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Prevalence of Onychomycosis among the Students of Joseph Sarwuan Tarka University, Makurdi

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Abstract
The increased cases of onychomycosis call for global health concern. Nail diseases should raise attention and receive proper care from both physicians and other health care providers. This study investigated the prevalence of fungi associated with nail infections among students of Joseph Sarwuan Tarka University, Makurdi, Benue State. A total of 300 samples were tested in the Microbiology laboratory, Joseph Sarwuan Tarka University, Makurdi using 20% KOH and culture plates of Sabouraud Dextrose Agar (SDA) which was mixed with streptomycin as an antibiotic. Results obtained, showed that 53.34% of the total sample were KOH positive and 26.66% were KOH negative. While 20.0% were negative for both culture and KOH. The predominant pathogen were dermatophytes (41.66%), followed by yeast (33.32%) and the moulds with (24.99%). Onychomycosis was observed to be common between the age of 24-28 with the occurrence of 60%, and 53.33% in female students while 46.67% were recorded for male students. Based on the result obtained, it was recommended that people should avoid going barefooted in public places, keep feet cool and dry. Students should avoid wearing the same closed footwear day by day, they should rotate footwear and they should also comply with all the preventive and treatment protocol

Keywords: Onychomycosis, Dermatophytes, KOH , Sabouraud Dextrose Agar, Nail,

Introduction

Onychomycosis is a fungal infection of the nail, which occurs worldwide with Dermatophytes as the most common causal agents although yeast and mould are also involved [1, 7].

The finger nails and toe nails are important organs of our body, serving as protection for the tips of fingers and toes. Fingernails also enhance fine touching and tactile sensivity, as well as aid in picking up of small objects [1, 5]. The fingernails are a part of the human body attached to the hand which are in most contact with the outside world. People use their hands for variety of activities everyday [7,9,10].

Onychomycosis is traditionally defined as a fungal infection of the nail [6, 12, 17]. Fungal nail infections are most commonly caused by anthropophilic fungi called dermatophytes. The genera, *Trichophyton, Epidermophyton rubrum, T. menagrophytes var interdigitale, and Epidermophyton floccosum* [2, 6, 13, 18]. Other fungi, moulds or yeast may be isolated, such as *Scopuariopsis brevicaulis, Aspergillus, Fusarium, Candida albicans. T. rubrum* is now regarded as the most common cause of onychomycosis worldwide [11, 19, 21,]. For some patients, nail disease is a cosmetic issue rather than medical problem and they seek advice for cosmetic reasons. However, it can cause pain, social, emotional and occupational discomfort, permanent damage to nail for patients and spread of the infection to other persons [11, 22, 24, 36]. It may need a long term treatment. Onychomycosis cover 50% of the nail infection [21, 26, 31] and [27, 38]. Onychomycosis is classified into several types like Distal subungal onychomycosis (DSO), white superficial onychomycosis (WSO), Proximal subungal onychomycosis (PSO), and Total Dystrophic onychomycosis (TDO), [37]. There is a long list of fungi which have a tendency to damage the nail, like dermatophytes (50%), Yeast(27%) and molds(23%), [9, 32, 35]. However, all the nail diseases are not fungal in origin, they are also caused by other clinical conditions [37, 41] like trauma, wet work (with the hand submerged in water), [23], HIV-AIDS, immunodeficiency which is due to organ or bone marrow transplantation, old age, psoriasis, atopic dermatitis [30, 40], diabetes with a predominance of *Candida* spp, renal transplant recepients [33, 34].

The *Dermatophytes spp*, are mostly distributed in the temperate western countries. The *candida spp,* (yeast) are mostly distributed in the tropical and subtropical countries and in persons whose hands are often submerged in water [25]. The common fungi from dermatophytes category are *Trichophyton rubrum, T. menagrophytes var interdigitale, and Epidermophyton floccosum* and the yeasts include *Candida albicans, Candida parapsilosis, C. tropicalis*, etc, while the molds include a wide range of fungi like *Scytalidium spp, Aspergillus spp, Geotrichum candidum and Fusarium spp. Scytalidium* is present in tropical regions. *Scytalidium* is a saprophytic fungus which is present in water, soil, plants and some decaying material and it is transmitted by direct contact [8]. Dermatophytes and

yeasts are present mainly in the middle age. The molds mainly affects older persons who are over 60 years of age. The dermatophytes are common in men in foot nail onhchomycosis because of the constant wearing of shoes, perspiration and exercise. The yeasts are common in women in fingernail onychomycosis because their hands are often submerged in water.

