. And ALERT Hospital. Each respondent was given the right to refuse to take part in the study and to withdraw at any time during the study period. All the information obtained from the study subjects was coded to maintain confidentially. When the participants were found to be positive for fungal pathogen, they were informed by the hospital clinician, information was read for study participants an assent form was be completed and signed by a family member and/or adult guardian for participants under the age of 18 years.

5. Results

5.1 Socio demographic characteristics

A total of 303 cases included in this study. Out of these, males 100(33%) and females 203 (67%) giving, rise to a male to female ratio of 1:2. The age range of the study participants were 2 minimum and 75 were the maximum. From these individuals with age 25-44 occurred most frequently (n=120, 76.4%) in both gender. The details were presented in (table 1.)

Table 1: Demographic characteristics sex age group study participant attending dermatology clinic ALERT Hospital, Addis Ababa, Ethiopia, (N=303)

		Age					Total
		1-14	15-24	25-44	45-64	>65	
sex	Male	11	27	34	21	7	100
		11.0%	27.0%	34.0%	21.0%	7.0%	100.0%
	Female	13	68	86	35	1	203
		6.4%	33.5%	42.4%	17.2%	0.5%	100.0%

5.2 Prevalence of onychomycosis

The overall prevalence of onychomycosis in this study was 185/303 (61.1%). While analyzing the prevalence of onychomycosis in different age groups, it was observed that the age group 45- 64 years (70.9%) were most commonly affected followed by the young age group 25-44 years (62.1%) table.2. Out 203 were females the rest were males. As table 2 showed when age increased the disease also increased.

Table 2: prevalence of onychomycosis study participant attending dermatology clinic ALERT Hospital, Addis Ababa, Ethiopia, (N=303)

Age	Disease	
	NO disease	With disease
1-14	12 50.0%	12 50.0%
15-24	36 37.9%	59 62.1%
25-44	4 39.2%	73 60.8%
45-64	16 29.1%	39 70.9%
>65	7 75.0%	2 25.0%

Age group- WHO age classification for health 2007

Upon microscopic examination by KOH and culture technique fungal pathogens among study participant attending dermatology clinic ALERT Hospital, Addis Ababa, Ethiopia,(n=303). From this we can see that 87.3% positive by microscopy resulted in positivity by culture 53.3% were positive in culture microscopic negative Table 3,each specimen was divided into two parts; one was take for direct microscopic examination after 20% KOH solution treatment and second was inoculated on Sabrouad Dextrose agar and SabrouadCycloheximide Chloramphenicol agar(Table3).

Table 3: microscopy and Culture result of study participant attending dermatology clinic ALERT Hospital, Addis Ababa, Ethiopia, (N=303)

Microscopy	Culture	
	Positive	Negative
Negative	128 53.3%	112 46.7%
Positive	57 87.3%	8 12.7%
Total	185 60.4%	120 39.6%

When we see mycological profile respect to site of infection toe nail infection is most frequent, Table4, (57.66.3%) and (128, 59.3%) toe and finger nail respectively from study participantClinical abnormalities were observed discoloration of the nail plate, trauma, cracked and bleeding.

Table 4: Infection with anatomical site of study participant attending dermatology clinic at ALERT Hospital, Addis Ababa, Ethiopia, (N=303)

Site	Disease	
	no disease	with disease
Finger	89 40.7%	128 59.3%
Toe	29 33.7%	57 66.3%
Total	118 38.7%	185 61.3%

5.3 Associated risk factors

Several risk factors are associated with onychomycosis .In our study we included associated factors related to onychomycosis, such as age, sex, HIV infection diabetes trauma and how often people have contact with risk factors like water, chemicals, and environmental contact with soil. From a total of 93 risk factor associated case (72,77.4%)were positive 32cases were trauma 23 water contact,16 contact with soil the rest were contact with chemical infection with HIV and diabetes ,positive with onychomycosis contact with water also showed 23 where examined and (Table 5)(n=19, 26.3%)were positive trauma (n=23, 12.4.9%) contact with soil(n=12 ,10,3%)were positive, HIV and diabetes 11 %and 6.9% respectively Truman was accounts higher from the total case logistic regression showed have association with fungal infection .

Table 5: Disease frequencies of onychomycosis with predisposing factors of study participant at ALERT hospital (N=303)

Pre disposing factors	Disease		Total Case
	With disease	No disease	
Trauma	23	9	32
Diabetes	8	1	9
HIV	5	2	7
Water contact	19	5	23
Chemical contact	4	2	6
Soil contact	12	2	6
Non risk cases	113	97	210
Total	185	118	303

5.4 Fungal pathogens group

Among the group of fungal etiology non dermatophytes the most frequent cultured from a total of 185 isolated (n63, 34. %) followed by dermatophytes (n51, 27.56.4%).and yeasts (n54, 29.2 %). In 17 patients (9.1%), were isolated mixed pathogen dermatophytes, non dermatophytes and yeasts were culture (figure 2).

