

Isolation and Identification of Fungi from Patients Attending Amanawa General Hospital Sokoto State, Nigeria

Shinkafi, S.A,¹ Mohammed, N¹ and Zainab, I.A.²

¹Department of Biological Sciences, Federal University Gusau

²Department of Microbiology, Usmanu Danfodiyo University Sokoto, Nigeria.

Corresponding E-mail: saadatushinkafi@rocketmail.com

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Abstract

The research was conducted to investigate the mycological etiology, prevalence and distribution of fungal pathogens among patients with dermatological conditions attending Amanawa Hospital, Sokoto. A total number of thirty 30 samples were collected. Eight (8) species of fungi and one (1) genus were isolated these include; Trichophyton rubrum, Trichophyton concentricum, Trichophyton mentagrophytes, Microsporium ferrugineum, Microsporium canis, Microsporium audouinii, Aspergillus flavus, Aspergillus niger, Trichoderma and Penicillium spp. Percentage frequency of occurrence of isolated fungi from female patients indicated that Microsporium canis had the highest percentage frequency of 18.2% and the remaining species of fungi had the least percentage frequency of 9.1% each. The percentage frequency of occurrence of isolated fungi from male patients indicated that Trichophyton concentricum, M. ferrugineum, Mucor spp and Penicillium spp had the highest percentage frequency of 12.5% while Trichophyton verrucosum, Trichophyton rubrum, Trichophyton schoenleinii, Microsporium canis, Microsporium audouinii, Aspergillus niger, Candida albicans had the least percentage frequency of 6.3%. Occurrence of isolated fungi was found to be much in age group 11-13. With regard to gender, female patients are mostly affected than the male patients. There is therefore, the need for improved social, economic, health care and hygiene practice.

Key words: Identification; Fungi; patients; Amanawa; Hospital.

1.0 INTRODUCTION

Skin is the largest organ in our body and it has the largest surface area when compared with other organs of the body. It is also the most exposed organ and as such the most vulnerable. Diseases affecting human skin, hair and nail diseases are common and may be caused by bacteria, fungi or viruses (Hainer, 2003; Everitt and Kendric, 2005). Fungal diseases are emerging significant infections in the world (WHO, 2005). Most of the fungal infections are found on the skin. Some of these skin microorganisms have the ability to form spores that are so resistant to adverse conditions of dryness and temperature (Sears, 2005; Hainer, 2003). In recent years fungal infections have become an important clinical condition that deserves public health attention (Cohen and Powdery, 2004).

In recent decade, the prevalence of fungi have significantly reduced in many developed nations of the world compared to the developing ones due to improved social, economic, health care and hygiene practice factors evident in the former (Havlickova *et al.*, 2008; Ilkit, 2010). Study of fungal diseases is a field ignored in medical research and this limit the availability of documented data on the overall prevalence of fungal infections in the world (Souza *et al.*, 2008). Recent literature indicated the prevalence of dermatological conditions is as high as 30% depending on the type of fungal agent and the country (Williams, 1993; Hashemiet *al.*, 2009; Schulz and Jorgensen, 2001).

Bacterial infections are difficult to establish infection in intact skin. They become opportunistic pathogens whenever they are given the right conditions like a crack, break or wound on the skin. Fungal infections help by aiding bacterial skin flora to establish opportunistic infections. (Rajan, 2012). Some of the fungal skin infections which can predispose humans to SSTIs include eczema psoriasis and superficial fungal infections which include; *tinea pedis*, *tinea versicolor*, *white piedra* *black piedra* and onychomycosis (Templer and Brito, 2009).

Fungal skin infections are of public health concern because; they can elicit social, economic and public health problems (Maselena and Hasan, 2013). Heavily infected persons with

visible lesions are often unconscious targets of social stigma of rejection/isolation because of their unsightly appearance. The cost of long term diagnosis and treatment can be over bearing for low income individuals who make up the bulk of developing countries where these infections occur in epidemic proportions due to poor hygiene. It therefore becomes more traumatic for such individuals when diagnosis or treatment becomes delayed or ineffective. The burden is more in developing countries and also ranges from one country to another, for example the prevalence of fungi in Tunisia is 30.3%, in Brazil 38.4% and in Iran 21.1 % (Souza *et al.*, 2008; Nejiet *al.*, 2008; Hashemiet *al.*, 2009).

