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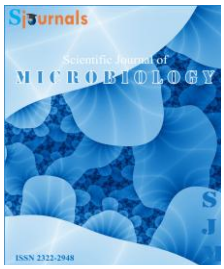

Original article

Evaluation of fungal (Onychomycosis) in Sokoto, Nigeria

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ABSTRACT

Onychomycosis is a fungal infection of the nail, which occurs worldwide with Dermatophytes as the most common causal agents although yeast and moulds are also involved. The diagnosis usually involves direct microscopy and culture to prove actual existence of onychomycosis. A total of 30 samples were tested using 20% KOH and culture plates of Sabourad Dextrose Agar (SDA) which was mixed with streptomycin as an antibiotic. A total of 80.0% samples were identified as positive by culture, among which, 46.7% were KOH positive and 33.3% were KOH negative. 20.0% were culture negative. The predominant pathogen was yeast 37.43%, followed by dermatophyte 33.33% and the moulds with 24.12% of the cases recorded. Onychomycosis was observed to be common between the age of 41-50 with the occurrence of 50%, and 50% in both male and female patients attending the hospital. The research highlighted that the yeast was a predominant pathogen in Ammannawa General Hospital Sokoto, patients should be well informed on the need to take their health and personal hygiene seriously, and also adhere to treatments. Based on the result obtained, it was recommended that people should avoid going barefoot in public places, and keep feet cool and dry. To educate patients on the need to improve their health and personal hygiene. Patients should endeavor to apply antifungal powder/spray to the inside of their shoes once a week or more, and they should also comply with all treatment protocol.

1. Introduction

Onychomycosis is a Greek word "*onyx"* meaning nail and "*mykes"* meaning fungus. It is traditionally defined as fungal infection of the nail (Chander, 2010). Toenail are about 25% more likely to be infected than the fingernails. Onychomycosis is a fungal infection of the nail caused by dermatophyte, yeast or non-dermatophyte molds and represent about 30% of mycoticcuteneous infections (Kaur et al., 2008). Onychomycosis though not life threatening (in immune competent individuals), can cause pain, discomfort and disfigurement. It may also produce serious physical and occupational limitations. Psychosocial and emotional effects resulting from onychomycosis are widespread and may have a significant impact on quality of life. Onychomycosis is worldwide in distribution. Prevalence rates from 2%-3% in temperate climates and 12% in tropical climates. Prevalence rate in children are 30 times less than adults, ranging from 0%-0.2% (Ngwogu and Otokunefor*,* 2007).

The most common symptoms of a fungal nail infection is the nail becoming thickened and discolored: white, black yellow or green, as the infection progresses, the nail becomes brittle with pieces breaking off or coming away from the toe or finger completely. If the left untreated the skin can become inflamed and painful underneath and around the nail, they may also be white or yellow patches on the nail bed, or scaly skin next to the nail and foul smell. There is no pain or other bodily symptoms unless the disease is severe (Karimzadegen-Nia et al., 2007). People with onychomycosis may experience significant psychological problems due to the appearance of the infection on the nail, particularly when the fingers which are always visible rather than the toenail are affected (Weinberg et al., 2003). For some patients, nail disease is a cosmetic issue rather than a medical problem and they seek advice for cosmetic reasons. However, the condition can be painful, cause a loss of dexterity and interfere with the patient's ability to stand, walk or exercise. Moreover fungal infections can cause psychosocial and emotional distress with implication on overall patients' health (Deberker, 2009).

A number of organisms are responsible for onychomycosis, including dermatophyte, non-dermatophyte molds, and candida. It is an exceptionally common problem with studies estimating its prevalence around 14% in general population (Ghannoum et al., 2000). Dermatophytes such as *Trichophyton rubrum*, *Trichophyton mentagrophyte* accounts for 80-90% of all cases. Non-dermatophyte molds that can cause onychomycosis include *Acremonium* spp. *Alternaria* spp. *Aspergillus* spp. *Fusarium* spp. *Scytalidium* spp. and *Scopulariopsis* spp. *Candida albican* accounts for approximately 70% of onychomycosis caused by yeasts (Thomas et al., 2010).