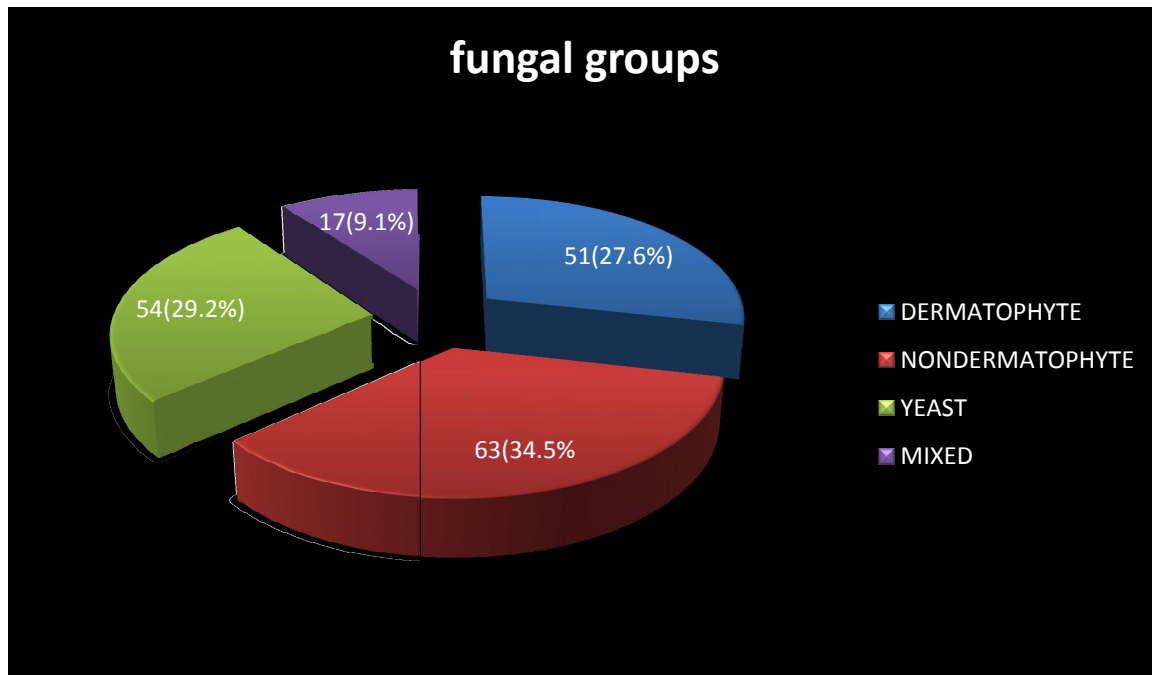


Figure 2: proportion of fungal groups isolated from study participant with onychomycosis at dermatology clinic ALERT hospital (N=185)

Table 6: Distribution of fungal pathogens in age group among study participant attending dermatology clinic at ALERT Hospital, Addis Ababa, Ethiopia, (N=185)

Isolated agents	Age					Total
	1-14	15-24	25-44	45-64	>64	
Mixed	3(17.6%)	5(29.4%)	3(17.6%)	6(35.3%)	0(0.0%)	17(100%)
<i>T. tonsurans</i>	0(0.0%)	7(35.0%)	9(45.0%)	4(20.0%)	0(0.0%)	20(100%)
<i>T. mentagrophytes</i>	0(0.0%)	8(61.5%)	4(30.8%)	1(7.7%)	0(0.0%)	13(100%)
<i>T. rubrum</i>	0(0.0%)	1(12.5%)	3(37.5%)	4(50.0%)	0(0.0%)	8(100%)
<i>Microsporum nanum</i>	0(0.0%)	0(0.0%)	1(50.0%)	1(50.0%)	0(0.0%)	2(100%)
<i>T. verrucosum</i>	0(0.0%)	0(0.0%)	1(50.0%)	1(50.0%)	0(0.0%)	2(100%)
<i>T. schoenleinii</i>	0(0.0%)	2(100.0%)	0(0.0%)	0(0.0%)	0(0.0%)	2(100%)
<i>T. violaceum</i>	1(25.0%)	0(0.0%)	1(25.0%)	2(50.0%)	0(0.0%)	4(100%)
<i>C. albicans</i>	0(0.0%)	9(34.6%)	12(46.2%)	5(19.2%)	0(0.0%)	26(100%)
<i>C. glabrata</i>	0(0.0%)	2(40.0%)	2(40.0%)	1(20.0%)	0(0.0%)	5(100%)
<i>C. krusei</i>	1(5.6%)	6(33.3%)	8(44.4%)	3(16.7%)	0(0.0%)	18(100%)
<i>C. tropicalis</i>	0(0.0%)	4(100.0%)	0(0.0%)	0(0.0%)	0(0.0%)	4(100%)
other yeasts	0(0.0%)	0(0.0%)	0(0.0%)	1(100.0%)	0(0.0%)	1(100%)
<i>Aspergillus spp</i>	3(10.0%)	5(16.7%)	17(56.7%)	4(13.3%)	1(3.3%)	30(100%)
<i>Cladosporium</i>	2(12.5%)	4(25.0%)	6(37.5%)	3(16.6%)	1(11.1%)	16(100%)
<i>Fusarium</i>	2(28.6%)	0(0.0%)	1(14.3%)	4(57.1%)	0(0.0%)	7(100%)
<i>Alternaria</i>	2(0.0%)	3(60.0%)	2(40.0%)	0(0.0%)	0(0.0%)	5(100%)
<i>Scopulariopsis sp</i>	0(0.0%)	2(66.7%)	1(33.3%)	0(0.0%)	0(0.0%)	3(100%)
<i>Hendersonula</i>	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	2(100%)	2(100%)

