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Prevalence and associated factors for isolated *Malassezia* species in patients with Dandruff in Mekelle City, Tigrai, Ethiopia

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Abstract

Objective Dandruff, a condition caused by lipophilic *Malassezia* fungi, is an excessive shed of dead skin cells from the scalp. Effective preventive and curative measures of the condition depend on knowledge and understanding of the prevalence of the condition, the common etiologic species, and the associated factors. This study aimed to investigate the prevalence, common etiologic species, and associated factors of *Malassezia* infection in Mekelle City, Ethiopia.

Method A facility-based cross-sectional study was conducted from February 2019 to June 2020 involving 217 participants who were visiting dermatology clinics to seek treatment for dandruff conditions. Information on the socio-demographic characteristics and hair care behaviors of the participants was obtained. Isolation and identification of *Malassezia* species from scalp scrapings using cultural and biochemical tests were carried out.

Results Out of the 217 participants with dandruff, 111 (51.15%) were positive for *Malassezia* fungi. One hundred forty (140) *Malassezia* isolates were collected from the 111 positive participants. Further study of the isolates yielded three etiologic species: *Malassezia globosa* (67.15%), *M. furfur* (21.70%), and *M. restricta* (12.15%). Demographic characteristics, namely gender (AOR = 2.605; 95%CI: 1.427 – 4.757) and age (AOR = 2.667; 95%CI: 1.046 – 6.795), as well as hair care behaviors, namely use of hair oil (AOR = 2.964; 95%CI: 1.288 – 6.820), were associated with the presence of *Malassezia* species. However, the use of anti-dandruff shampoo (AOR = 2.782; 95%CI: 1.301 – 10.993) was negatively associated with the presence of *Malassezia* species among the participants with dandruff conditions. These findings open opportunities to devise effective prevention, management, and control measures for *Malassezia*-based dandruff conditions.

Keywords Dandruff, *Malassezia* spp., Mekelle, Associated factors

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Introduction

Dandruff is considered one of the most common dermatology conditions that affect a vast proportion of the global population [1, 2]. It is one of the known scalp disorders, affecting 50% of the world population [3, 4]. It often affects people at puberty, and males are more susceptible than females [5]. Dandruff is characterized by a large amount of white to silvery, powdery scales on the scalp with abnormal flaking, moderate to unpleasant itching, and a feeling of dry or tight scalp [1]. Dandruff can be severe due to various environmental conditions [6]. Microbial metabolism, sebum production (oily hair), and individual susceptibilities promote dandruff severity [6–10]. Major risk factors for dandruff include excessive sebum over the scalp, gender, age, anatomic region of scalp formation, and microorganisms [11]. Even though there is a paucity of studies, documented associated factors are excessive exposure to sunlight, irritation of the scalp due to excess shampooing, frequent combing, use of certain cosmetic products, and exposure to dust and dirt [12, 13].

The most widely known microorganisms associated with dandruff are lipophilic yeasts belonging to the genus *Malassezia* [12, 14]. *Malassezia* includes lipid-dependent basidiomycetous yeasts that live in the skin and mucosal sites of humans and other warm-blooded vertebrates [15]. *Malassezia* is the only fungal genus associated with dandruff [16]. It comprises 19 species [17], and seven of them, namely: *M. furfur*, *M. globosa*, *M. pachydermatis*, *M. sympodialis*, *M. obtuse*, *M. slooffiae*, and *M. restricta*, are dandruff-causing agents [18, 19]. *M. globosa* and *M. restricta* are the most common species [20–22]. All *Malassezia* species are lipid-dependent, except *M. pachydermatis* [23]. Changes in normal skin flora and the hosts' defense mechanisms can lead to infections and colonization by the *Malassezia* species [24]. Individuals with genetic predispositions, enhanced sebum secretions, hyper-hydrosis, and immunocompromised immune systems are prone to developing infections due to *Malassezia* [9, 24, 25]. The fungi prefer areas with numerous sebaceous glands because they need a high amount of lipids for their growth [26]. They convert the sebum lipid into fatty acids and triglycerides, leading to accelerated proliferation (i.e., hyperproliferation) of the keratinocytes [11, 27].