There are four classic types of onychomycosis. Distal Subungal Onychomycosis (DSO) is the most common form of tineaungium and it is usually caused by Trichophyton rubrum, it invades the nail bed and the underside of the nail plates (Westerberg and Voyack, 2013). White Superficial Onychomycosis (WSO) is caused by fungal invasion of the superficial layers of the nail plate to form "White island" on the plates of the nail. It accounts for only 10% of onychomycosis cases (A.A.P.A., 2010). Proximal Subungal Onychomycosis (PSO) is a fungal penetration of the newly formed nail plate through the proximal nail fold. It is the least commonly form of tineaungium in healthy people, but it is found commonly when the patient is immune compromised (Westerberg and Voyack, 2013). Candidal Onychomycosis is a candida special invasion of the fingernails, usually occurring in persons who frequently immerse their hands in water. This normally requires the prior drama of the nail by infection or trauma (Hall and Brain, 2012).

Aging is the most common risk factor for onychomycosis due to diminished blood circulation. Longer exposure to fungi and nails which grow more slowly and thicken Increasing susceptibility to infection. Nail fungus affect men more than women and is associated with a family history of this problem. Other risk factors include perspiring heavily, being in a humid or moist environment, psoriasis, wearing shoes and socks that hinder ventilation and do not absorb perspiration, going barefoot in damp public places such as swimming pools, gyms and shower rooms, having athlete's foot (Tinea pedis), minor skin or nail injury, damaged nail or other infection and having diabetes, circulation problems which may also lead to lower peripheral temperatures on hands and feet, or a weakened immune system (Weinberg et al., 2003).

The prevalence of onychomycosis has been reported to be as high as 23% across Europe and 20% in East Asia. In North America, the incidence of onychomycosis is up to 14%, in Nigeria most of the research work done on the superficial mycoses has been on the skin, with fungal infection responsible for 50% of nail disease around the world and with millions of dollars being spent annually on oral and topical prescriptions, laser treatments, over-the-counter products and home remedies, it is obvious that people are still bothered by their fungal toenail infections and are determined to get rid of them.

Due to the increase in the incidence of onychomycosis in the past few decades and also due to the involvement of climatic, occupation, socio-economic status, age, gender, genetic and immune factors. It is necessary to determine the fungal agents and their prevalence. The worldwide incidence of onychomycosis is increasing and it continues to spread and persist. Knowledge of the epidemiology and mycological characteristics is an important tool for control of this infection. According to WHO 4% of drugs that are prescribed to patients for various indications are not scientifically proven effective. It is especially clearly manifested in the treatment of certain infections.

The aim of this research is to provide knowledge of the incidence of mycological disease characteristics of onychomycosis involving toenails among patients attending General Hospital Ammannawa in Sokoto metropolis. The objectives of this research are:

- ✓ To isolate and identify the fungi associated with onychomycosison the toenails of patients attending General Hospital Ammannawa
- ✓ To provide a background study on the prevalence rate of onychomycosis on toenails
- ✓ To find out the association of demographic factors with the occurrences of onychomycosis

2. Materials and methods

2.1. Sample collection

A total of 30 samples were collected from patients attending Ammannawa General Hospital Sokoto using the formula:

$$n = \frac{p(100-p)}{(SE)2}$$

Scrapings were collected from patients between 19 years to 50 years of age and from both male and females. The collected samples were obtained from patients that have not been on any antifungal drugs (Alexopoulus et al., 2002).

2.2. Collecting nail scrapings

The nails of the patients were made sterile by applying spirit (70% ethyl alcohol) to them before the sample collection, to avoid bacterial contamination. The nail scrapings were collected using a new razor blade which was used to scrap the nail plate by sweeping the sharp edge of the blade back and forth, the scrapings were then put in envelopes and were transported to the laboratory (Alexopoulus et al., 2002).

2.3. Microscopy

Direct microscopic examination was carried out on the specimens by dissolving a portion of each sample in freshly prepared 20% KOH (Potassium hydroxide) for 60 minutes to be examined under high power objective for the presence of fungal elements such as hyphea, yeast cells, pseudohyphea, budding cells, spores and the blastoconidia (Alexopoulus et al., 2002).

2.4. Preparation of media

2.4.1. Suspension

The medium used in this study was Sabourad Dextrose Broth. The media was prepared in accordance to the manufacturer's instruction (Oxoid ltd Basingstoke Hants England) sixty-two (62g) gram of Sabourad dextrose broth was weighed and dissolved in 100mls of distilled water, and the broth was poured into 30 test tubes, the antibiotic streptomycin was added which was well shaded and placed into a beaker, the media was then autoclaved at 121^{o}C for 15 minutes. The sterile medium was allowed to cool down. And then the nail scrapings were then inoculated into the medium and were incubated at 25^{o}C-28^{o}C for growth for 7-14 days (Cheesbrough, 2002).