T=trycophyton C= Candida

The present study indicated that mold *aspergillus* 30(16.2%) *t.tonsurans* 20(10.9%) *t.mentagrophytes*13(7.1%) and yeast genus *Candida albican* 26(14.2%) was a predominant pathogen in our study (Table6). This provided useful guidelines for the appropriate management of the cases. A high frequency of finger and toe nail onychomycosis was observed among women study participants and so, they were advised to avoid or minimize contact with risk factor and to improve their health and personal hygiene. This study suggests that the diagnosis of nail diseases not only depend on the clinical patterns of the nail changes. It also performed a mycological confirmation.

According to (table 7)all fungal groups manifestation observed higher in among woman's The most common fungi cultured from infected Finger and toe nail in woman's were yeasts genus *Candida*(n, 18;14.24)and*aspergillus*,(n,18,14.24%)dermatophyte*T.tonsurans*(n 15,11.9%)*T.men tanegrophyte* (n10,7.9%) and In 10 patients (7.9%)mixed dermatophytes with yeasts, non dermatophyte and dermatophyte ,yeast with non dermatophyte were isolated, culture positive results .The most often observed non dermatophytes were *scopularisis* and *Hendesolun spp*.

Table 7: Distribution of fungal isolates with gender among study participant attending dermatology clinic ALERT Hospital, Addis Ababa, Ethiopia, (N=185)

Fungal agent isolates	Frequency %		Sex			
			Male		Female	
	Frequency	%	Frequency	Percent (%)	Frequency	Percent (%)
Mixed	17	9.2 %	7	12.2 %	10	7.9%
<i>T.tonsurans</i>	20	10.9%	5	8.7 %	15	11.9 %
<i>T. mentagrophytes</i>	13	7.1%	3	5 %	10	7.9%
<i>T. rubrum</i>	8	4.3%	1	1.7 %	7	5.5 %
<i>Microsporumnanum</i>	2	1.09%	0	0 %	2	1.5%
<i>T.verrucosum</i>	2	1.09%	0	0 %	2	1.5%
<i>T.shoenleinii</i>	2	1.09%	0	0 %	2	1.5%
<i>T, violaceum</i>	4	2.18%	1	1.7 %	3	2.3%
<i>C albicans</i>	26	14.2%	8	14.0 %	18	14.24%
<i>C. glabrata</i>	5	2.73%	1	1.7 %	4	3.1 %
<i>C kurusei</i>	18	9.83%	6	10.5 %	12	9.5 %
<i>C tropicalis</i>	4	2.18 %	2	2.4 %	2	1.5%
Other yeasts	1	0.54 %	1	1.7 %	0	0 %
<i>Aspurgilluspp</i>	30	16.4%	12	21.05 %	18	14.24%
<i>C ladosporium</i>	16	8.7 %	6	10.5 %	10	7.9 9%
<i>Fusarium</i>	7	3.8 %	1	1.7 %	6	%
<i>Alternaria</i>	5	2,7 %	1	1.7 %	4	3.1 %
<i>Scopularipsisspp</i>	3	1.6%	2	2.4 %	1	0.79%
<i>Hendesoluna</i>	2	0.54%	1	0.42	1	0.79%
Total	185	100%	58	100%	127	100%

T=Trycophyton C=Candida

6. Discussion

Onychomycosis is a fungal infection of the nail and common nail manifestation in the general population worldwide. Many epidemiological and prevalence studies have reported the high frequency of fungal infection, however the prevalence varies with many factors like demographic climates, the selected study participant and immune status. Onychomycosis is the most common superficial infection and represents a major public health problem of over the world.

