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Virulence Factors and Antifungal Resistance in Dermatophytes Associated with Dandruff among University Students and a Tertiary Institution in Nigeria

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ABSTRACT

Incidence dandruff among students of a tertiary school was assessed in this study. A standard questionnaire was used to get demographic data while standard microbiological methods were used to identify organisms associated with the infection. Virulence factors and resistance of the isolates to different antifungal agents were also determined. Seventy-eight (65.55 %) out of 119 students sampled had dandruff while dermatophytes were isolated from only 32 (41.07 %). The highest occurrence of dandruff was observed among the age group of 21-25 years with 32 (71.11%) followed by 20 (60.61%) found among 31-35 age group. A total of 108 (90.76 %) of the subjects were single while 9.24 % (n=11) were married. Dry flakes with itching are the highest sign and symptoms among the subject with 71 (91.03 %). A total of 23 (29.29 %) used shampoo as an intervention for dandruff treatment. Two genera of dermatophytes were recovered in this study. The occurrence of *Trichophyton* was more than *Microsporum*. The isolates presented different virulence factors. None of the isolates was susceptible to clotrimazole while all *Trichophyton* were resistant to fluconazole. Nystatin followed by ketoconazole had good inhibitory effects on the isolates. The knowledge of the efficient screening, management, reduction, and treatment of the dermatophytic infection should be fruitful in the future.

INTRODUCTION

Skin is the largest organ of the body, its infection has an important influence on the quality of life and psychology (Hall, 2006; Hay *et al.*, 2014). Skin diseases could be either infectious or non-infectious. The causative agents are mainly fungi while the non-infectious one could be inflammatory dermatoses. Dermatomycoses are infections of the skin, hair, and nail caused as a result of colonization of the keratinized layers of the body (Park *et al.*, 2012; Narshana and Ravikumar, 2018). The infections are predominant in the tropical and subtropical countries due to the hot climate and humid weather. Also, climatic factors, social practices, age, genetic makeup, poor hygiene conditions, diabetes melittus, and immunodeficiency among other related factors have been identified to be predisposing factors to the infections **(**Ranganathan and Mukhopadhyay, 2010; Ismail and Al-Kafri, 2016**).**

Hair and scalp infection is not usually associated with death however, it has serious psychological effects like confidence in social settings and low self-esteem (Cardin, 1998). The scalp of humans has a very high follicular density and a high rate of sebum production than any parts of the body. Scalp present favorable growth conditions for the growth of pathogens (Grimalt, 2017).

Dandruff is a fungal infection that leads to a high rate of shedding of dead skin cells especially from the scalp (Faergemann *et al.,* 2007). It affects almost half of the post-pubertal population regardless of sex and race (Misery *et al.,* 2013; WHO 2013). Dandruff is a fungi disease that affects both males and females and the disease is characterized by flaky white scales usually accompanied by itching (Misery *et al.,* 2013). There are two main classes of dandruff based on its pathology, it may either caused by microbe or by some other conditions other than microbial (Weedon, 2007). Non-microbial dandruff has been reported and attributed to prolonged exposure to sunlight, overexposure to some cosmetic products, imbalanced diet inappropriate methods of covering of heads, and frequent contacts with dust and dirt (Goldberg, 2010; Berger, 2011). The incidence of dandruff is high among individuals with an oily face, eyebrows, and eyelashes.

Trichophyton rubrum is the major fungal pathogen associated with skin infections, it occurs in 8 out of every ten cases of dermtophytoses. The infection is very common among immunocompromised patients (Gupta and Nakrieko, 2015). The objective of this study is to evaluate the prevalence of dandruff among the students of Ekiti State University, Ado-Ekiti.

MATERIALS AND METHODS

Setting, Subjects, and Sampling:

This study was conducted in person at different faculties of Ekiti State University, Ado-Ekiti, Nigeria. The aim of the study was explained and consented subjects were made to sign the consent forms. Subjects that have visible signs of dandruff (which include dry white flakes on combing hair, itchy scalp, and hair fall) were included in this if they have not been on antifungal treatment more than two months and not less than the age of 15. The subject had to be students of staff of institution currently running either a full time of a part-time programme. Inclusion in the study was not restricted based on ethnicity, course of study, marital status, and religion.

Instruments:

In the study, a semi-structured self-administered questionnaire interview was used to collect primary data. The questionnaire designed for this study was evaluated, reviewed, and then pre-tested. The questionnaire comprises of three main parts. Demographic information of the subjects was contained in part one while part two was the signs and symptoms of dandruff in the subjects. The third part of the survey was based on the preventive or treatment measured the subjects have taken in the control of the infection.

