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Prevalence of Etiological Bacteria and Fungi of Dandruff in Al-Gabal Al-Gharbi, Libya

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انتشار العوامل البكتيرية والفطرية المسببة لقشرة الرأس في منطقة الجبل الغربي، ليبيا

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Abstract:
The skin hosts a diverse range of microorganisms that influence both its health and disease. Dandruff, a common scalp condition, affects approximately 50% of the global adult population. This study aimed to examine the prevalence of bacteria and fungi in dandruff samples.
Materials and Methods: Fifty-two dandruff samples were collected from volunteers in Al-Gabal Al-Gharbi, aged 11–62 years. The samples were cultured for bacterial and fungal growth, followed by microbial identification and sensitivity testing as per routine microbiological procedures.
Results: Bacteria were identified in 50 cases (96.15%) and fungi in 35 cases (67.30%). The most common bacterial isolates were *Staphylococcus aureus* (37.11%) and *Staphylococcus epidermidis* (24.74%). The most common fungal isolate was *Aspergillus niger* (29.89%), followed by *Aspergillus flavus* (3.10%) and *Malassezia spp*. (5.15%). *S. aureus* and *S. epidermidis* were more prevalent in females (58.33% and 54.16%, respectively), while *Aspergillus niger* and *Malassezia spp*. were more common in males (44.83% and 60%, respectively). Chloramphenicol exhibited 100% sensitivity against both *S. aureus* and *S. epidermidis*, while penicillin and ampicillin showed complete resistance in *S. epidermidis*. Regarding antifungal activity, *Malassezia spp*. exhibited full resistance to ketoconazole, while *Aspergillus niger* was more susceptible to miconazole, with ketoconazole showing 100% resistance.

Keywords: Dandruff, Bacterial Infections, Fungal Infections, Staphylococcus Aureus, Antibiotic Susceptibility.

الملخص
يعيش على البشرة مجموعة متنوعة من الكائنات الدقيقة التي تؤثر على سلامة الجلد ومرضه. قشرة الرأس هي حالة شائعة تصيب فروة الرأس، وتؤثر على حوالي 50% من السكان البالغين في العالم. هدفت هذه الدراسة إلى فحص انتشار البكتيريا والفطريات في قشرة الرأس.
المواد وطرق العمل: جمعت 52 عينة من المتطوعين في منطقة الجبل الغربي، تتراوح أعمارهم بين 11 و 62 سنة. جمعت العينات، وتم تشخيصها والتعرف عليها وإجراء الاختبارات الحساسية وفقًا للإجراءات المعمول بها في تشخيص الأحياء المجهرية.

النتائج : وجدت البكتيريا في 50 حالة (96.15%) والفطريات في 35 حالة (67.30%). كانت أكثر العزلات البكتيرية شيوعًا هي *Staphylococcus aureus* (37.98%) و*Staphylococcus epidermidis* (26.26%). أما أكثر العزلات الفطرية شيوعًا فكانت *Aspergillus niger* (28.42%)، تليها *Aspergillus flavus* (3.15%) و *Malassezia spp.* (5.26%). كانت *S. aureus* و*S. epidermidis* أكثر انتشارًا بين الإناث (58.33% و 54.16% على التوالي)، بينما كانت *Aspergillus niger* و*Malassezia spp.* أكثر شيوعًا بين الذكور (44.83% و 60% على التوالي). أظهر الكلورامفينيكول حساسية بنسبة 100% ضد كل من *S. aureus* و *S. epidermidis*، في حين أظهر البنسلين والأمبيسيلين مقاومة كاملة ل *S. epidermidis*. أظهرت *Malassezia spp.* مقاومة كاملة للكيتوكونازول، بينما كانت *Aspergillus niger* أكثر حساسية للميسونازول (Miconazole)، مع إظهار الكيتوكونازول مقاومة بنسبة 100%.

الكلمات المفتاحية: قشرة الرأس، الالتهابات البكتيرية، الالتهابات الفطرية، المكورات العنقودية الذهبية، الحساسية للمضادات الحيوية.