The present prevalence study Out of 303 subjects 66.9% were females while 33.1% were males the age range was minimum 2 maximum 75 the mean age group was 25-44 years this is consistent with study conducted in Ethiopia a total of 305, study participants were enrolled in the study which of 97 (31.8%) were males and 208 (68.2%) females. The ages of study subjects ranged from 1 to 80 year with a mean age of 26 years [19] in other study male higher (317 males and 273 females [22]females were more commonly affected than males which agree with some reports [19] . But there was no significant relationship in the occurrence of mycoses with respect to the sex and these results. This may be caused by with different predisposing risk more among women. Cosmetics reasons repeated aggressive manicure and pedicure, long hours contact with water frequent housework, and using detergents that cause nail trauma and generally females seek advice more frequently for onychomycosis. However, several studies concluded that males are more infected than females due to the fact that males are more exposed to nail trauma and using occlusive footwear [25].

Our results also showed that children the age group 1-14 years and elders > 64 years are less infected with onychomycosis; this frequency is in accordance with results observed in school children in Spain Barcelona [23] onychomycosis infections in children not common can be due to many reasons, rapid growth of the nail, have less exposure to fungal infection risk factors than adults such as aggressive pedicure and manicure, frequent housework. Other study Hong Kong disagreed more male and elderly patients were affected except that the sex prevalence in toe nail onychomycosis was not shown to be significance. (26).This differences the prevalence and fungal distribution different worldwide.

Both site toe and finger nail manifestation of onychomycosis have been reported in the literature. In the present study toe nail most frequent affected, (n57.66.3%) and (n128, 59.3%) toe and finger nail respectively this work; agree with similar study in Europe and USA prevalence of onychomycosis. It is more frequent in males, [14, 21] and toenails.

In investigating the causative agents of onychomycosis we found that the most common isolated pathogens were non dermatophytes among them, *Aspergillus* was the most common causative agent followed *Cladosporium*, *Fusarium* and *Aternaria*. These results are agreed with other studies. Non dermatophyte molds were isolated from 19 cases [59.4 %] *Aspergillus* species being El Batawi [10, 16] interpreted that the prevalence of molds was greater. The second agent responsible for onychomycosis was dermatophytes, with a high frequency of *T. tonsurae* and *T. mentagrophytes*. These results were agreed by *T. mentagrophytes* was the most common species) 20 (52.6%) cases 18 (47.4%) respectively [16] isolated among the dermatophytes This confirmed with the study in Egypt however the most dermatophyte, Isolate were *M. canis* and *t. violaceum* [10] and can be explained by the fact onychomycosis have variation of prevalence among world population in many reason.

Molds are saprophyte cosmopolitan filamentous fungi; most of them are and can be contaminants; however they become opportunistic under unfavorable conditions. In addition to the causative dermatophytes and yeasts, in the present study among molds *Aspergillus* (n30, 16.4%) isolated 21% case male and 14.2% in female, this showed that molds are become public health importance [14] Moulds were found on average in 13.3% and yeasts in 21.1%. Traditionally, these molds have been considered as secondary pathogens of nails which affect a keratin. This study contradict with recent survey of foot mycoses in Tunisia were observed in 8.02% most frequent cases *Fusarium* spp [25]

In our prevalence study, we found a low incidence of mixed fungal pathogen agents and the most prevalent species were *Fusarium* with yeast, *T. mentagrophyte* with yeast spp. (9.2%) This result agreed with the study reported. [12]

In our result among fungal etiology group (n64, 35. %) followed by dermatophytes (n52, 28.4%). and yeasts (n 48, 26.2. %). In 19 patients (10.4%), was isolated mixed pathogen dermatophytes, non dermatophytes and yeasts were culture, from yeast the most frequent cultivate were genes *Candida albican*. This were conformed an investigation on Onychomycosis in Eastern India [17] *Candida albican* was important (79.2%) amongst the yeast isolates

The association between clinical manifestations, onychomycosis and risk or predisposing association factor was tested by Chi-square and logistic regression tests. It was found to have significant association ($P = 0.05$) onychomycosis contact with water showed (26.3%) were positive trauma 31.9%) contact with soil 16, 6%) were positive, HIV and diabetes 11 % and 6.9%

respectively this have similarity with A. Gupta in. HIV positive patients 10.40% Truman was accounts higher from the total case an increased prevalence of onychomycosis in certain patient populations may be attributed to impaired immunity reduced peripheral circulation and alterations to the nail plate which render these patients more susceptible to infection [24, 26]

The main objective of the study was to determine the prevalence of onychomycosis, collected nail scraping from, finger and toe nail. Clinical specimens examined microscopically and culture, 185/303 (61.1%) result obtained dermatophytes, non Dermatophyte, yeasts and mixed fungal pathogens were isolate [14, 16, 21].

7. Limitation and Strength of the study

7.1 Strength

- ✓ In our study we included predisposing factors and possible fungal etiology included
- ✓ The findings of the study may serve as a baseline data for further study; the study addresses possible sites of onychomycosis and potential fungal pathogens.

7.2 Limitations

Due to resource limitation time, financial and other constraint drug susceptibility test and other non-fungal infection affected nail parts such as nail matrix bed and plate were not performed in our study this will be our active site in future study.

8. Conclusion

This is a recent study of onychomycosis prevalence and associated factors on study site the studies can be useful to eradicate these infections and to provide further measures of hygiene education to avoid or minimize risk factors.