Mekelle, the capital of Tigrai National Regional State of Ethiopia, is a tropical city in the Sub-Sahara where environmental and socioeconomic factors that worsen dandruff are prevalent [6, 12, 13, 28, 29]. It is a gusty city, with much dust, excessive sunlight, and high diurnal temperature. Moreover, the socioeconomic conditions and educational levels of the majority of its residents are low, leading to poor environmental and personal hygiene. However, with very limited empirical research under similar conditions [e.g., 30], the prevalence of dandruff as well as its causative agents and associated factors are barely known. Thus, devising effective management and control of the condition and the resulting disease becomes difficult. Hence, the present study was aimed at examining the prevalence, etiologic agents, and associated factors of dandruff in Mekelle City. The findings will assist the general public, in general, and patients and healthcare professionals, in particular, in devising effective measures to manage and control the condition.

Materials and methods

Location of the study

Flakes (scale specimens) were collected from six dermatology clinics in Mekelle City, Ethiopia. The city is located about 200 km southeast of the twin historic cities of Aksum and Adwa (alt.: 1,900 to 2,300 masl; lat.: 13° 29″ 45′ N; long.: 39° 28″ 28′ E). The climate of the city is semi-arid, with a mean daily temperature fluctuating between 11.5 and 31.7 °C, a mean monthly temperature of 17.6 °C, and a mean annual rainfall of 600 mm [7]. According to a 2016 estimate, the population of Mekelle city was 310,436 (males: 140,067, females: 170,369) [31].

Research participants and data and specimen collection

The study was a health facility-based cross-sectional study design, conducted from February 2019 to June 2020. The researchers obtained approval from the Health Research Ethical Review Committee of the College of Health Sciences, Mekelle University (Ref. No.: ERC 1500/2020). The source population was clients visiting six dermatology clinics in the city operating during the study period. The diagnosis criteria for dandruff were the presence of white to silvery-colored powder on the scalp and itchy, flaking skin without visible inflammation to the naked eye. From 9 to 64 years old patients with white to silvery-colored powder and itchy flaking skin on their scalp, with minimum scalp inflammation, and consented to participate in the study were included. Patients with additional skin conditions, and who did not consent to participate were excluded. This exercise yielded 540 patients. Then, the single proportion and adjustment formulas were used and a sample size of 225 patients was fixed. The 225 samples were, then, distributed into the six dermatology clinics through the proportionate allocation technique. Full information was collected for 217 patients.

Socio-demographic data of the participants, namely: age, gender, educational level, occupation (job), marital status, and place of residence (urban or rural), were collected using a structured questionnaire developed in *Tigrinya*, the mother tongue of the study participants. Hair care behaviors of the participants, namely: bathing frequency, hair-washing frequency, use of hair shampoos,

use of anti-dandruff shampoos, use of hair oils, and use of head cover, were also collected using the same questionnaire. Finally, flakes or scalp specimens were collected from the scalps (heads) of the participants. The collection of the flakes or scale specimens was carried out by partitioning the hair with a sterile comb and scraping the head (scalp) using a sterile blunt scalpel. The specimens were placed in sterile plastic bags and shipped to the microbiology laboratory in the College of Veterinary Medicine, Mekelle University, for further studies.