2.4.2. Sabourad dextrose agar

This preparation was also done according to the manufacturers' instruction (Oxoid Ltd Basingstoke Hants England); 62g of the powdered medium was weighed and dissolved into 100mls of distilled water, the mixture was then heated using hot plate to dissolve completely and then autoclaved at 121^{o}C for 15 minutes, the flask was then

incorporated with the antibiotic streptomycin and was allowed to cool down to 45^{o}C before it was poured into sterile Petri-dishes (Cheesbrough, 2002).

2.4.3. Inoculation

The prepared media Sabourad Dextrose Agar was allowed to solidify. A small portion of each of each of the fungal colony from the Sabourad Dextrose Broth was singly placed at the center of each of the SDA plates and was incubated at room temperature (28^{o}C±2^{o}C) for another 7-14 days (Manga and Oyeleke, 2008).

2.4.4. Sub-culturing

After the 14th day more Sabourad Dextrose Agar plates (SDA) plates were prepared and allowed to solidify. A portion of each different fungal colony from the plates that have mixed growth was singly placed in the center of SDA plates and was incubated at room temperature for another 14 days. This was done to obtain pure culture of the isolates (Cheijinna, 2006).

2.4.5. Fungal identification

The pure cultures of the isolates obtained were subjected to microscopic examination with the aim of identifying the organisms that are associated with onychomycosis. Clean grease-free glass slide was used for the identification. A drop of distilled water was placed in the center of the slide, a small portion of the fungal culture was with an inoculating needle, which was made sterile by heating till red hot and then cooled. The piece was put directly into the water and emulsified. A cover slip was then placed on the slide; it was mounted on the stage. It was viewed under low (10×) and high-power objective (40×) to observe the morphological features. The isolates were identified based on morphological characteristics in accordance with a mycological atlas (Table 4) (Alexopoulos et al., 2002).

3. Results and discussion

A total of 30 samples were tested in the microbiology laboratory, 24 samples were identified as positive by culture among which 14 (46.70%) samples were found to be KOH positive and 10 (33.30%) samples were found to be KOH negative, 6 (20.00%) samples were identified as negative by both the methods i.e both culture and KOH.

Onychomycosis affected all age groups with the highest frequency of 15 (50%) recorded for ages between 41 and 50 years. The current study also shows that both males and females have even distribution of 15 (50%) each. The predominant pathogen which was identified in this study was the yeast species which represent 37.43% of the culture positive samples and this includes yeast such as *C. tropicalis* 8.30%, *C. parapsilosis* 8.30%, *C. pseudotropicalis* 4.16% and *Torulopsisdattila* 16.67%, which constitute of the 37.43% of yeast. The yeast species was closely followed by the dermatophyte, *T. rubrum* which was present in 33.30% cases. Moulds were present in 29.12% cases which included moulds such as *Cladosporium*spp 12.50%, *Aspergillusniger* 8.30%, *A. terreus* 4.16% and *A. fumigatus* 4.16% of the culture positive cases.

The result from Table 1 obtained with direct microscopy did not correlate with the result during culture in some cases involving moulds. This is not surprising because reports have shown that KOH preparations of specimens have up to 30% false-negative rates. False-negative findings had also been observed in previous studies (Baran et al., 2001).

The result from Table 2 shows that toenail onychomycosis was found to be more common in the aged between the ages of 41 and 50 years consisting of (50%), maybe due to a low immunity, a poor peripheral circulation, a poor personal care and the presence of some systemic disease like diabetes. These findings were in accordance with those of other studies (Cursi, 2011). An even distribution rate was noticed in women and in men (50%) each of all cases, which showed that toenail onychomycosis is a disease of both men and women. This might be caused by walking barefoot in public places, or precipitation when one wears covered shoes always. The same result was obtained by other authors (Neupane et al., 2009).

Dermatophytes are the most encountered organisms in onychomycosis. They cause (90%) toenail and (50%) fingernail. The yeast represented (37.43%) of cases, which include *C. tropicalis* (8.30%), *C. pseudotropicalis* (4.16%), *C. parapsilosis* (8.30%) and *Toruplosisdattila* (16.67%), followed by the dermatophyte *T. rubrum* which was present in (33.33%) cases. Moulds were present in (29.12%) cases which included *Cladosporium* spp. (12.50%),

Aspergillusniger (8.30%), *A. terreus* (4.16%) and *A. fumigates (*4.16%) (Table 3). The same matter was reported by previous research (Kaur et al., 2008).