The prevalence of onychomycosis that caused by different fungal pathogen was similarly with others study which was 185/303 (61.1%) Though non dermatophyte, 63,(34%) yeasts 54 (29.2) dermatophytes,51(27.6)mixed 17(9.1) fungal agents were the predominant group isolated fungi was non dermatophyte followed by dermatophytes and yeasts was also considerable indicating that the spectrums of fungi causing onychomycosis.

Several risk factors are associated with onychomycosis. Having Knowledge of these risk and predisposing factors is important when treating and educating patients with onychomycosis to take appropriate measurements wearing personal protective to minimize workplace hazards Truman chemical contacts and etc.

9. Recommendations:

- We recommend and encourage direct microscopy and the mean time fungal culture together help patients get the appropriate diagnosis and empirical treatment.
- As our study has indicated the prevalence of fungal infection same as higher compared to other studies and hence we recommend avoid risk factor for fungal infection to minimize onychomycosis.
- As our study provides baseline information, predisposing factors hence, we recommend further study for non-fungal agents, affects nail have similar clinical feature with onychomycosis.
- Nail changes are not always a reliable marker for predicting the causative organism, and relying only on the clinical manifestation. Pattern of nail changes in the diagnosis of onychomycosis can cause misleading. The present study highlights the need for microbiological confirmation in case of onychomycosis before under anti-fungal treatments.
- This is a prevalence study for onychomycosis in a study site. Epidemiological, survey and anti-fungal drug susceptibility studies can be useful to eradicate these infections and to provide further managements of empirical drug therapy, hygiene and education.

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Annex I

1. Participant information sheet (English version)

Participant information sheet Department of Medical Laboratory Science, Collage of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

Title: Onychomycosis: Prevalence and spectrum of fungal etiological agents among patients attending the dermatology clinic at All African Tuberculosis and Leprosy Rehabilitation Training Center.

Introduction First of all we would like to thank you in advance for your cooperation and consent in participation in this study. Please listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely. **Background information:** Onychomycosis is a denomination used to describe fungal infection of one or more of the nail units and can be caused by dermatophytes, yeasts or non-dermatophytic molds. It accounts for up to 50% of all nail diseases and 30% of all mycotic infections of nail the world's population, and their incidence and prevalence is constantly increasing particularly in developing nations.

Aim of the study: The objective of this study is to determine the prevalence of onychomycosis and associated fungi implicated in causing various onychomycosis at ALERT hospital Addis Ababa, Ethiopia. Procedure of the sample collection Sample will be collected from the specified site affected the site is cleaned with 70% ethanol and some part of the specimen collected will be used for investigation to help the physician in managing his patients' treatment protocol and the remaining part of the sample will be used for research purposes after patients or guardian's willingness to participate is confirmed in their signature. The consent agreement will be made by the principal investigator in the laboratory when fungal infection suspected patients come to ALERT hospital laboratory.

Benefits for participants: Study participants will not have any financial incentives or other inducements from participating on this study. However, their results will be given and will be treated by the prescribing physician based on the results and depending on the nature of the disease and the physicians decision; patients may be appointed to await culture results for better treatment.

Risks and complication there is no considerable risk to the study subjects in participating in the study.

Confidentiality

In order to maintain the confidentiality of participants' information, the name will not be given and the samples will be coded. Participants will not be prohibited to stop or withdraw at any time from the study. Only interested participants can retrieve their own lab result using their code number. The physician will be responsible for the interpretation of the results and providing treatment. No personal identifier will be disclosed to third party or will not appear in any report from this study.

2. የተሳታፊ መረጃ (Amharic Version (ትርጉም))

2. የተሳታፊ መረጃ (Amharic Version(ትርጉም))

የህክምና ላቦራቶሪ ሳይንስ ጤና ሳይንስ ኮሌጅ የአዲስ አበባ ዩኒቨርሲቲ አዲስ አበባ ኢትዮጵያ የጥናቱ ርዕስ:-በአለርት ሆስፒታል ለጥፍርና ለቆዳ ህክምና የሚመጡታካሚ ላይ በፈንገስ የሚታመሙ ሰዎች በዛት አዲስ አበባ ኢ.ዮ.ጵ.ያ.። በመጀመሪያ በጥናቱ ላይ ለመሳተፍ ፍቃደኛ ስለሆኑክልብምስጋናዬን አቀርባለውእባክዎን ይህንን የተሳታፊ መረጃ ክልብአንዲያዳምጠ -በትህትና እንጠይቃለው።ስለጥናቱ ያለዎትን ጥያቄበማንኛውም ጊዜ መጠየቅ ይችላሉ ።ስለጥናቱ መረጃ ዘወትር በፈንገስስለሚጠቁ የሰውነት ክፍሎቻችን ውስጥ ፣ ንዱ ጥፍር ዋና ነው።በርከት ያሉ ጥናቶች እንደ ሚያሳዩት ከ2-50 ፐርሰንት የሚሆነው የአለማችንህዝብ በዚህ በሽታች ይጠቃል።ይህ በሽታች በተለይም በማደግ ላይ ባሉ አገሮች በመስፋፋት ላይ ይገኛል። የጥናቱ አላማ በአለርት ሆስፒታል ለፍጥርና ለቆዳ ህክምና የሚመጡታካሚ ላይ በፈንገስ የሚጠቁ ሰዎች ብዛትና ስርጭት። ናሙና የመስጠት.ፍቃደኝነት የሚጠይቅ ተማራማሪው ሲሆን ከተገባውን የሰውነት ክፍል በ 70 ፐርሰንት አልኮል ከፀዳ በኋላ ናሙና ይወሰዳል ናሙናውም ሃኪም ላዘዘው መረጃ የሚረዳ፤የሚያገለግልና የተሳታፊ ፍቃደኝነት ከተረጋገጠ በኋላ የተረፈውን ናሙና ደግሞ ለጥናቱየሚያገልግልይሆናል።