Isolation and identification of Malassezia fungi from participants with dandruff

Clinical specimens were inoculated into Dixon agar plates combined with chloramphenicol to inhibit the growth of bacterial contaminants and incubated at 32 °C for 7 days [32]. The agar plates were examined every day for fungal growth. Pure colonies were prepared from plates with positive growth by subculturing on Sabouraud dextrose agar (SDA) overlaid with olive oil before performing physiological tests. Colonies were stained with lactophenol cotton blue (LPCB) stain for microscopic study of the morphology of the fungal cells. A drop of LPCB was placed at the center of a clean glass slide. Part of the fungal colony was taken using a sterile wire loop, put onto a slide, mixed gently with the stain, and covered with a coverslip. Such preparations were examined under the microscope to study the cells' morphologies [27]. Then, the isolates were further subjected to catalase and Tween assimilation tests to differentiate the species [33]. Gas bubble production signifies a positive reaction for the catalase test. Catalase test was carried out using well-established methods [18, 32]. The catalase reaction was determined by applying a drop of 3% H_2O_2 solution onto a culture smear glass slide and mixing it gently using a plastic loop. The catalase production test differentiated *M. globosa* and *M. furfur* (catalase positive) from *M. restricta* (catalase negative) [34, 35].

A Tween assimilation test was carried out to differentiate between *M. globosa* and *M. furfur* using the methods of Guillot and co-workers [25]. A 2.0 mL suspension of fungal isolate was added to the medium. The fungal suspension was obtained by inoculating 5.0 mL of sterile distilled water with a loop full of actively growing fungus. The concentration was adjusted to about 1×10^6 to 5×10^6 CFU/mL. The suspension was poured into a plate containing SDA with 0.05% chloramphenicol and cooled to 50 °C. The inoculum was spread evenly. After the medium was solidified, four holes were made using a 2.0 mm diameter punch, and the first, second, third, and fourth holes were filled with 5.0 µL of Tween 20, 40, 60, and 80, respectively. The plates were incubated for one week at 32 °C. Tween utilization was assessed by the degree of growth or reaction (precipitation) of the lipophilic fungi around the wells [18, 36].

Statistical analyses

The collected quantitative data were analyzed using appropriate statistical methods using SPSS Version 21. Data on the socio-demographic characteristics of the study participants, hair care, hygiene practices, and isolated *Malassezia* species were summarized using frequency distribution and compared. The analysis of associated factors for the presence of *Malassezia* species in the participants with dandruff was carried out using the crude odds ratio (COR) and adjusted odds ratio (AOR) at a 95% confidence interval (95%CI). Comparisons of COR and AOR were carried out at an a priori established significance level of $p \leq 0.05$.

Results and discussions

Socio-demographic characteristics of the study participants

The gender ratio of the study participants was about 1:1, with males constituting slightly more than 50% ($n=114$) (Table 1). The ages of the participants ranged from 9 to 65 years old. But about 75% ($n=162$) of them were 9 to 30 years old. Likewise, about 68% ($n=148$) of the participants were single or divorced. Furthermore, nearly 91% ($n=197$) have some level of formal education, while about 70% ($n=151$) have some type of occupation or business. The larger majority of the participants (81%; $n=176$) were urban dwellers. Thus, most of the research participants were urban dwellers who were relatively younger, single, or divorced, with some formal education and occupation.

Malassezia infection prevalence as a function of socio-demography of the participants

Out of the 217 participants with dandruff conditions, *Malassezia* species were isolated in 111 (51.15%) in them (Table 1). It is known that the condition affects up to 50% of the general adult world population [1–4] and, in some places, more than 50% because of environmental factors and socio-demographic characteristics [e.g., 37]. The prevalence of such infections showed variations as a function of the socio-demographic characteristics of the study participants. The rate of infection was comparably higher in the participants aged 20 to 30 years (63.2%) as compared to that of other age groups (39.1 to 46.9%). One study conducted in similar settings in Ethiopia reported comparable prevalence among the youngest age group [30]. Likewise, the rate was higher in males (58.8%) as compared to that in females (42.7%). This finding is similar to the universal observation with rare exceptions [37]. High infection rates in males are believed to be due to higher sebum production and behavioral factors in

Table 1 Sociodemographic characteristics and distribution of *Malassezia* species participants: frequency and proportions