Introduction

The human skin is a complex ecosystem inhabited by a diverse range of microorganisms, including archaea, viruses, bacteria, and fungi. These skin microorganisms contribute to both health and disease [1]. Human hair dandruff is a common and undesirable scalp condition that affects a large proportion of the global population [2]. Dandruff affects approximately 50% of the global adult population, with a higher prevalence in males compared to females [3,4]. It is a widespread scalp condition that affects nearly half of the post pubertal population across all ethnicities and genders and is frequently associated with itching [5]. Various intrinsic and environmental factors including sebaceous gland secretions, fungal colonization of the skin surface, individual susceptibility, and interactions between these factors, collectively contribute to the pathogenesis of dandruff [6,7]. A recent study has indicated that dysbiosis, characterized by an altered ratio of predominant bacterial and fungal populations, is associated with the development of dandruff [8]. Fungi contribute to skin diseases, acting as either primary or secondary pathogens. Both inflammatory and non-inflammatory skin conditions are associated with various mold and/or dermatophyte genera [9]. The primary bacterial species identified on the scalp surface are *Propionibacterium acnes* and *Staphylococcus epidermidis*, while *Malassezia restricta* is the predominant fungal species present [8]. Although the exact relationship between *Malassezia* and dandruff is not fully understood, *Malassezia* is widely acknowledged as a major contributor to the condition as thoroughly reviewed by Shuster [10]. In a recent study conducted in Canada, Mas-Ud et al. [2] demonstrated that the bacterial colonies isolated from hair dandruff samples exhibited approximately 99% genomic similarity to *Staphylococcus aureus* as determined by PCR amplification. These colonies were identified as Gram-positive, small, round-shaped, and purple in color colonies. Another study by Mas-Ud et al. [2] also identified these bacterial colonies in hair dandruff samples. Park et al. [11] found that *Filobasidiumfloriforme* was the main fungal species inhabiting dandruff-afflicted scalps, while Clavaud et al. [8] reported that *Propionibacterium acnes* and *Staphylococcus epidermidis* along with *Malassezia restricta* were the main microbial inhabitants on the scalp surface. A study conducted in Nigeria by Obum-Nnadi et al. [9] found that the most commonly isolated dermatophyte was *Microsporumspp.* (41.7%), and dandruff cultures revealed a high proportion of species from a non-dermatophyte mold, *Aspergillus* with *Aspergillus niger* (25%) being the most commonly isolated species followed by *Aspergillus fumigatus* (12.5%) and *Aspergillus flavus* (12.5%) in dandruff-afflicted scalps. *Malassezia* species are considered the etiological agents of pityriasis versicolor and *Malassezia* folliculitis. They are also associated with seborrheic dermatitis and are considered contributing factors in several other skin conditions including atopic dermatitis, psoriasis, confluent and reticulate papillomatosis, and neonatal pustulosis [12]. This study aims to investigate the prevalence, common etiologic species, and associated factors of bacterial and fungal infections in Um Al-Jarsan and Zentan Cities, Libya.

Material and methods

Study area description

Al Jabal Al Gharbi region (Um Al-Jarsan and Zentan) is located in northwest of Libya (32°07N and 12°58E) region is 135 kms far from Tripoli, which has mountainous climate with an annual rainfall of 168 mm, a relatively humidity of 52.2%, and an average annual temperature of 19.4°C.

Collection and Examination of Samples

This study was conducted in the Jabal al-Gharbi region (specifically in the cities of Um Al-Jarsan and Zintan), where samples were collected from 52 volunteers suffering from dandruff. A total of 52 samples were collected by cotton swabs and scrapings onto filter paper over a period from July 7th to August 12th, 2024. The volunteers were individuals of both genders, aged between 11 and 62 years.

Once collected, the samples were promptly transferred to the laboratory for further processing. Each sample was divided into two parts: one for direct microscopic examination (D.M.E) and the other for fungal culture. Subsequently, the cotton swabs were cultured on bacterial media to isolate and grow bacteria, while the scrapings were cultured on fungal media to isolate and grow fungi. The isolates were then subjected to diagnostic procedures for microbial identification and sensitivity tests were performed on both bacterial and fungal isolates [13].

Methodology

Sterilization of Materials

The materials employed in this study were sterilized according to their specific characteristics. Glassware was subjected to sterilization in a hot air oven at 160°C for 60 minutes, while culture media were autoclaved at 121°C for 15 minutes. Plastics and the laboratory work surface were disinfected with alcohol, and the inoculation loop was sterilized using a direct flame in accordance with the protocols outlined by [15].

Direct Microscopic Examination (D.M.E): The first part of each sample was prepared for D.M.E by placing the collected material on a microscopic slide. The slide was treated with 10% potassium hydroxide (KOH) solution to help dissolve tissue elements and make fungal elements like hyphae, spores, or yeasts more visible under the microscope. The slides were then examined using a light microscope at magnifications of 10x and 40x to detect fungal elements [16].

Fungal Culture: The second part of each sample was cultured on Sabouraud Dextrose Agar (S.D.A) plates supplemented with 0.05 mg/mL of chloramphenicol to inhibit bacterial growth. The inoculated plates were incubated at 30°C for one week to allow fungal growth. After the incubation period, the colonies formed were examined for their morphology [17,18].

Colony Examination: In addition to macroscopic evaluation, a portion of the colonies was further examined microscopically. Lactophenol Cotton Blue (LPCB) stain was applied to clear the material, making it easier to observe the structural features of the fungi. Microscopic observations were conducted using 10x and 40x objectives to identify fungal species. Pure isolates of the fungal species were recorded and stored in the refrigerator for future reference [14,15].

