

## Isolation and Identification of fungi from suspected fungal skin infections in patients attending the Dermatology Clinic at University Teaching Hospital

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### Abstract Objective

The purpose of this study was to determine the fungal species causing skin infections among patients attending the dermatology clinic -UTH in Lusaka, Zambia.

### Methodology

A Cross-section and descriptive case study conducted to quantifying the distribution of certain variables among patients with tinea infection which include clinical presentation, age, gender and fungal species

### Results

*Aspergillus niger* was the most common fungal species isolated accounting for 42%, followed by *Cladosporium species* 10%, *Trichoderma species*, *Acremonium species* and *Aspergillus flavus* accounted for 5% each while *Scopularopsis species*, *Sporothrix schonkii* and *Aspergillus fumigatus* accounted for about 2% each.

Most of the fungal skin infections were found to be from the trunk. Age group of 21 to 40 years was the more affected by the fungal. Female patients were more affected in the study

### Conclusion

Non Dermatophte Fungal agents have shown to be the major cause of superficial infections in patients attending skin clinic at UTH. These include *Aspegillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Cladosporum species*, *Sporothrix schonkii*, *Scopularopsis species*, *Trichoderma species* and *Acremonium species*

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## I. INTRODUCTION

There are different types of fungi responsible for a variety of skin fungal diseases that can affect glabrous skin, nails and hair. These fungi can collectively be known as keratinolytic fungi (2). The common keratinolytic fungi that cause skin infections are the dermatophytes which can be classified according to the genera *Trichophyton*, *Microsporum* or *Epidemophyton* and by primary habitant which include geophilic, anthropophilic and zoophilic (4,1). Other causes of skin infection include yeasts, candida and non-dermatophyte fungi (25). Keratinolytic fungi routinely affect individuals who are otherwise healthy, but people with compromised immune systems are particularly susceptible (1,20). The fungal species causing skin infection and the subsequent clinical presentation varies from one geographical area to another (4; 14) and within the same geographical area the species causing infections may frequently shift to another (15,13). The estimated lifetime risk of acquiring a skin infection is between 10 to 20% (19). Prevalence of infections is influenced by factors such as the social-demographic background such as sex and age (25), cultural practices, public practices and climate of a particular region (1,28). The knowledge of social-demographic background of individuals infected with skin infections has not been the focus of most sub-Sahara disease control programs impacting negatively to the well being of the individuals at risk (8). Besides, most African countries have either scanty knowledge or have not identified the fungal species causing skin infections in their communities which is an essential component in management of this condition (3, 5) as well as in selection of therapy as treatment options may be altered based on the causative agent identified (8). The purpose of this study was to determine the fungal species causing skin infections among patients attending the dermatology clinic -UTH in Lusaka, Zambia..

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## II. RESEARCH METHODS

The research method in this study was related to the purpose of the study. This section has aimed at providing detailed information regarding study design, study site, sample size, study population, details of methods used to obtain results and framework for data analysis.

### 2.1 Study design

A Cross-section and descriptive case study conducted to quantifying the distribution of certain variables among patients with tinea infection which include clinical presentation, age, gender and fungal species at one Hospital (28, 8).

### 2.2 Measurement Variables

| Variable              | Measurement |
|-----------------------|-------------|
| Clinical presentation | Categorical |
| Age groups            | Categorical |
| Gender                | Nominal     |
| Species               | Categorical |

### 2.3 Study site

The study was conducted in the dermatology clinic at The University Teaching Hospital in Zambia from June 2012 to may 2013.

### 2.4 Study population

All the patients that clinically present with skin infection and were sent to the clinic-3 microbiology laboratory

### 3.5 Sample size

The sample size was 83, as estimated using the formula of the sample size calculation. A hypothetical prevalence of 50% was taken owing to lack of current data on prevalence of the skin infection at the UTH dermatology clinic. The standard error was calculated using:

$$e = \frac{\sqrt{P(Q-P)}}{n} = \frac{\sqrt{50(100-50)}}{50} = 7.03$$

The sample size was therefore calculated as

$$n = \frac{P(Q-P)Z^2}{e^2} = \frac{50(100-50)(1.28)^2}{7.03^2} = 83 \text{ samples}$$

Where n= sample size, P = prevalence, Q = 100, Z = Confidence interval at 90% and e = standard error.