Sample Size Determination

The method of Smith (2013) was used to calculate the sample size. The accepted value error or precision in this study was put at 50 %. The sample size was calculated using the following formula:

The specimen size was determined using the formula described by Mugo (2008).

$$N = \frac{Z^2 PQ}{D^2}$$

Where:

N=Minimum number of samples to be collected

Z=1.96 (standard normal distribution at 95 % confidence limit)

P= Local prevalence rate of the previous study on HIV and HBV co-infection among pregnant women = 50 % = 0.5 (Manuel and Ranganathan, 2011)

Q= (1-P) =1-0.5 =0.5, D=Tolerable error (5 %)

$$N = \frac{1.96^2 \times 0.5 \times 0.5}{0.05^2}$$

$$N=\frac{3.8416 \times 0.25}{0.0025}$$

$N \approx 384$

$N = 384$

Thus, the total sample size was calculated to be 395. Therefore, 395 non-repeat samples were collected from students of Ekiti State University, Ado-Ekiti.

Data Analysis:

Data from the study were analysed using descriptive statistics and Chi-squared analysis was done to test for significance in the resulting data. The confidence interval was set at $p \leq 0.05$.

Collection of Samples:

Questionnaires were given to 395 students that had earlier given their verbal consent to participate in the study. The scalp of the subjects that reported any signs and symptoms of dandruff aside from itching was collected into a sterile foil paper and transported to the laboratory for immediate processing as described by Azab *et al*. (2011).

Sample Processing:

The amount of scrapping of the scalp or flakes was prepared using 10 % potassium hydroxide according to Quinn *et al*. (1994. A small piece of the sample was placed on a clean grease-free slide and a drop of 10 % KOH was added and covered with coverslip avoiding over floating. It was then observed under the microscope at x 10 and x 40 objectives for the presence of fungal elements. Part of the sample was aseptically cultured on Sabouraud dextrose agar and was then incubated at 25 ± 2 ^{o}C for 3 weeks, examined at 2 days interval for fungal growth as recommended by Azab *et al*. (2011).

Characterization and Identification:

The isolates were examined using a visual examination of the fungal growth on SDA. Colonial morphology, colour, and presence of pigmentation in the medium were recorded as described by Jagdish (1995). The needle mounting method was used to observe the microscopic features of the fungi. Under the microscope, different features which include macro- and microconidia, chlamydosphore, and hyphae structures were lookout for as reported by Mackie *et al.* (1996).

Determination of Virulent Factor:

Urease test was done using Christensen's urea agar as described by Ochei and Kolhaktar (2000). The fungi isolates were inoculated on the Christensen urea agar slant and were incubated at 30 ^{o}C for 5 days. The agar slant was viewed for the pink colour which indicated a positive urea test. For the detection of proteolytic activity of the isolates, the egg surface was sterilized with 1 % hypochlorite and thereafter with 75 % ethanol prior to breaking of the shell. The yolk was added to Sabouraud dextrose agar, containing 1.17 g NaCl, earlier sterilized at 121 ^{o}C for 15 min, and allowed to cool to about 50 ^{o}C. The fungal blocks aseptically cut at advancing edge of the agar were inoculated on the plate and then incubated at 37 ^{o}C for 48 hr. The production of whitish coloration around the colony after incubation shows proteolytic production.

Hair Perforation Test:

Virgin (unconditioned) hair from a subject that is not on the antifungal drug was collected and cut into an average length of 30 mm. The hairpieces were washed and rinse severally in water and later sterilised in the autoclave at 121 °C for 15 min. About 10 mg of hair sample was flooded in a Petri dish with 25 mL of sterile distilled water and 2 drops of 20 % yeast extract (Himedia, India) were introduced into the dish. The Inoculated with cells of growing fungi. A plug of hyphae of 2 weeks old culture of test fungi was cut at the growing edge and was introduced into the plate. The plate was then incubated for 4 weeks at 27 °C. Hairpieces were sampled at an interval of 5 days. The sample was mounted in lactophenol cotton blue and examined under a light microscope. The Perforation of the hair shaft was looked out for according to AL-Janabi *et al.* (2016).