Complications have arisen in the diagnosis and treatment of fungal skin infection as a result of interference from opportunistic pathogens of other microorganisms which can either prolong or reduce the efficiency of diagnosis and treatment. Therefore, this has necessitated the need to identify other microorganisms associated with these fungal skin infections in order to facilitate quicker and more efficient diagnosis and treatment. The objective of this study was therefore to isolate and identify fungal agents of dermatological conditions among patients presented with suspected clinical symptoms of fungal infections in Amanawa Hospital, Sokoto.

Fungal infections has increased greatly over the last recent years (Williams, 1993), yet the prevalence and distribution of fungal disorders and the etiological agents are largely undocumented. There is only scanty information in various hospitals which is disorganized and undocumented. This study aimed at describing fungal dermatological conditions in terms of prevalence and distribution among patients attending Amanawa Hospital, Sokoto. The study also described and documented the etiological agents associated with dermatological conditions in Amanawa Hospital, Sokoto. Fungal infections for a long time have not been given much attention by medical personnel due to the assumptions that they are not serious infections (WHO, 2005). Health care providers rarely request for fungal investigations on their patients, possibly delaying effective treatment. There is also lack of proper documentation of the most common etiologies and the prevalence

within the country. This means that the populations at risk of fungal infections are

2.0 MATERIALS AND METHODS

2.1 Sample Collection

A total number of thirty (30) samples (skin scrapping and nails) were collected from patients attending Amanawa General Hospital, Sokoto. The samples from the skin were obtained from patients with signs of erythema, alopecia, scaling, crusting, ciacinate lesions or follicular inflammations and pruritus. Infected tissues were first cleaned with 70% ethanol and then collected using sterile razor blades and epilator forceps respectively into folded aseptic papers. Each of these papers were appropriately labeled with the patients names, date of collection and site of infection after which the sample were taken to the Mycology laboratory of Usman Danfodiyo University, Sokoto for analysis.

2.2 Collection of Nail Scrapings

The clinical appearance and location of onychomycosis (toenail and/or fingernail) were recorded. 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 specimens were obtained from clinically abnormal nails by a vigorous scrapping of the nail bed, underside of the nail plate and the hyponychium using a new razor blade. Nail scrapings and/or clippings were collected in sterile Petri dishes and a portion of specimens was dissolved in 20.0% potassium hydroxide (KOH) overnight and subjected to direct microscopy for identification according to previously reported procedure of (Alexopoulos *et al.*, 2002).

2.2 Microscopic Examination

Slides were prepared using the procedure of (Havold, 1999).

A drop of fungal stain was placed on clean glass slide with the use of a mounting needle and was carefully teased (spread). A drop of lacto phenol cotton blue was placed on the clean grease free glass slide, and a tuft of fungal filament was picked up from the culture plate using teasing

3.0 RESULTS

The results of isolation and identification of fungi carried out revealed the presence of eight (8) species of fungi and one (1) genus. These

unknowingly increasing.

needle. Then the filaments were transferred to the lacto phenol stain and gently teased and a clean cover glass was carefully placed over the preparation without any air bubbles. This preparation was then examined under high power objective. The spore structure and their arrangement, hypae were observed.

2.4 Inoculation and Isolation of Fungi

0.1ml each from 10^{-4} and 10^{-5} dilution were inoculated in Sabouraud Dextrose Agar plates and swabbed to ensure even distribution of inoculum in the medium. 0.5ml each was taken directly from the stock suspension and inoculated in SDA agar plates and swabbed as above. All plates were labeled and incubated at room temperature for 3 weeks. After incubation, the fungal colonies that developed were counted (Oyeleke and Manga, 2008).

2.5 Sub-Culture

After 3 weeks, 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)

2.6 Identification of Fungi

The samples were cultured on SDA for 3-4 weeks after serial dilution after which the isolates were identified using slide culture technique (Oyeleke and Manga, 2008). The cultural and morphology characteristics for 1-3 weeks cultures were observed. A small portion of the fungal growth from the representative culture was picked using a sterile inoculating needle and placed on a sterile glass slide containing a drop of normal saline. Smears were made and covered with a cover slip and viewed under the light microscope first with (x10) and then with (x40) objective lens to detect spores, hyphae, and other special structure.

include; *Trichophyton rubrum*, *Trichophyton concentricum*, *Trichophyton mentagrophytes*, *Microsporium ferrugineum*, *Microsporium canis*, *Microsporium audouinii*, *Aspergillus flavus*,