### 2.6 Method of determination of fungal species causing skin infection using culture media .

Skin scraping was collected from patients who attended the dermatology clinic at the University Teaching Hospital. The samples were collected after the patients were assessed by a clinician and suspected that the lesions could be due to fungal infection. Samples were collected using a sterile scapel blade and the skin scraping were collected onto prepared sabouraud media in duplicate. One culture media was incubated at 37°C while the other was incubated at room temperature (25°C). Incubated plates were inspected for fungal growth on a daily basis. Fungal isolate that existed as mixed growth were subcultured each on a different plates so as to obtain pure growth. Having obtained pure growth (which took about 3 to 7 days). The macroscopic features on the forward as well as the reverse side of the plate were observed under a Biosafety Cabinet Class-II. Thereafter a drop of lacto phenol cotton blue was put on a clean slide and using a piece of cello tape, the fungi was gently touched and fixed on a slide containing lacto phenol blue. The slide were viewed under low power (10x objective) and then under medium or 40x objective lens. The microscopic appearance was recorded by drawing. Using an atlas (odds R.J, 1983, Ernest et al, 1980). the fungal agent was identified by matching the recorded macroscopic and microscopic appearances.

### 2.7 Identification of clinical presentation of fungal skin infection in patients

The participant's information regarding clinical presentation was recorded in a research book as it appeared on the laboratory forms.

### 2.8 Identification of gender and sex mostly affected with skin infections

The information regarding sex and gender was obtained from the laboratory forms of the participants

### 2.9 Statistical analysis

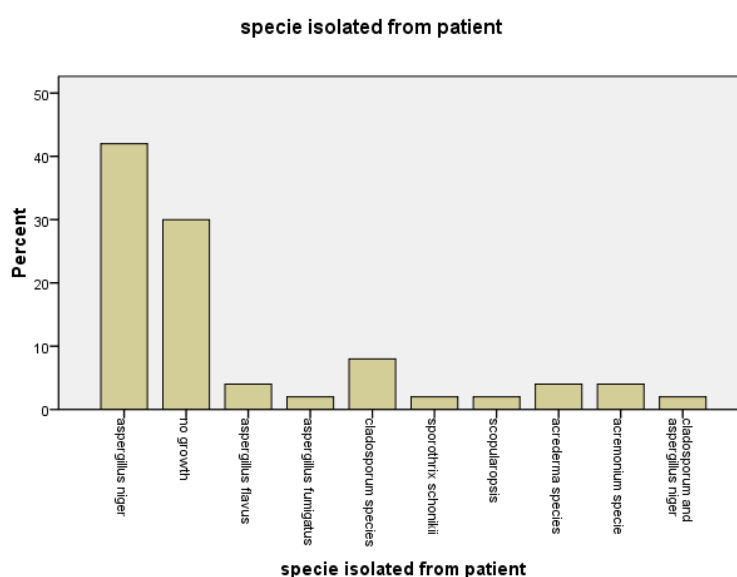
| Variable              | Statistical measurement                |
|-----------------------|--|
| Clinical presentation | Frequency                              |
| Gender                | Frequency and sex Ratio (Male/female). |

|                       |                  |
|-----------------------|------------------|
| Age groups            | Frequency        |
| Species               | Frequency        |
| Species and age group | Cross Tabulation |
| Species and gender    | Cross Tabulation |

To find out the significance of the differences in rates of skin infection among gender and age groups, a chi squared test with P-values of  $P \leq 0.05$  being significant (20). A Statistical Package for Social Sciences (SPSS) will be used to analyze the frequency of clinical presentations, frequency of the infection in various age groups and the species that will be isolated on culture media. The results will be presented in dummy tables, frequency tables and bar charts.

### III. 3. RESULTS

A total of fifty (50) skin scrapings were collected from patients with suspected fungal skin infection who attended the dermatology clinic at UTH between December 2012 and March 2013. Fungal species isolated and their frequencies are shown in figure 1 below:



**Figure 1.** Species isolated from the patient

*Aspergillus niger* was the most common fungal species isolated accounting for 42%, followed by *Cladosporium species* 10%, *Trichoderma species*, *Acromonium species* and *Aspergillus flavus* accounted for 5% each while *Scopularopsis species*, *Sporothrix schonkii* and *Aspergillus fumigatus* accounted for about 2% each. These fungi were isolated from different areas of the body. Samples were collected from the head, face, feet, nails and the whole body. The fungal agents isolated from these different areas of the body are shown in Table 1.