የጥናቱ ተሳታፊ ጥቅም

ተሳታፊዎች በጥናቱ በመሳተፍ ምንም አይነት የገንዘብ ጥቅም አያገኙም ነገር ግን ተሳታፊዎች የናሙና ምርመራና ውጤት ተቀብለው ተገቢውን ህክምና በሐኪማቸው በኩል እንደበሽታው ሁኔታ እንደ ሀኪም ውሳኔ ለተሻለ ህክምና ውጤት ጠብቀውእንዲታከሙ ይደረጋል።

ከጥናቱ ሊመጡ የሚችሉ የጎንዮሽ ጉዳቶች

በዚህ ጥናት የሚሳተፉ ሰዎች ምንም አይነት ጉዳት የማይደርስባቸው መሆኑን እንገልጻለን ።

የጥናቱ ምስጢራዊነት.

የተሳታፊዎችን መረጃ ምስጢራዊነት ስለመጠበቅ ይረዳ ዘንድ የጥናቱ ተሳታፊዎች ስምበጥናቱ ላይ አይገለፁም። በስም ፋንታ መረጃቹ በምስጢራዊ ቁጥር/ኮድ/ ይመዘገባሉ። ኖእንዲሁም ተሳታፊዎች በፈለጉ ሰዓት ከጥናቱ መውጣት ይችላሉ። ፈቃደኛ የሆነ ታካሚ ለሚሰጣቸው ኮድ ውጤታቸውን ማየት ይችላሉ። ጥናቱን የሚያካሂደው ሰው ማረጋገጫ ለዚህ ጥናት ሃላፊቱን

ለመውሰድ ማንኛውም ጥናቱን የሚመለከት ጉዳይ ለሚመለከተው አካል መግለጫ ለመስጠት በፊርማዬ አረጋግጣለሁ።

ስንቅነሽ ወልደ ፊርማ ----- ቀን -----

Annex II

1. Informed consent [English version]

I, the undersigned individual, I am oriented about the objective of the study. I have informed that all of my information will be kept confidential and used only for this study.

Your signature below indicates that you have read /or listened, and understand the information provided for you about the study. Before you sign, please understand purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have any question.

I have read /or listened to the description of the study and I understand what procedures are and what will happen to me in the study.

Based on the above information I agree to participate in the research

Signature: _____ Date: _____

Name of Data collector _____ Signature _____

If you have any question you can ask the principal investigator

Principal investigator [M.Sc. candidate]

Mobile 0911592995

E-mail sinkine_shkejka@yahoo.com.

2. Informed consent [Amharic version]

ከእኔ፡የሚወሰደው፡ናሙና፡ለጥናቱ፡አላማ፡ብቻ፡እንደሚወሰድ፡ተረድቻለሁ።

ሁሉም፡መረጃዎች፡እና፡የናሙና፡ወጤቱ፡ምስጢራዊ፡መሆኑን፡ተገንዝቤአለሁ።፡፡በጥናቱ፡ላይ፡በመሳተፊ

ምንም፡የገንዘብ፡ክፍያ፡እንደማላገኝ፡ተረድቻለሁ።፡፡በጥናቱ፡ያለመሳተፍ፡እንዲሁም፡በማንኛውም

ጊዜ፡የማቋረጥ፡መብት፡እንዳለኝ፡አወቁአለሁ።፡፡

ስለዚህ፡ህጥናት፡ማንኛውም፡ጥያቄ፤ካለዎት፡በማንኛውም፡ጊዜ፡ከዚህ፡በታች፡በተጠቀሱት፡አድራሻዎች፡መጠየቅ፡ይችላሉ።፡፡

እኔም፡የጥናቱ፡ተሳታፊ፡ይህንን፡በመገንዘብ፡ጥናቱ፡ላይ፡ለመሳተፍ፡ተስማምቼያለሁ።፡፡

ፊርማ -----ቀን-----

መረጃውን፡የሰበሰበው፡ግለሰብ፡ስም-----

ፊርማ -----

የዋና፡ተመራማሪው፡አድራሻ

ኢ-ሜይል e-mail sinkwolde@gmail.com

ስልክ፡0911592995

Annex III.