Harvesting of Fungal Spores:

Fungal spores were harvested by adding 10 mL of sterile Tween 80 (1 %) to each of the Petri dishes containing 5-day old culture. The dish was gently the agar surface

was scraped with a long-handled sterile rod to dislodge the spores and then homogenized manually by shaking and later filter through a sterile cheesecloth as described by Egbontan *et al*. (2013). The spores concentration was reduced to 6 x 10^3 CFU/mL. Spore viability was assessed under the microscope as spores with clear, whitish to creamish color, and a dense, granulated content was considered viable. While spores with either transparent content or with dark brown membrane were termed dead. Spore suspensions used for this work had 90 ± 5 % viability.

Antifungal Susceptibility Test:

Preparation of Antifungal Agent:

All stock solutions were prepared in 100 % dimethyl sulfoxide at a concentration of 1000 µg/ml. They were labeled accordingly until when needed. The drugs were diluted to achieve different concentrations and impregnated into sterile Wharman No. 1 filter paper disc as described by EUCAST (2017). Standardized fungal spores were seeded on Sabouraud dextrose agar and antifungal-impregnated discs were aseptically placed on the agar incubated at 37 ^oC for 48 h. The zone of inhibition was then measured and interpreted according to EUCAST (2017) guideline.

Statistical Analysis:

All categorical variables were pre-coded in the datasheet and were presented as percentages in the results. Categorical (qualitative) data were compared by the chi-square test for correlation. The value of significance was fixed at the 5 % level significance of result were considered as significant at $P<0.05$.

RESULTS

Table 1 shows the demography of and prevalence of dermatophytes among students of Ekiti State University, Ado-Ekiti, Nigeria. Out of the hair scalped samples collected from 119 students only 78 (65.55 %) had dandruff while 46 (58.97 %) yielded no growth. The number of females screened was more than the male in this study. The percentage of occurrence of dandruff in the two sexes was 65.96 % and 65.28 % for males and females respectively. Among the subject screened, age groups 21-25 years had the highest number, 45 (37.82 %) followed by 26-30 years with 28 (23.53 %). The highest occurrence was also observed among the age group of 21-25 years with 32 (71.11) followed by 20 (60.61) recorded among 31-35 age group. A total of 108 (90.76 %) of the subjects were single while 9.24 % (n=11) were married. The signs and symptoms of the infection in the subjects ranged from dry flakes and itching which occurred in 71 (91.03 %) of the subjects, followed by white flakes with an occurrence of 67 (85.90 %). Oily scalp and facial skin with dry flakes were reported by 12 subjects accounted for 15.39 %. The reported that they have been infected with less than 1 year to four years. The subjects used sampoo [23 (29.29 %)], medicated cream [12 (15.38 %)], medicinal plants [5 (6.41 %)] and their combinations as shown in Table 2.

Table 1: Demographic distributions of subjects recruited for the study

Demography	Number screened [n (%)]	Number positive for dandruff [n (%)]
Sex		
Male	47 (39.50)	31 (65.96)
Female	72 (60.50)	47 (65.28)
Age in Years		
15-20	8 (6.72)	5 (62.50)
21-25	45 (37.82)	32 (71.11)
26-30	28 (23.53)	19 (67.86)
31-35	33 (27.73)	20 (60.61)
> 35	5 (4.31)	2 (40.00)
Marital status		
Single	108 (90.76)	75 (69.44)
Married	11 (9.24)	3 (27.27)

Table 2: Signs and duration of dandruff in the subjects and intervention taken since detection

Parameter		Occurrence [n (%)]
Signs and symptoms	White flakes	67 (85.90)
	Itchy scalp	52 (66.67)
	Dry flakes and itching	71 (91.03)
	Oily scalp and facial skin with dry flakes	12 (15.39)
	Hair fall	32 (41.03)
Duration of the sign (yr)	≤1	17 (21.80)
	2	31 (39.74)
	3	19 (24.36)
	4	11 (14.10)
Interventions	Medicated oils	12 (15.38)
	Medicinal plants	5 (6.41)
	Shampoo	23 (29.29)
	Shampoo and conditioner	35 (44.87)
	Shampoo and medicated oils	3 (3.85)

As shown in Table 3, two genera of dermatophytes which were *Microsporum* (n=1) and *Trichophyton* (n=4) were isolated in the subjects. *Trichophyton mentagrophytes* had the highest occurrence [n=12 (31.57 %)], followed by both *T. rubrum* and *T. violaceum*. Thirty-one (31) out of the total 38 isolates recovered produced proteolytic enzymes while 24 and 21 were positive for urase production and keratinase (assessed by hair penetration test) respectively. Three antifungal agents were tested against the isolates. All the isolates were resistant to clotrimazole while all *Trichophyton* were resistant to fluconazole. Nystatin followed by ketoconazole was effective in inhibiting the growth of the isolates as shown in Table 4.