**Table 2 Different Fungi Isolated from different areas of the Body and their Frequencies**

| Fungus                       | Number of isolates |          |            |          |             |
|------------------------------|--------------------|----------|------------|----------|-------------|
|                              | Head               | Face     | Whole body | Feet     | Nail / Hand |
| <i>Cladosporium species</i>  | 1                  | 0        | 3          | 1        | 0           |
| <i>Scopularopsis species</i> | 0                  | 0        | 1          | 0        | 0           |
| <i>Trichoderma species</i>   | 0                  | 0        | 2          | 0        | 0           |
| <i>Sporothrix schenkii</i>   | 0                  | 0        | 1          | 0        | 0           |
| <i>Acromonium species</i>    | 0                  | 0        | 2          | 0        | 0           |
| <i>Aspergillus flavus</i>    | 2                  | 0        |            | 0        | 0           |
| <i>Aspergillus fumigatus</i> | 0                  | 0        | 1          | 0        | 0           |
| <i>Aspergillus niger</i>     | 3                  | 5        | 10         | 1        | 2           |
| <b>Total</b>                 | <b>6</b>           | <b>5</b> | <b>20</b>  | <b>2</b> | <b>2</b>    |

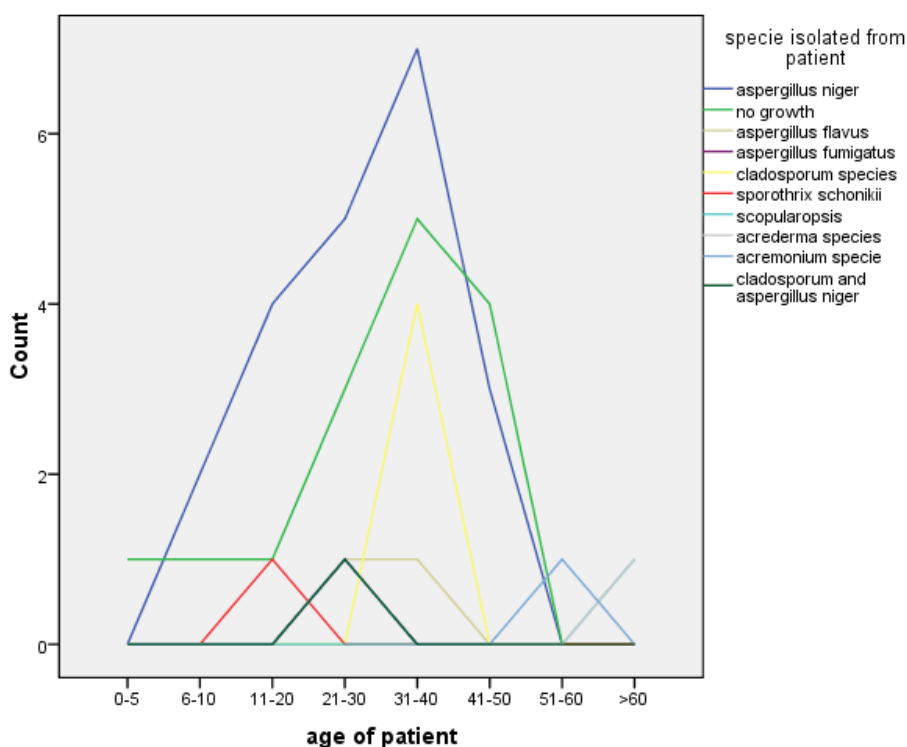
Most of fungal species were isolated from trunk (56%). Most of the fungal species isolated in this study were isolated from the trunk (generalized body infection).

Patients from different age groups were seen at the dermatology clinic. Fungi isolated from various age groups are shown in the table below.