Socio-demographic variable and categories	Dandruff positive participants ($n=217$)		*Malassezia* positive participants ($n=111$)		*Malassezia* species frequency ($n=140$)		Malassezia species distribution as per socio-demographic characteristics [*n* (%)]		
	n	%	*n*	%	*n*	%	M. globosa	M. furfur	M. restricta
1. Age									
9 – 19	67	30.9	27	40.3	35	25.0	25 (71.4)	9 (25.7)	1 (2.9)
20 – 30	95	43.8	60	63.2	74	52.9	51 (68.9)	14 (18.9)	9 (12.2)
31 – 41	32	14.7	15	46.9	19	13.6	10 (52.6)	4 (21.1)	5 (26.3)
42 – 65	23	10.6	9	39.1	12	8.6	8 (75.0)	2 (12.5)	2 (12.5)
2. Gender									
Female	103	47.5	44	42.7	57	40.7	36 (63.2)	17 (29.8)	4 (7.0)
Male	114	52.5	67	58.8	83	59.3	58 (69.9)	12 (14.4)	13 (15.7)
3. Marital status									
Single	138	63.6	70	50.7	90	64.3	62 (68.9)	19 (21.1)	9 (10.0)
Married	69	31.8	35	50.7	44	31.4	29 (65.9)	8 (18.2)	7 (15.9)
Divorced	10	4.6	6	60.0	6	4.3	3 (50.0)	2 (33.3)	1 (16.7)
4. Educational level									
No formal education*	20	9.2	11	55.0	11	7.9	8 (72.7)	2 (18.2)	1 (9.1)
Primary school	72	33.2	31	43.1	44	31.4	30 (68.2)	8 (18.2)	6 (13.6)
Secondary school	63	29.0	36	57.1	43	30.7	29 (67.4)	7 (16.3)	7 (16.3)
College and above	62	28.6	33	53.2	42	30.0	27 (64.3)	12 (28.6)	3 (7.1)
5. Occupation									
Merchant (petty)	63	29.0	34	54.0	43	30.7	29 (67.4)	8 (18.6)	6 (14.0)
Office work	46	21.2	25	54.3	32	22.9	19 (59.4)	10 (31.3)	3 (9.3)
Students	66	30.4	24	36.4	37	26.4	29 (78.4)	6 (16.2)	2 (5.4)
Others	42	19.4	28	66.7	28	20.0	17 (60.7)	5 (19.9)	6 (21.4)
6. Residence									
Urban	176	81.1	90	51.1	112	80.0	76 (67.9)	22 (19.6)	14 (12.5)
Rural	41	18.9	21	51.2	28	20.0	18 (64.3)	7 (25.0)	3 (10.7)
Total	**217**	**100.0**	**111**	**51.2**	**140**	**100.0**	**94 (67.15)**	**29 (20.70)**	**17 (12.15)**

*: Can read and write

hair care practices [5, 8, 38–44]. The rates of infection among singles and married participants were comparable (50.7%), while those among the divorced ones were much higher (60%). Participants with primary school education had a lower rate of infection (43.1%) compared to the other groups (53.2−57.1%). With regard to occupation, students had a lower rate of infection (36.4%) than petty merchants (54.0%) and office workers (54.3%). Finally, the infection rates among rural and urban are dwellers comparable.

Malassezia species isolated from Malassezia positive participants

The study of flakes (i.e., scale specimens) collected from the 111 *Malassezia*-positive participants yielded 140 isolates (species). High proportions of infections with multiple *Malassezia* species seem to be generally prevalent among rural-dwelling younger female participants (Table 1). Out of the 19 *Malassezia* species, *M. furfur*, *M. globosa*, *M. pachydermatis*, *M. sympodialis*, *M. obtuse*, *M. slooffiae*, and *M. restricta* are known to be associated with dandruff; the most dominant being *M. globosa* and *M. restricta* [18, 25–27]. The study of the 140 isolates in the present research revealed that they belong to *M. globosa* ($n=94$; 67.15%), *M. furfur* ($n=29$; 20.70%), and *M. resrticta* ($n=17$; 12.15%). A study conducted in a similar setting to ours reported these species to be more prevalent [30]. With three exceptions, the proportions of the prevalences of the three species were consistently similar within all socio-demographic groups of the participants. In line with this study, research in Bosnia and Herzegovina reported *M. globosa* as the dominant fungus in patients with dandruff [45]. However, studies conducted in other countries gave different results. For example, one study in the USA [46] and another in China [47] showed that *M. restricta* was predominant, followed by *M. globosa*. Likewise, a study in Bangladesh and Iran showed *M. furfur* as the most common agent associated with the condition [48, 49]. Furthermore, studies in Korea showed