Ascent form (English)

Parental/Guardian Consent Form (for ages less than 18 years old)

I was informed take whatever time I need to discuss the study with my family and friends, or anyone else I wish to. The decision to let my child join, or not to join, is up to me, and, my child can stop participating at any time and will not lose any benefits as thereof. As parent or legal guardian, I assure in my signature to become my child a participant in the research study described in this form. Guardian's name-----Signature/fingerprint: ----- Date -----
 ----- Witness's name----- signature: -----Date ----- Investigator's name-----
 ----- signature: ----- date -----

Ascent form (Amharic version)

የወላጅ፡ወይም፡ያሳዳጊ፡ፈካደኝነ፡ትቅፅ/ከ18 አመትእድሜበታች፡ያለታዳጊዎችበቻ

የጥናቱ፡ርዕስ፡በአለር፡ሆስፒታል፡

ለጥፍርእናለቆዳህክምናየሚመጡፈንገስታማሚየጥፍርፈንገስተጠቂሰዎችበዘትለማወቅየሚሰራጥናትነዉ።በዚህ፡ጥናት፡ውስጥ፡
 የእርሶ፡ልጅ፡ሰለተመረጠ፡እባኮዎን፡ሰለልጅዎ፡በዚህ፡ጥናት፡የመሳተፍ፡ፍቃደኝነትዎን፡ያሳውቁን፡ዘንድ፡እርስዎ፡ፍቃደኛ፡ከሆኑ፡
 ልጅዎ፡ከጥፍር፡ናመና፡እንድንወስድ፡ይኸውም፡ከጥቂት፡ደቂቃ፡በላይ፡የማይወስድ፡መሆኑን፡በተጨማሪ፡ህመም፡የሌውና፡
 እንዲሁ፡ምበፈገው፡ጊዜ፡ከጥናቱ፡መውጣት፡እንደሚችል፡በመውጣቱም፡ምንም፡ጉዳትና፡ከህክምናም፡ምንም፡ጉዳት፡እንደሌው፡
 እንገልጻለን።

የተመራማሪውስም----- ቀን ----- ፊርማ-----

የተሳታፊውስም----- ቀን-----ፊርማ-----

የእማኝነትስም----- ቀን -----ፊርማ-----

Annex IV: Questionnaire

Addis Ababa University Collage of Health Sciences Department of Medical Laboratory Science,,
questionnaires for the demographic and risk factors for the prevalence of onychomycosis among
patients visiting dermatology clinic at ALERT, Hospital Addis Ababa, Ethiopia.

Code number _____

I. Socio-demographic factors

1. Age _____

2. Sex: 2.1 male _____ 2.2 Female _____

II. Health status

1. Pathological/Physiological

1.1. Normal receptive states: (Pregnancy)

A. Yes B. No

1.2. Severe or chronic underlying infections

1. HIV/AIDS 2. Endocrine disorder: e.g., diabetes mellitus, 3. Malignancies
(Carcinoma and leukemia)

1.3. Mechanical (Trauma including burns)

A. Yes B. No

1.4. Drug therapy

1. Antibiotics, 2. Oral contraceptives,

1.5. Surgical procedures

1. Heart, 2. Gastro- intestinal, 3. Brain 4. Transplantation surgery

1.6. Frequency of nail manicure, pedicure and chemicals _____

1.7. Frequency of contact with water and soil per day _____

Annex V: Sample collection, storage and transportation

Annex VI: Reagent prep Nails Clean nail with ethanol. Scrape and discard outer portion of nail. Collect scrapings from inner nail and put a portion of it in glass slides for wet mount KOH preparation. Send an entire nail, if it has been removed, in a sterile Petri dish container. Store and transport at room temperature. Processing Nails must be ground in a mortar if the procedure need before inoculated on a culture medium.

Preparation (Stain, wet mount solutions and culture Media)

A. 20% POTASSIUM HYDROXIDE

Formula;

Potassium hydroxide (KOH)	10g
Glycerol	20ml
Distilled Water	80 ml

Dissolve the potassium hydroxide in distilled water, and then add glycerol. Mix well, Filter and Store in sterile amber colored bottle for 3 months.

Purpose: To digest or clear organic material e.g. keratinized tissue cells artifacts in a specimen in order to allow fungal elements (structures) to be more easily demonstrated.

Principle: Fungi are unaffected by KOH. Glycerol prolongs shelf-life by preventing crystallization and preserves the slides for a few days.