Culture and Identification of bacterial species

Each specimen was divided into two parts, one portion used for a primary Gram stain, while the other portion was used for culture inoculations onto Peptone water, MacConkey Agar, Eosin methylene blue, Mannitol Salt Agar and Blood Agar (Oxoid, UK). These cultures were then incubated aerobically at 37°C for 24 to 48 hours. Gram-positive isolates were underwent to catalase and coagulase tests, while Gram-negative bacteria were subjected to oxidase Sulfur Indole Motility (SIM), urease production [19], and citrate utilization tests antibiotic sensitivity testing and interpretation of results were done according to CLSI guidelines.

Results and discussion

The Figure (1) presents the distribution of causative agents, highlighting the number of cases and their respective percentages. Bacteria were identified as the causative agent in 50 cases, accounting for 96.15% of the total cases, while fungi were responsible for 35 cases, representing 67.30%.

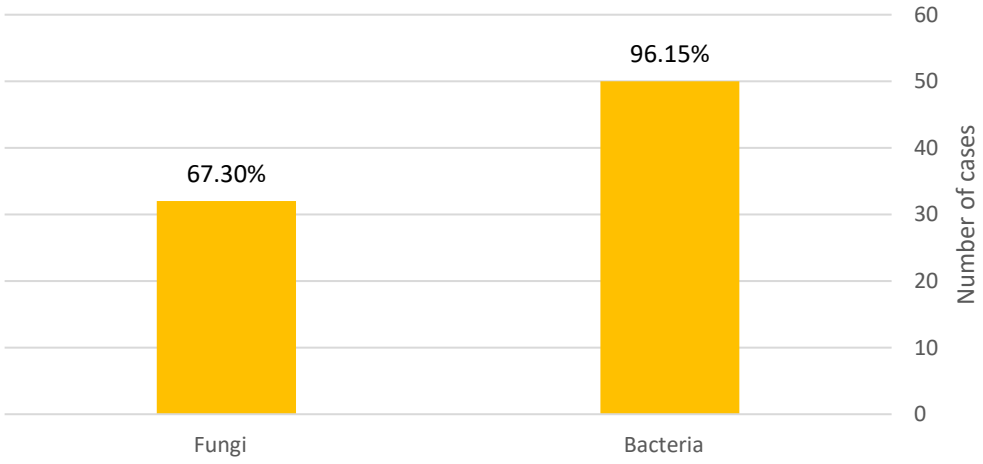

Figure (1) shows the number and percentage of the prevalence of bacteria and fungi.

As shown in Table (1), A total of 97 isolates (bacterial and fungal) were obtained from 52 cases, with bacterial isolates comprising 61.86% (60 isolates) and fungal isolates representing 38.14% (37 isolates) of the total. Among the bacterial isolates, *Staphylococcus aureus* was the most frequently identified species 37.11% (36 isolates), followed by *Staphylococcus epidermidis*, which constituted 24.74% (24 isolates). Among the fungal isolates, *Aspergillus niger* was the most prevalent species representing 29.89% (29 isolates), followed by *Aspergillus flavus* (3.10%, 3 isolates) and *Malassezia* spp. (5.15%, 5 isolates). These findings highlight the predominance of *Staphylococcus aureus* among bacterial isolates and *Aspergillus niger* among fungal isolates in the studied cases.

Table (1): Isolated bacteria and fungi genera (n= 97).

Bacterial and fungal genera	**Number of isolates I**	**Percentage (%) I%**
Bacteria		
Staphylococcus aureus	36	37.11%
Staphylococcus epidermidis	24	24.74%
Total Bacteria	**60**	**61.86%**
Fungi		
***Aspergillus** niger*	29	29.89%
***Aspergillus** flavus*	3	3.10%
*Malassezia **spp**.*	5	5.15%
Total Fungi	**37**	**38.14%**

I, Incidence. **(I%)**, Incidence %.

The data represented in **Table (2)** show the prevalence of isolated bacteria and fungi in relation to gender. *S. aureus* was identified in 36 cases, with 15 males (41.67%) and 21 females (58.33%). *S. epidermidis* was identified in 24 cases, with 11 males (45.83%) and 13 females (54.16%). The total number of bacterial isolates was 60, with 26 males (43.33%) and 34 females (56.67%). Regarding the fungi, *Aspergillus niger* was identified in 29 cases, with 13 males (44.83%) and 16 females (55.17%). *Aspergillus flavus* was identified in 3 cases, with 2 males (66.67%) and 1 female (33.33%). *Malassezia spp.* was identified in 5 cases, with 3 males (60%) and 2 females (40%). The total number of fungal isolates was 37, with 18 males (48.65%) and 19 females (51.35%).