Table 3: Prevalence of dermatophytes in cases of dandruff and occurrence of virulence factors in the isolates

Fungal isolates	Occurrence [n (%)]	Hair Perforation test		Urease test		Proteolytic test	
		+ve	-ve	+ve	-ve	+ve	-ve
M. canis	6 (15.79)	4	2	5	1	5	1
T. concentricum	6 (15.79)	2	4	2	4	5	1
T. mentagrophytes	12 (31.57)	10	2	9	3	12	0
T. rubrum	7 (18.42)	3	4	2	5	5	2
T. violaceum	7 (18.42)	2	5	6	1	4	3

Table 4: Frequency of antifungal resistance among the dermatophytes isolated

Fungal isolates	Clotrimazole	Fluconazole	Ketoconazole	Nystatin
M. canis (n=6)	6	5	2	1
T. concentricum (n=6)	5	5	4	3
T. mentagrophytes (n=12)	12	12	6	4
T. rubrum (n=7)	7	7	4	4
T. violaceum (n=7)	7	7	5	5

The incidence of dandruff detected in this study was not higher than the earlier reported ones. It is clear that the genus of *Trichophyton* is the main circulation genus in the study area. The treatment of this disease should be targeted toward the genus. The organisms isolated from the subjects appear to have been exposed to common antifungal agents hence subjects should be educated not to practice self-medication.

DISCUSSION

Out of 119 subjects recruited only 78 (65.55 %) had dandruff. Subjects within 21 and 25 years age range had the highest occurrence of dandruff with 32 (71.11 %) followed by subject within age 31 and 35 years [n=20 (60.61 %)]. Nita and Rashmika (1999) reported the highest occurrence within the age group. However, lower incidence rates within the age group were reported by Sumana and Singaracharya (2004) and Sen and Rasul (2006) with 52 % and 44 % respectively. The higher incidence of dermatophyte at a young age may be due to increased physical activity increased opportunity for exposure and hormonal pattern.

In this study, the number of female subjects infected was more than male. This result is different from the report of Acharya *et al*. (1995) who reported a higher incidence of dermatophyte in males (65 %) than in females (35 %). In the same vein, Singh and Beena (2003) and Neetu *et al*. (2008) reported a higher occurrence of dermatophytes in males than females. The predominance of dermatophyte in female students was observed probably due to hairstyle and lifestyle of the female students. Some of them that plaits their hair may not wash for some weeks if not months and also some of them used artificial wigs which can harbor fungal pathogens. Only 11 (9.24 %) of the subjects were married while 108 (90.76 %) of the subjects were singles.

Dry flakes and itching were reported by 71 (91.03 %) of the subjects, followed by 67 (85.90 %) which reported flakes with occurrence. This agrees with the reports of Nematian *et al.* (2006) and Rockoff and Shiel (2013) who reported flaking and hair loss as the predominant symptom of dandruff. In this study, 78% of the subject had they have been infected with less than 1 year to four years. The subjects used sampoo [23 (29.29 %)], medicated cream [12 (15.38 %)], medicinal plants [5 (6.41 %)]. Ahmed *et al*. (2007), found that most of the individuals (93.4%) used different hair oils and household remedies for the treatment of dandruff.

Trichophyton species were most encountered species with *Trichophyton mentagrophytes* having the highest occurrence [n=12 (31.57 %)], followed by both *T. rubrum* and *T. violaceum*. Circulating species or strains of dermatophytes appears to be varied from one location to the other considering the reports of Mohanthy *et al*. (1998), Bindu, *et al*. (2002) and Singh *et al*. (2003). The high prevalence of *T. mentagrophytes* in this study is at variance with earlier reports from other parts of Nigeria and Africa where similar studies have been conducted. In Northern Nigeria, Halner (2003) reported the predominance of *Trichophyton schoenleinii* while *Trichophyton violaceum* is the main species reported in the middle belt of Nigeria as reported by Enweani *et al*. (2005). Nweze (2007) reported *Trichophyton tonsurans* as the main circulating strain in the South Eastern part of Nigeria and

All the isolates were resistant to cotrimazole while all *Trichophyton* were resistant to fluconazole. This is in agreement with the work of Magagnin *et al*. (2011) who reported clotrimazole and fluconazole as being the least active among the evaluated antifungal agents. Galuppi *et al*. (2010) reported that fluconazole was the least effective against dermatophytes. Nystatin followed by ketoconazole was effective in inhibiting the growth of the isolates.

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