**Table 2 Frequencies of Fungi Isolated from different Age Groups.**

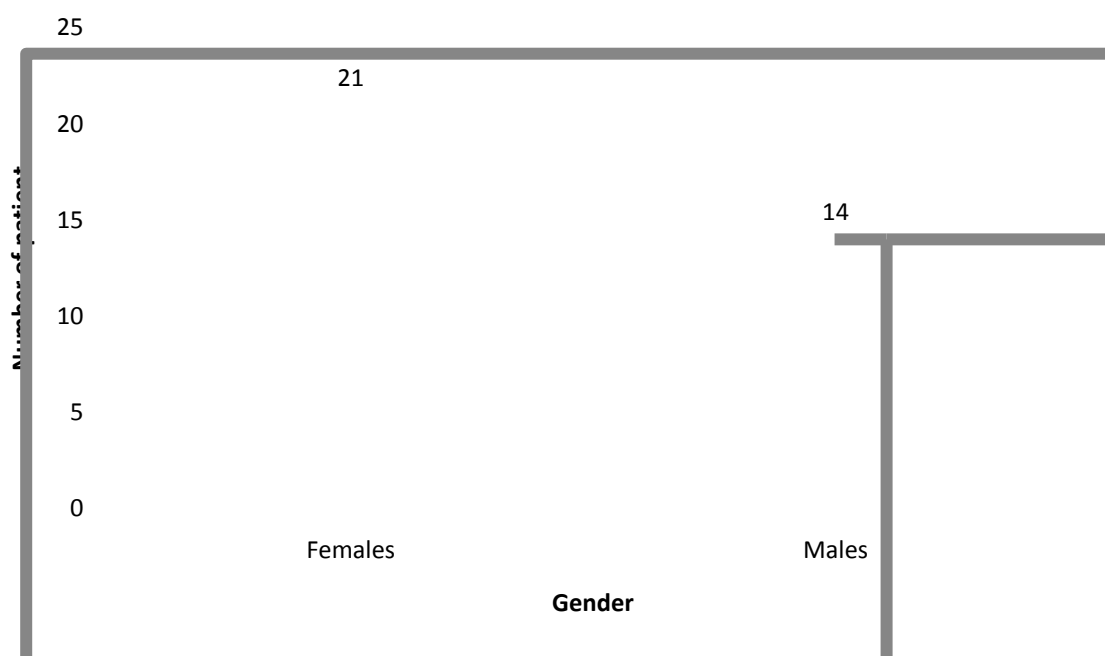
|       |       | Frequency | Percent | Valid Percent | Cumulative Percent |
|-------|-------|-----------|---------|---------------|--------------------|
| Valid | 0-5   | 1         | 2.0     | 2.0           | 2.0                |
|       | 6-10  | 3         | 6.0     | 6.0           | 8.0                |
|       | 11-20 | 6         | 12.0    | 12.0          | 20.0               |
|       | 21-30 | 13        | 26.0    | 26.0          | 46.0               |
|       | 31-40 | 17        | 34.0    | 34.0          | 80.0               |
|       | 41-50 | 7         | 14.0    | 14.0          | 94.0               |
|       | 51-60 | 1         | 2.0     | 2.0           | 96.0               |
|       | >60   | 2         | 4.0     | 4.0           | 100.0              |
|       | Total | 50        | 100.0   | 100.0         |                    |

Most of the fungi were isolated from middle age of 21 to 30 and 31 to 40 years and all the fungal species identified in this study were represented in this age groups as shown below;



**Figure 2.** Fungal species isolated from different age groups.

The study included males and females and the rates of infection is illustrated in the bar graph below.



**Figure 3.** Graph of number of patients fungi isolated against gender  
The graphs indicate that there were more female from which fungi was isolated than males.

### DISCUSSION

This section discusses the current results in this research and it is discussed in relation to the problem that has been investigated and in light of other published results. It consists of subsections focusing on Clinical presentation of patients with skin infections, gender and age is more affected with fungal skin infections, Fungal species causing skin infections and a recommendation regarding superficial infections at the University Teaching Hospital.

#### Fungal species causing skin infections

From the specimen received, the fungal agents isolated from patients with skin infections included *Aspergillus niger* isolated in 42% ,*Cladosporum species* 10%, *Aspegillus fumigatus* 2%, *Aspegillus flavus* 5%, *Acremonium species* 5%, *Sporothrix schonikii* 2%, *Scopulariopsis* 2% and *Trichoderma species* were isolated in 5 % of the cases. The findings in this study do not agree with previous researchers who concluded that the leading cause of skin infections were dermatophytes, Candida and yeast with non-dermatophyte molds accounting for the least of superficial infections (2 ,25, 23 ). This study reviews that at The University Teaching Hospital, the leading cause of skin infections are the non-dermatophyte molds. This can be attributed to the fact that the prevalent fungal agents causing skin infection varies due to climate of the geographical region and may shift to another due to social demographic factors such as migration and health condition of individuals. (30). Besides that, the increase in the immune suppressive conditions such as AIDS and the use of chemotherapies may account for the isolation of the NDM as the most common cause of skin infections.