There is a problem of increased cases of onychomycosis in the past few decades and also due to the involvement of climate, occupation, socio-economic status, gender, age and genetic and immune factors, it is necessary to determine the fungal agents and their prevalence.

It is doubtful if any recent studies have been carried out in Joseph Sarwuan Tarka University, Makurdi, Benue State to provide adequate information to help identify different fungi in nails of students of the institution. Hence the need to investigate the occurrence of onychomycosis among students in the University, with the view to suggest control for the infection and to advice on ways to prevent the disease.

Materials and Methods

Study area

This research was carried out in Joseph Sarwauan Tarka University, Makurdi.

Benue State located on latitude $7^0$34N and longitude $8^0$34E.

Ethical approval

The Ethical approval for this study was obtained from the Ethical Committee on Research of Infectious Diseases of Federal Medical Center, Makurdi, Benue State. Consent was also obtained from the students that presented themselves for sample collection. The approval was on the agreement that participants' anonymity will be maintained, good laboratory practice/quality / control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only. However, students that desire to know the results, would be given (verbally) free of charge.

Sample collection

Samples used in this study were collected from students of Joseph Sarwauan Tarka University, Makurdi. Study population include the male and female students of the institution. A total of 300 samples will be collected from the students of the University using the formula :

$n = p(100 - p)/(SE)^2$ (1)

Scrapings were collected from the students between 17 years to 50 years of age. The collected samples were obtained from patience that have not been expose to any antifungal drugs [14].

Collection of nail scrapings

The nails of the students were made sterile by applying spirit (70% ethyl alcohol) to them before the sample collection, to avoid bacterial contamination [8, 16]. The scrapings were collected using a sterile 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 sterile envelops.

Microscopy

Direct microscopic examination was carried out on the specimen by dissolving a portion of each sample in freshly prepared 20% Potassium hydroxide (KOH) for 60 minutes and examined under high power objective for the presence of fungal elements such as hyphae, yeast cells, pseudo-hyphae, budding cells, spores and the blast conidia [3].

Preparation of media suspension

The medium used in this study was Sabouraud Dextrose broth (Oxoid Ltd Basingstoke Hants Englands). The media was prepared in accordance to the manufacturer's instruction, Sixty-two (62g) gram of Sabouraud dextrose broth was weighed, dissolved in 1000ml of distilled water, the mixture was heated using hot plate to dissolve completely and poured into 30 sterile test tubes. Streptomycin (antibiotic) was added into each test tube to inhibit the growth of bacteria and autoclaved at 121^0C for 15 minutes. The sterile medium was allowed to cool down. And then the nail scrapings were then inoculated into the medium and was incubated at 27^0C for growth for 14 days [8].

Sabourad dextrose agars

This preparation was also done according to the manufacturers instruction (Oxiod ltd. Basingstoke hants Enland); 62g of the powdered medium was weighed and dissolved into 1000ml of distilled water, the mixture was heated using hot plate to dissolve completely and then autoclaved at 121^0C before it was poured into sterile Petri dishes.

Inoculation

The prepared media Sabouraud Dextrose Agar was allowed to solidify. A small portion of each of the fungal colony from the Sabouraud Dextrose broth was singly placed at the centre of each of the SDA plates and incubated at room temperature (27^0C ± 2^0C) for another 14 days [14].

Sub-culturing

After the 14th day more Sabouraud Dextroses Agar plates (SDA) plates were prepared and allowed to solidify. A portion of each different fungal colony from the plates that have mixed growth were singly placed on the centre of SDA plates and was incubated at room temperature for another 14days. This was done to obtain pure culture of the isolates [11].

Fungal identification

The pure cultures of 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 centre of the slide, a small portion of the fungal culture was picked 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 placed on the slide; It will be mounted on the stage. It was viewed under low (10x) and high power objective (40x) to observe the morphological features. The isolates were identified based on

morphological characteristics in accordance with a mycological atlas [20].