Table 2 Prevalence of *Malassezia* isolates in participants in relation to hair care behaviors

SN	Hair care behaviors	Categories	Participants [*n* (%)]	Isolates [*n* (%)]
1	Bathing frequency	1–2 times/week	173 (79.7)	117 (83.6)
		3 – 4 times/week	44 (20.3)	23 (16.4)
2	Hair washing frequency	1–2 times/week	127 (58.5)	87 (62.1)
		3 – 4 times/week	90 (41.5)	53 (37.9)
3	Antidandruff shampoo use	No	188 (86.6)	127 (90.7)
		Yes	29 (13.4)	13 (9.3)
4	Regular combing	No	82 (37.8)	50 (35.7)
		Yes	135 (62.2)	90 (64.3)
5	Regular use of hair oil	No	38 (17.5)	17 (12.1)
		Yes	179 (82.5)	123 (87.9)
6	Use of head cover	No	163 (75.1)	102 (72.9)
		Yes	54 (24.9)	38 (27.1)
	Total		**217 (100)**	**140 (64.50)**

Table 3 Multivariate analysis to identify factors associated with *Malassezia* infections

SN	Variables	Malassezia isolates		COR (95% CI)	*p*	AOR (95% CI)	*p*
		Negative	Positive				
1.	Gender						
	Male	47 (41.2)	67(58.8)	1.912 (1.114–3.280)	0.019	2.605 (1.427–4.757)	0.002*
	Female	59(57.3)	44(42.7)	1			
2.	Age						
	9 – 19	40 (59.7)	27 (40.3)	1.050 (0.398 – 2.768)	0.921	1.048 (0.374 – 2.935)	0.929
	20 – 30	35 (36.8)	60 (63.15)	3.040 (1.18 – 7.84)	0.040	2.667 (1.046 – 6.795)	0.049*
	31 – 41	17 (53.1)	15 (46.9)	1.373 (0.462 – 4.073)	0.568	1.279 (0.405– 4.036)	0.675
	42 – 65	14 (60.8)	9 (39.1)	1			
3.	Use of anti-dandruff shampoo						
	No	89 (45.9)	105 (54.1)	3.343 (1.264 –8.84	0.015	3.782 (1.301 – 10.993)	0.015*
	Yes	17 (73.9)	6 (26.1)	1			
4.	Use of hair oil						
	No	27 (71.1)	11 (28.9)	1			
	Yes	79 (44.1)	100 (55.9)	3.107 (1.452– 6.648)	0.003	2.964 (1.288– 6.820)	0.011*

CI: 95% Confidence Interval; COR: Crude Odds Ratio; AOR: Adjusted Odds Ratio; *: Statistical significance at $p \leq 0.05$

M. restrict to be dominant [50], while in Iran, *M. Furfur* was dominant [51].

Malassezia infection prevalence as a function of hair care behavior

Antidandruff products and practices are believed to abate dandruff conditions associated with fungal infections. This study observed that the prevalences of dandruff-causing *Malassezia* species among the participants who reported frequent bathing, frequent hair washing, use of antidandruff shampoos, regular combing, and use of head covers was much lower than the prevalences of those who have limited or no hair care habits, use no antidandruff products, and use hair oils (Table 2). Hair care behavior did not change the relative prevalences of the three *Malassezia* species.