*Aspergillus* species which accounted for most of fungal agents isolated can be attributed to the ubiquitous distribution of the fungi (27). Besides that, studies have reviewed that *Aspegillus niger*, *Aspegillus flavus* and *Aspergillus fumigatus* are known to cause superficial infection in immunocompromise individuals (1, 20). The *Scopulariopsis* and *sporothrix schonikii* are reported to cause superficial infection that are resistant to broad spectrum antifungal agents (17). This would be one of the reasons behind the cases of fungal skin infections reported be resistant to treatment. *Acremonium species*, *Cladosporum species* and *Trichoderma species* isolated were previously regarded as environmental contaminants. These fungal agents have recently been included among the emerging fungal pathogens and have been reported to cause superficial infections of the skin and nails in healthy and immune compromised individuals (7), hence their isolation as fungal species causing skin infections at the Dermatology clinic. There was no *Candida* species as well as *Dermatophytes* isolated in this study.

### **Clinical presentation of patients with skin infections**

According to the rate of skin infections on an anatomic site, most of the fungal skin infections were found to be from the trunk (generalized body). These findings are in line with Mohammed (14) and Das (10) who reviewed that individuals with superficial infections predominantly presented with generalized skin infections clinically. This is because there are multiple etiologic agent capable of causing fungal infection of the whole body. As shown in table 1.

### **Gender and age is more affected with fungal skin infections**

Regarding the age more affected with superficial infections among patients from whom fungal agents were isolated, individuals who were in the age group of 21 to 40 years accounted for most of fungal agents isolated. The frequency of the skin infections decreased with either the decrease or increase in age of patients, which agrees to the research findings of Nasreen (25). Among the 35 fungal agents isolated in the research, most isolates were from female patients in the study. This data suggested that females are more affected with superficial infections than males. Scientific literature citing the reasons justifying the findings are unclear however, the findings can be attributed to females being more conscious with their skin health hence seek prompt medical attention. Not only that, females are more in the habits of applying bleaching and other corticosteroids containing lotions which weakens the skin layer predisposing themselves to superficial fungal infections. Diagnosis of superficial fungal infections at the University Teaching Hospital is currently being done using microscopy alone, this is indeed an economical and efficient way which enables patient to get results within a short time. However, considering the findings in this research such as the fungal agents isolated, would recommend that Clinic-3 works in conjunction with Microbiology laboratory so that mycological analysis involve Identification of fungal agents to the species level. This may be useful in initiating an appropriate treatment among patients bearing in mind the emergence of drug resistance. Susceptibility testing of fungal agents would be also be appropriate at the referral hospital as it would assist in monitoring treatment of patients and would facilitate early detection of resistant fungal agents.

### **CONCLUSION**

Non Dermatophyte Fungal agents have shown to be the major cause of superficial infections in patients attending skin clinic at UTH. These include *Aspegillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Cladosporum species*, *Sporothrix schonkii*, *Scopularopsis species*, *Trichoderma species* and *Acremonium species*. The fungal isolated are affecting the females more than males and the frequency of the skin infections decreased with either the decrease or increase in age of patients. With the emergence of previously avirulent species to virulent fungi, mycological analysis which include identifications of the specific fungal agents causing a superficial infection cannot be over-emphasized. We recommend the routine use of mycology analysis involving the identification of species and susceptibility testing to monitor treatment of patients and facilitate early detection of resistant fungal agents.

### **FUTURE STUDIES**

Having identified the age group which is mostly affected with superficial infection, further study would involve identifications of the risk factors to acquiring superficial fungal infection and how they are related to different age groups. This includes the evaluation of whether the fungal skin infection are due to immunocompromised state of the patients. The future studies to include patients in other location such as rural areas so as to capture a variety of social- demographic variables which will strengthen the analysis.

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