Aspergillusniger, *Trichoderma* and *Penicillium*spp as the genus. The results obtained for the percentage frequency of occurrences of isolated fungi based on the age groups of the patients attending the clinic revealed that *Trichophyton rubrum* was found to be positive in all the age groups with the highest percentage frequency of occurrence of 26.7%, followed by *Microsporumcanis* with percentage frequency of occurrence of 20%, then fungi with the least frequency of occurrence was *Penicillium*spp with 6.7%. However, from the result obtained for percentage frequency of occurrence of isolated fungi from female patients (Figure 1) indicated that *Microsporumcanis* had the highest percentage frequency of 18.2% and *Trichophyton rubrum*,

Trichophyton concentricum, *Trichophyton mentagrophytes*, *Microsporumferrgineum*, *Microsporumaudounii*, *Aspergillusflavus*, *Aspergillusniger*, *Penicillium*spp and *Trichoderma* had the least percentage frequency of 9.1% respectively. The result obtained for percentage frequency of occurrence of isolated fungi from male patients indicated in figure 2 showed that *Trichophyton concentricum*, *M. ferrgineum*, *Mucorspp* and *Penicillium*spp had the highest percentage frequency of 12.5% while *Trichophyton verrucosum*, *Trichophyton rubrum*, *Trichophyton schoenlenii*, *Microsporumcanis*, *Microsporumaudounii*, *Aspergillusniger*, *Candida albicans* had the least percentage frequency of 6.3%, respectively.

Table 1. Percentage frequency of occurrence of isolated fungi based on the age groups of the patients.

Organisms	Age Groups (Years)				Frequency	% Age
	5-6	7-8	9-10	11-13		
<i>Trichophyton rubrum</i>	+	+	+	+	8	26.7
<i>Microsporoncanis</i>	+	+	+	+	6	20
<i>Aspergillus niger</i>	-	+	-	+	4	13.3
<i>Candida albicans</i>	-	-	-	+	3	10
<i>Mucor racemosus</i>	-	-	+	+	4	13.3
<i>Penicillium Spp</i>	-	-	-	+	2	6.7
<i>Trichophyton mentagrophyte</i>	-	-	-	+	3	10
Total					27	100

Key: + = Positive
 - = Negative

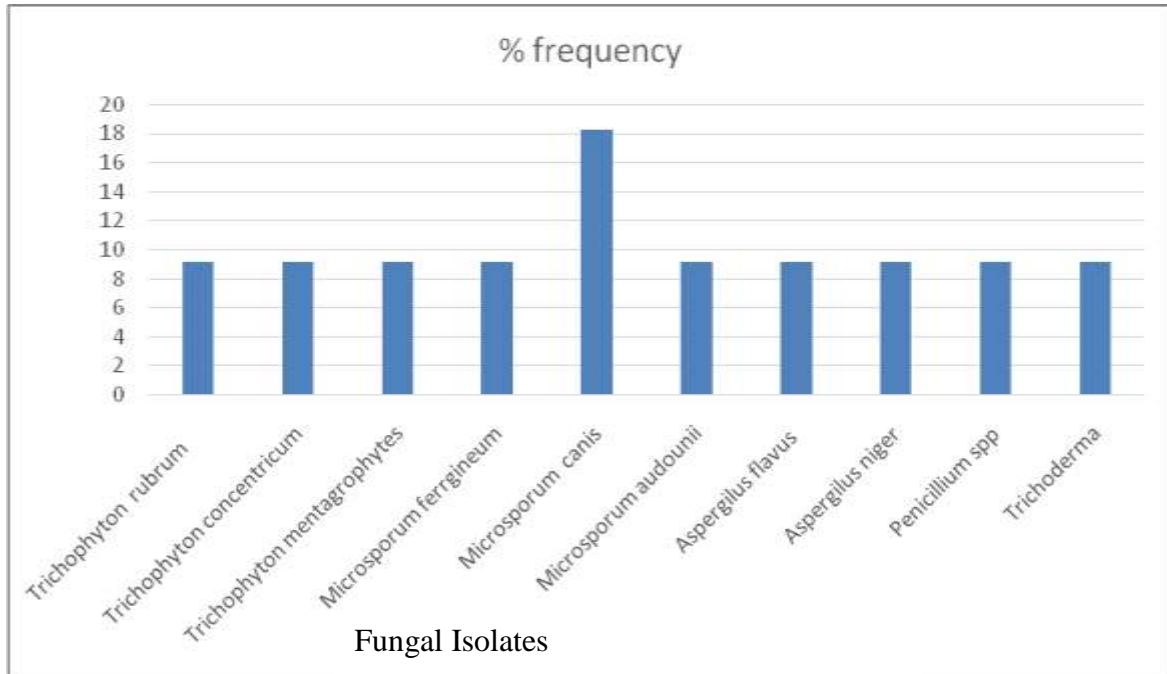


Figure 1. Percentage frequency of occurrence of fungi isolated from female patients