Table 1
Testing methods and results.

	KOH+VE		KOH-VE		Total	
Test	**No.**	**%**	**No**	**%**	**No**	**%**
Culture+VE	14.00	46.70	10.00	33.30	24.00	80.00
Culture-VE	00.00	00.00	06.00	20.00	06.00	20.00
Total	14.00	46.70	16.00	53.30	30.00	100.0

Table 2
Distribution of patients according to age group and gender.

	Male		Female		Total	
Age	**No.**	**%**	**No**	**%**	**No**	**%**
19-30	03.00	10.00	04.00	13.30	7.00	23.30
31-40	04.00	13.30	04.00	13.30	8.00	26.70
41-50	08.00	26.70	07.00	23.30	15.00	50.00
Total	15.00	50.00	15.00	49.90	30.00	100.0

Table 3
Frequency of distribution and percentage of frequency of fungus isolate.

Category	**Fungal species**	**Frequency**	**Percentage of frequency (N=24)**
Yeast	*Candida tropicalis*	2	8.30
	C pseudotropicalis	1	4.16
	C parapsilosis	2	8.30
	Torulopsidattila	4	16.67
Dermatophyte	*Trichophyton rubrum*	8	33.33
Moulds	*Cladosporium spp*	3	12.50
	Aspergillus niger	2	8.30
	Aspergillus terreus	1	4.16
	Aspergillus fumigatus	1	4.16
Total		24	100

Table 4
Macroscopy and microscopy of isolated fungal species.

Fungus	**Colonial and morphological characteristics**
Aspergillus fumigatus	Colonies on SDA at room temperature for 7 days consisted of s dense felt of dark green conidiophores' intermixed with aerial hyphea, bearing Conidiophores'. Conidial heads typically columnar conidiophores' are short smooth walled, green particularly in the upper part.
Aspergillus niger	Colonies on SDA at room temperature after 7 days consisted of a compact white or yellow basal felt with a dense layer of dark brown to black conidiophores'. Conidial heads radiated tending to split into loose columns with age conidiophores stripes are smooth-walled, hyaline but often in brow colors.
Aspergillus terreus	Colonies on SDA at room temperature for 7 days mostly consisted of dense felt of yellow-brown conidiophores, becoming darker with age, conidial head are compact and columnar, and conidiophores hyaline are smooth-walled.
Cladosporium spp	Grew in 10 days, velvety, locally powdery due to conidia, olivaceous-green to olivaceous-brown. Greenish-black conidiophores mostly arising laterally from the hyphea with terminal and intercalary swellings, and geniculate elongations, pale to mid-olivaceous brown smooth-walled conidia in long and branched chains.

Trichophyton rubrum	Grew moderately quick after 7 days, radiating mycelium with elevated center, after 2 weeks it had velvety to fluffy surface, central hat-shaped elevation was noticed, upper side was white to light yellow, fine radiating extensions, slight color diffusion hyphea ramified and septets, micro conidia was longish, smooth-walled with multiseptet blunted ends.
Candida parapsilosis	Colonies were flat, finely wrinkled irregularly fringed, cream to yellow-white, semi-glossy. Blastophores are oval to long oval shape arranged like flower rosette. Pseudomycellium present in addition to septet mycelium.
Candida pseudotropicalis	Round, smooth-walled colonies, cream-yellow. Blastophores are short and oval, Pseudomycellium are also present.
Candida tropicalis	Flat to slightly doomed, and slightly wrinkled also smooth to fringed margin. Pseudomycellium forms grey-yellow matt. Blastophores are squall oval to round, pseudomycellia are plentiful.
Toruplopsis dattila	Round smoothly demarcated colonies, cream in color. Blastophores are only elongated and oval.

4. Conclusion

This research highlighted that the yeast was a predominant pathogen in Ammannawa. This will provide useful guidelines for the appropriate management of the cases. A high frequency of toenail onychomycosis was observed among the aged, between ages 41 and 50 years. The same frequency rate of toenail onychomycosis occurred among both male and female patients, so they were advised to improve their health and personal hygiene. The study suggests that the diagnosis of nail disease can't rely only on the clinical patterns of nail changes it also requires a microbiological confirmation. Based on the result obtained, it was recommended that people should avoid going barefoot in public places, and keep feet cool and dry. To educate patients on the need to improve their health and personal hygiene. Patients should endeavor to apply antifungal powder/spray to the inside of their shoes once a week or more, and they should also comply with all treatment protocol.

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