Procedure

1. Add a drop of 20% KOH to specimen on slide and Cover by cover slip.
 - If needed Gentle heating may aid in dissolving debris
 - if specimen is thick, it may take 15-30 minutes to dissolve
2. Observe under low light microscope

B. Lacto Phenol cotton Blue (LPCB)

Formulae:

Distilled water:	_____	_____	20.0ml
Lactic acid	_____	_____	20.0ml
Phenol Crystals	_____	_____	20.0g
Cotton blue	_____	_____	0.05g
Glycerol	_____	_____	140ml.

Dissolve phenol in the lactic acid, glycerol, and water by gently heating. Then add aniline blue.

Purpose: Used for wet mount preparations of fungal cultures.

Sabrouad Dextrose Agar with Chloramphenicol and cyclohedmid

The SDA media is comprised of enzymatic digest of casein and animal tissues which provide a nutritious source of amino acids and nitrogenous compounds for the growth of fungi and yeasts.

Dextrose is the fermentable carbohydrate incorporated in high concentration as a carbon and energy source. Agar is the solidifying agent. Addition of antibiotics like Chloramphenicol and/or tetracycline acts as broad spectrum antimicrobials to inhibit the growth of a wide range of gram-positive and gram-negative bacteria. cyclohedmid is added to further inhibit the growth of gram-negative bacteria.

Approximate Formula per Liter Purified Water (DW)

Pancreatic Digest of Casein-----	5.0 g
Peptic Digest of Animal Tissue-----	5.0 g
Dextrose-----	40.0 g
Agar-----	15.0 g
Chloramphenicol-----	0.05 g

Storage Instructions: store plates in the dark at 2 – 8°C ready for use.

For slopes: Dispense 10 ml. amounts into UGB bottles.

Plates : Dispense 40ml of each 100x90ml plate

Annex VII. Principles of the procedure

Sabrouad Dextrose Agar-is a peptone medium supplemented with dextrose to support the growth of fungi. The peptones are sources of nitrogenous growth factors. Dextrose provides an energy source for the growth of microorganisms. Chloramphenicol is a broad-spectrum antibiotic which is inhibitory to a wide range of gram-negative and gram-positive bacteria. cycloheximide is an Eukaryote protein synthesis inhibitor.

For slopes: Dispense 10 ml. amounts into UGB bottles then Autoclave 121°C for 15 minutes and Store at RT final pH 7.0 at 25°C.

Mycosel agar

Purpose

- To isolate pathogenic fungi (onychomycosis) mainly dermatophytes from contaminated specimens it inhibits bacteria and most fungi saprophytic fast growing.
- To determine Cycloheximide resistance of fresh isolates as a screening test for pathogenic fungi.

Procedure

1. Suspend or re dehydrate 36 g of the powder in 1 L of purified water (DW). Mix thoroughly.
2. Heat with frequent agitation just until the medium boils; completely dissolve the powder to homogenize.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Slope: Dispense 10 ml. amounts into 25-ml UGB bottles, Autoclave 15 min at 121°C, Cool on a slant then Store at room temperature final pH 6.9 ± 0.2.

Plates: 40 Dispense ml of each 100x90ml plate

Annex VIII: Chromo agar Candida

Intended use

CHROM™ Candida is a selective medium recommended for the isolation and identification of yeasts. This medium also allows for the differentiation of *C. albicans*, *C. tropicalis* and *C. krusei* based on differences in colony morphology and color. This medium facilitates the detection of mixed yeast cultures.

Summary and principles

CHROM™ Candida is a selective and differential medium containing chromogenic substrates. After degradation by specific enzymes, the substrates release different colored compounds. Certain species or groups of organisms can then be differentiated with a minimum number of confirmatory tests. Colonies of *C. albicans* appear green to dark metallic green, *C. tropicalis* colonies appear medium blue to dark metallic blue with a blue halo, and *C. krusei* colonies appear flat, often rough or crenated, and pink to medium pink in color. Other species appear pink, often with a darker mauve center (*C. glabrata* and other species). Other yeasts may appear white to pink.

When HardyCHROM™ Candida is used as the primary plating medium, only colonies that morphologically (pink, often with a darker mauve center) resemble *C. glabrata* should be tested for trehalose assimilation.

HardyCHROM™ Candida contains glucose and selected peptones as a nutrient supply. Chromogenic substrates are incorporated to enable the production of different colored compounds when degraded by specific enzymes formed by the yeast. Chloramphenicol is added as an inhibitory agent against the growth of most bacteria, which may be present in the sample.

Formula

Ingredients per liter of demonized water (DW)

Glucose -----	20.0gm
Peptone -----	10.0gm
Chromogenic Mixture -----	2.0gm
Chloramphenicol -----	0.5gm
Agar -----	15.0gm

Storage and shelf life

Storage: Upon receipt store at 2-8°C. Away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination.

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Sinkinesh Wolde (B.Sc.)

Signature: _____

Date of submission July 2018

This thesis has been submitted with my approval as advisor.

Advisor: Adane Bitew (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.