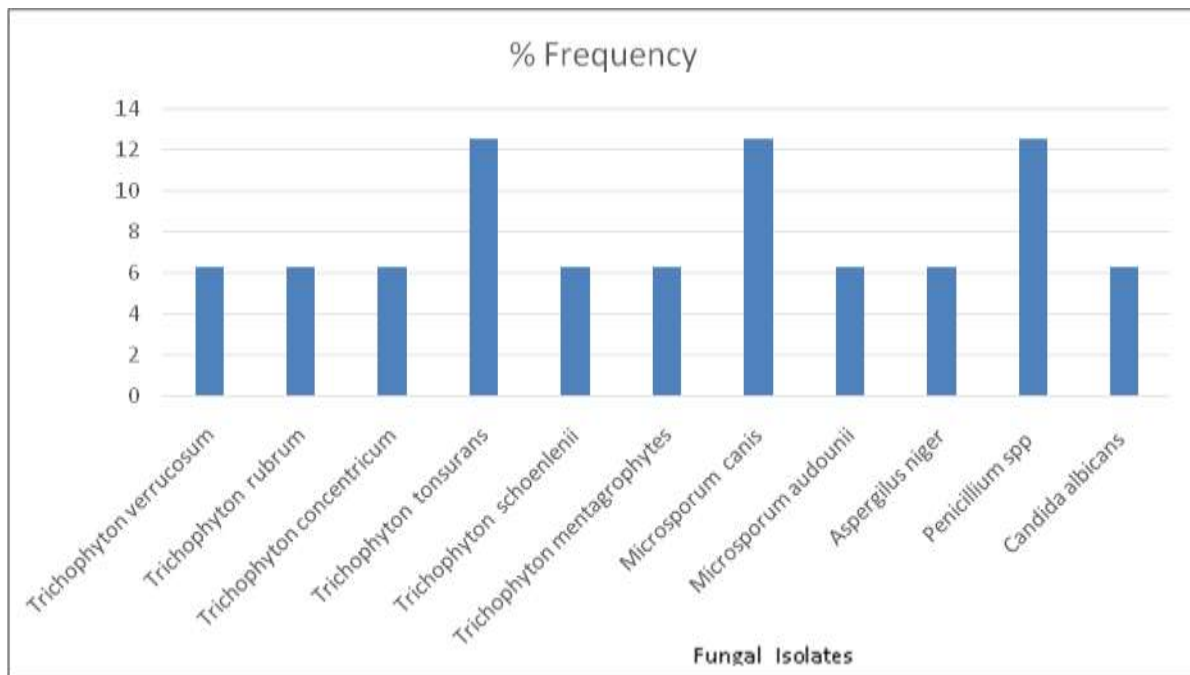


Figure 2. Percentage frequency of occurrence of fungi isolated from male patients.

4.0 DISCUSSION

In many clinical and epidemiological studies, fungal infections of the skin and scalp represent a relatively common problem especially in the tropical and subtropical regions of the world where warm and humid climate provides a favorable environment for fungi. They have become a significant health problem affecting children, adolescents and adults. These factors influence the demographic pattern of the etiology and epidemiology of fungi while also underpinning the need for their strategic management (Sahinet *al.* 2004). This study was carried out in Amanawa General Hospital, Sokoto; prevalence of fungi infections was recorded. This is however higher compared to the frequency reported in Nigeria and other parts of Africa which ranged from 5 – 20% (Enweaniet *al.*, 1996; Nweze, 2001; Ogunbiyiet *al.*, 2005; Ngwogu and Otokunefor, 2007; Adefemiet *al.*, 2011). The reason for this may be linked to several factors such as the host 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 and nature of health care system that facilitate the transmission of fungal infections (Efuntoye and Fashanu, 2002; Khosravi and Mahmoudi, 2003; Nweze, 2010b). This observation therefore debauches earlier studies linking high fungalinfection prevalence to rural and suburban communities (Ngwogu and Otokunefor, 2007; Nweze, 2010a, 2010b). The scarcity of adequate comparative data equally confounds this line of thought. The failure of 9% fungi show visible sign of growth in culture among the sampled population may be due to reasons ranging from poor inocula viability to weak nutrient utilization and competitive growth capacities (Nweze and Okafor, 2005).