Results and Discussion

A total of 15 samples were tested in the microbiology laboratory, Joseph SarwuanTarka University, Makurdi. 12 (80%) samples were identified as positive by culture, among which 8 (53.34%) samples were found to be Potassium hydroxide (KOH) positive and 4 (26.66%) samples were found to be KOH negative. 3 (20%) samples were identified as negative by both the methods i.e both culture and KOH as has been shown in (Table 1).

Table 1: Testing Methods and Results

Test	KOH+Ve Frequency (%)	KOH-Ve Frequency (%)	Total Frequency (%)
Culture +VE	8 (53.33)	4 (26.67)	12 (80)
Culture -VE		3 (26.67)	3 (20)
Total	8 (53.33)	7 (46.67)	15 (100)

Key: +Ve = Positive, -Ve = Negative .

Table 2, the observed differences among the male and female students who were involved in KOH and culture testing methods were not statistically significant. In KOH testing ($p<0.05$, df = 1, $\chi^2 = 0.077$). In the culture test ($p<0.05$, df = 1, $\chi^2 = 0.268$). The result shows that there are no significant difference among the males and females who were positive and negative for KOH as well as for culture.

Table 2: Sex distribution in KOH and Culture Testing Methods

Sex	KOH Test		Culture Test	
	Positive (+)	Negative (-)	Positive (+)	Negative (-)
Male count	4	3	6	1
Expected count	3.7	3.3	5.6	1.4
Female count	4	4	6	2
Expected count	4.3	3.7	6.4	1.6
Table	8	7	12	4

For KOH, $p<0.05$, df = 1, $\chi^2 = 0.077$ Not significant; For culture, $p<0.05$, df = 1, $\chi^2 = 0.268$, Not significant

Table 3 shows the Age (in years) of the number of students who were positive and negative for KOH test as well as those for culture test. The table shows that there is no significant difference among the Age (in years). For KOH, $p<0.05$, df = 4, $\chi^2 = 6.964$), for culture test, $p< 0.05$, df = 4, $\chi^2 = 2.500$.

Table 3: Age Distribution of Students involved in KOH and Culture Testing Methods.

Age (in years)		KOH Test positive (+)	Negative (-)	Culture Test positive (+)	Negative (-)
23	Count	4	0	4	0
	Expected count	2.1	1.9	3.2	0.8
24	Count	1	0	1	0
	Expected count	0.5	0.5	0.8	0.2
25	Count	1	3	3	1
	Expected count	2.1	1.9	3.2	0.8
27	Count	1	3	3	1
	Expected count	2.1	1.9	3.2	0.8
29	Count	1	1	1	1
	Expected count	1.1	0.1	1.6	0.4
Total		8	7	12	3

For KOH, $p< 0.05$, df = 4, $\chi^2 = 6.964$, Not significant; For Culture, $p<0.05$, df = 4, $\chi^2 = 2.500$, Not significant

Table 4 shows the different age groups and the gender which were involved in this study. In the current study, females (53.33%) were involved in Onychomycosis more than males (46.67%). Highest numbers of patients (60%) were between 24-28 years of age, followed by the age group 19-23 (26.67%) and the least age group was 29-33 (13.33%).

Table 4: Distribution of Students according to Age group and Gender.

Age	Male		Female		Total	
	Frequency	Percentage (%)	Frequency	Percentage (%)	Frequency	Percentage (%)
19-23	2	13.33	2	13.33	4	26.67
24-28	4	26.67	5	33.33	9	60
29-33	1	6.67	1	6.67	2	13.33
Total	7	46.67	8	53.33	15	100

Table 4.5 presents the fungi which were identified from various samples. The predominant pathogen which was identified in this study was the Dermatophytes which represent 41.66% of the culture positive samples and this includes dermatophyte such as *Trichophyton rubrum* (25%), *Trichophyton mentagrophytes* (8.33%) and *Epidermophyton floccosum* (8.33%). The dermatophyte species was followed by the yeast species 33.32% which includes yeast such as *Candida albicans* (16.66%) and *Candida parapsilosis* (16.66%). Moulds were present in 24.99% cases which included mouds such as *Aspergillus terreus* (16.66%) and *A. niger* (8.33%) of the culture positive cases.

Table 5: Frequency of distribution and percentage of frequency of fungus isolate.