Associated factors for Malassezia infections in the participants with dandruff

The study has shown that some socio-demographic factors and hair care behaviors are important factors that affect or determine *Malassezia* infections. Multivariate analyses showed that males with dandruff had a significantly higher likelihood of being *Malassezia* positive than females, with an AOR of 2.605 (95%CI: 1.427–4.757; $p \leq 0.05$) (Table 3). This finding agreed with the results of many other researchers [52–54]. Such observations are attributed to the higher sebum production in adult males than in females of the same age range [53, 55]. In line with such proposition, study participants aged 20 to 30 (most likely single and independent) showed a significantly higher association with *Malassezia* infection compared to the reference group (42–65 years) (AOR = 2.667; 95% CI: 1.046 – 6.795; $p \leq 0.05$). One study in the USA demonstrated a similar trend where younger and older groups showed lower rates of infection [53]. The lower

sebum production in the pre- and post-puberty stages is the plausible factor for such a pattern [8, 38–40].

As is quite expected and often observed [56], study participants who do not use anti-dandruff shampoos had a significantly higher likelihood to be *Malassezia* positive (AOR = 2.782; 95%CI: 1.301 − 10.993; $p \leq 0.05$). Likewise, participants who use hair oil products were significantly more likely to be *Malassezia* positive compared to those who do not (AOR = 2.964; 95%CI: 1.288 − 6.820; $p \leq 0.05$). The hair oil products used by the study participants might have been favorable for the growth of the *Malassezia* species. In line with this proposition, an Indian study concluded that hair styling products exacerbate the dandruff condition [42]. In addition to demographic characteristics and hair care behaviors, *Malassezia* infections are affected by many factors, such as diet and stress, environmental factors (like humidity), genetic predispositions, and neurological factors [9, 24, 25, 43, 57, 58].

Concluding remarks

The study revealed that more than half of the participants visiting dermatology clinics in Mekelle City for dandruff had *Malassezia* isolates. The *Malassezia* fungi isolated from the participants with dandruff were *M. globosa*, *M. furfur*, and *M. restricta*. *M. globosa* was greater than three times more prevalent than *M. furfur* and nearly six times more prevalent than *M. restricta*. The *Malassezia* isolates were more common in males than in females and participants aged 20 to 30 years. Likewise, more *Malassezia* isolates were observed among the group of participants who use hair oil products. However, the group of participants who use antidandruff shampoo had fewer isolates. These findings are very helpful for the general public, dandruff patients, and healthcare service delivery agencies in the city and its nearby rural districts to devise better prevention, management, and control measures for *Malassezia* infections and dandruff diseases.

Limitations

This study was restricted to three dandruff-causing *Malassezia* fungi only. No comparison studies were also made between dandruff patients and healthy individuals. Moreover, since no molecular techniques were used, some unculturable species might have been missed in the culture-based isolation and morphological and biochemical studies. Hence, future works have to consider these limitations.

Abbreviations

alt.	Altitude
AOR	Adjusted odds ratio
CFU	Colony forming units
CI	Confidence interval
COR	Crude odds ratio
E	East
H_2O_2	Hydrogen peroxide
km	Kilometer
lat.	Latitude
long.	Longitude
LPCB	Lactophenol cotton blue
masl	Meters above sea level
mL	Milliliter
mm	Millimeter
µL	Microliter
n=	Number
N	North
SD	Standard deviation
SDA	Sabouraud dextrose agar
SPSS	Statistical package for social sciences

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Author contributions

B.G.G., D.B.S., and M.A. were involved in conceiving the study; B.G.G., M.A, D.B.S., E.T., and G.G.B. were involved in designing and planning the study; B.G.G. carried out the collection of specimens, the experimentation, data collection and analyses, and draft manuscript write up; M.A. and E.T. offered administrative support; D.B.S. and B.G.G. reviewed the manuscript for content, language, and style; B.G.G. and D.B.S. prepared the manuscript for publication. All authors approved the submitted manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Attached.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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