The incidence of fungi among the sampled population of patients was insignificant compared to microscopic and culture results from those within ages 11 to 13. Dermatophytosis is also low (7.7%; $n = 91$)

within this age group largely as a result of close parental care and guardianship to which these patients are subjected in patients. This can be attributed to transitional puberty and concurred with previous works within and outside Nigeria (Ngwogu and Otokunefor, 2007; Nweze, 2010). *M. ferrugineum* (9.1%), *M. canis* (18.2%), *M. audouinii* (9.1%) and *T. concentricum* (9.1%) constituted the predominant representative fungi species recorded during the study. It is imperative to emphasize that *M. ferrugineum* and *T. concentricum* were scarcely reported in many related studies from other parts of Nigeria despite being implicated a spectrum of fungalinfections including *tinea imbricata*, *faciei*, *corporis* and *capitis* (Ajello, 1974). This present study may have been the first time account of these fungi among patients in the northwestern part of Nigeria despite also recording dermatophytes of anthropilic, geophilic and zoophilic origins. Unlike the *M. ferrugineum* with a wider range of geographic distribution, *T. concentricum* was reported to be limited in distribution to south-east Asia and Central America by Weitzman and Summerbell (1995) and have been recently introduced to the metropolis by human and/or animal immigrants. The positive absence of *Epidermatophyton* species, especially *E. floccosum* reported by Nweze (2010a) and Adeleke *et al.* (2008) on related studies in Northern Nigeria was not yet understood. It might however be attributed to unfavorable growth conditions which repressed arthroconidia germination or hyphal growth. Furthermore, all the etiological agents that were positively identified from the study have been reported in Nigeria and other parts of the world (Barbić-Erceg *et al.*, 2004; Sahinet *al.*, 2004; Nweze and Okafor, 2005; Popoola *et al.*, 2006; Mbata and Nwajagu 2007). High frequencies of *M. canis*, *M. tonsurans* and *Penicillium* (Fig. 2) concurred with Popoola *et al.* (2006) and may be attributed to the constant exposure of patients sampled. *A. flavus* (9.1%), *A. niger* (9.1%), *Penicillium*spp. 9.1% (fig. 1) and *Candida albicans* 6.3% (fig. 1) were the most predominant fungi identified by microscopy and culture. This result concord with the reports of Rahbar *et al.* (2010) and Mbata and Nwajagu

(2007) (yeast and conidial forms) synergize with fungi in a yet undefined phenomenal interaction to cause symptomatic superficial fungal infections. Ample literature exists on the zoophilic, geophilic and anthropophilic sources of fungal infections but little is presently known on the spectrum of zoonotic fungi in Nigeria to illicit the best sustainably affordable epidemiological control strategy. This observation was corroborated by Nweze (2011).

The confirmation of fungal isolates from the sampled population suggests a relationship that requires further investigation and whose complete understanding has a strong implication for the control of fungi in patients from Amanawa General Hospital, Sokoto. This study also recorded high prevalence of *M. ferrugineum* and *T. concentricum* which are uncommon fungi in Nigeria. Furthermore, many patients depending on their conduciveness i.e. state of infrastructure, facilities and location are potential hot spots for the spread of fungi and their reinfections. This study incidentally provided new insight into some epidemiological facilitator of dermatophytosis and transmission portals while also contributing to existing

5.0 CONCLUSION

From the results obtained in this research it is concluded that the occurrence of isolated fungi was found to be much in age group 11-13. With regard to gender, female are mostly affected than the male patients.

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knowledge useful for the efficient screening, management, reduction and treatment of the fungi.

In conclusion, the finding of this study suggests that fungi are significant cause of morbidity in Amanawa General Hospital affecting all ages and gender. The isolation rate of 84.1% compared well with those observed in other developed and developing countries. *Trichophyton spp* is the major cause of fungal dermatological conditions in Amanawa General Hospital. This is in agreement with other findings in work done in other countries. There was no significant association in isolation between different ages, gender and occupation, however the rate of isolation suggest that fungal skin infection are a major infection and need to be given more attention. The results in the wake of HIV/AIDS meaning that they can no longer be assumed as minor infections and there should be allocation of resources to control them. I do reject the null hypothesis and accept the alternate hypothesis that fungi are a significant cause of fungal dermatological infections in patients visiting Amanawa General Hospital Sokoto.

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