Category	Fungal species	Frequency	Percentage (%) of frequency (N=24)
Dermatophytes	*Trichophyton rubrum*	3	25
	T. mentagrophytes	1	8.33
	Epidermophyton floccosum	1	8.33
Yeast	*Candida albicans*	2	16.66
	Candida parapsilosis	2	16.66
Moulds	*Aspergillus niger*	1	8.33
	Aspergillus terreus	2	16.66
Total	7	12	100

Table 6 shows the distribution of fungi isolates identified in the different sexes i.e the male and female students. The result shows that the observed differences in the fungi categories among the sexes was not statistically significant. In dermatophytes, $p<0.05$, df = 3, $\chi^2 = 2.277$. For the yeast, $p< 0.05$, df = 2, $\chi^2 = 2.033$. For mould, $p<0.05$, df = 2, $\chi^2 = 4.286$.

Table 6: Distribution of fungus isolate among the Sexes

		Dermatophyte			Yeast		Moulds		Total
		T. rubrum	T. mentagrophytes	E.floccosum	C.albicans	C. parapsilosis	A. niger	A. terreus	
Male	count	1	1	0	1	0	1	2	6
	E. count	1.4	0.5	0.5	0.9	0.9	0.5 nt	0.9	42
Female	count	2	0	1	1	2	0	0	6
	E. count	1.6	0.5	0.5	1.1	1.1	0.5	1.1	6.4
Total		3	1	1	2	2	1	2	12

Key: E. count = Expected count; For Dermatophyte, $p<0.05$, df = 3, $\chi^2 = 2.277$, Not significant; For the Yeast, $p<0.05$, df = 2, $\chi^2 = 2.033$, Not significant; For Moulds, $p<0.05$, df = 2, $\chi^2 = 4.286$, Not significant.

Discussion

The current study highlighted that if the diagnosis of onychomycosis would rely on the clinical pattern of the nail changes, we would miss about 80% of the fungal causes. In the present study, 20% samples were identified as false negative (KOH negative and culture positive). 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 [28].

In this study, onychomychosis was found to be more common in the age group between 24 and 28 years (60%), followed by the age group between 19 and 23 years (26.67%) and the least was the age group between 29 and 33 years (13.33%). Onychomycosis affected all age groups with the highest frequency recorded for ages between 24 and 28 years, this may be 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 [38]. A slightly higher difference in distribution rate was noticed in females (53.33%) than in males (46.67%), which showed that onychomycosis is a disease of both males and females. This might be caused by walking barefoot in public places, or precipitation when one wears covered shoes always. Similar findings were shared by other authors [29]. Dermatophytes cause 90% toe nail and 50% finger nail onychomycosis [20]. However, some studies show equal incidences between dermatophytes and yeasts [15].
The predominant pathogen was dermatophyte (41.66%) which included (25%) cases with *Trichophyton rubrum,* (8.33%), *Trichophyton mentagrophytes* and *Epidermophyton floccosum* respectively. Yeast were present in 33.32% cases, which included (16.66%) cases with *Candida albicans* and (16.66%) cases with *Candida parapsilosis.* This was due to repeated contact with water which is the common mode of transmission of Candida spp. Moulds were present in 24.99% cases which included *Aspergillus terreus* (16.66%) and *Aspergillus niger* (8.33%), as reported by [20]. The observed difference in the distribution of fungi isolates among the male and female students was not statistically significant implying that fungal infection of the nail is not depended on sex. The Pearson Chi-square value for the fungi isolates among the sexes included the following; Dermatophytes ($p<0.05$, $df = 3$, $\chi^2 = 2.277$), Yeast ($p<0.05$, $df = 2$, $\chi^2 = 2.033$), Mould ($p<0.05$, $df = 2$, $\chi^2 = 4.286$).

Conclusion

This research highlighted that dermatophyte was a predominant pathogen associated with Onychomycosis in Joseph Srwuan Tarka University, Makurdi. Onychomycosis is age dependent, male and female disease. The predominant pathogen was dermatophyte which included *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum* respectively. Yeast were present which included *Candida albicans* and *Candida parapsilosis.* This was due to repeated contact with water which is the common mode of transmission of *Candida* spp. Moulds were present which included *Aspergillus terreus* and *Aspergillus niger* . The observed difference in the distribution of fungi isolates among the male and female students was not statistically significant implying that fungal infection of the nail is not depended on sex. The diagnosis of nail disease cannot rely only on the clinical patterns of nail changes, it also requires a microbiological confirmation.

Declaration of conflicting interests

The authors declared no potential conflicts of interest.

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