Table (2): Prevalence of isolated bacteria and fungi in relation to gender.

Identified organism		**Male (n = 23)**		**Female (n = 29)**	
	Total Number of Affected	No. of identified organism.	Percentage(%)	No. of identified organism	Percentage (%)
S. aureus	36	15	41.67	21	58.33
S. epidermidis	24	11	45.83	13	54.16
Total bacteria	**60**	**26**	**43.33**	**34**	**56.67**
***Aspergillus** niger*	29	13	44.83	16	55.17
***Aspergillus** flavus*	3	2	66.67	1	33.33
Malassezia spp.	5	3	60	2	40
Total fungi	**37**	**18**	**48.65**	**19**	**51.35**

Chi-square analysis indicated no statistically significant difference in the distribution of bacterial and fungal species across different gender types (since the p-values for the Pearson Chi-Square (0.750) and (0.91), respectively, are both greater than 0.05.

The results in Table 3 illustrate the prevalence of various bacterial species across distinct age groups among individuals diagnosed with dandruff. In this study, the total number of bacterial isolates was 60. These isolates were distributed across different age groups as follows: 10 isolates (16.67%) in the 11–20 years age group, 28 isolates (46.67%) in the 21–30 years age group, 9 isolates (15%) in the 31–40 years age group, 4 isolates (6.67%) in the 41–50 years age group, and 9 isolates (15%) in the 51–62 years age group.

Among the bacterial species identified. *aureus* was the most predominant, affecting 36 individuals in total. The highest prevalence of *S. aureus* was observed in the 21–30 years age group, where it affected 17 individuals (28.33%). This species was also present in other age groups: 8 cases (13.33%) in the 11–20 years group, 5 cases (8.33%) in the 31–40 years group, 2 cases (3.33%) in the 41–50 years group, and 4 cases (6.67%) in the 51–62 years group.

Another notable bacterium, *S. epidermidis*, affected 24 individuals in total and was most prevalent in the 21–30 years cohort, where 11 individuals (18.33%) were affected. This bacterium was also observed in other age groups: 2 cases (3.33%) in the 11–20 years group, 4 cases (6.67%) in the 31–40 years group, 2 cases (3.33%) in the 41–50 years group, and 5 cases (8.33%) in the 51–62 years group.
In terms of overall prevalence, the 21–30 years age group exhibited the highest frequency of cases, with 28 individuals (46.67%) affected by either *S. aureus* or *S. epidermidis*. The 11–20 years group had 10 individuals (16.67%), followed by the 31–40 years group, which had 9 individuals (15%). The 51–62 years and 41–50 years groups had 9 (15%) and 4 (6.67%) individuals, respectively.

Table (3): Prevalence of Bacteria Across Different Age Groups with dandruff.

Fungal Species	Dry Dandruff		Oily Dandruff		Total
	Number (N)	Percentage (%)	Number (N)	Percentage (%)	
A. niger	12	30%	17	42.5%	**29**
A. flavus	2	5%	1	2.5%	**3**
Malassezia spp	1	2.5%	4	10%	**5**
Total	**15**	**37.5%**	**22**	**55%**	**37**

Chi-square analysis indicated no statistically significant difference in the distribution of bacterial species across different age groups with dandruff ($\chi^2 = 2.8207$, $p > 0.05$, critical value = 9.488 at df = 4). $p > 0.05$.

The table (4) presents the prevalence of different fungal species across various age groups among individuals suffering from dandruff. A total of 37 participants were included in the study, with varying degrees of fungal infection across five age categories: 11–20 years, 21–30 years, 31–40 years, 41–50 years, and 51–62 years. ***A. niger*** was the most prevalent fungal species overall, affecting 29 individuals in total. The highest frequency of ***A. niger*** was observed in the **21–30 years** age group, where it affected 12 individuals (30%). This species was also found in other age groups, with 6 cases (15%) in the **11–20 years** age group, 5 cases (12.5%) in the **31–40 years** group, 4 cases (10%) in the **41–50 years** group, and 2 cases (5%) in the **51–62 years** group. ***A. flavus***, another fungal species, was less common, with a total of 3 affected individuals. It was observed in the **11–20 years** group (2 cases, or 5%) and the **21–30 years** group (1 case, or 2.5%). No cases of ***A. flavus*** were reported in the older age groups. ***Malassezia spp.***, a third fungal species known for its association with dandruff, affected 5 individuals in total. This species was observed in the **11–20 years** and **21–30 years** age groups, each with 2 cases (5%) and 1 case (2.5%) respectively. Additionally, 1 case (2.5%) of ***Malassezia spp.*** was reported in both the ***41–50 years*** and ***51–62 years*** age groups. In summary, ***A. niger*** was the most prevalent fungal species across all age groups, while ***A. flavus*** and ***Malassezia spp.*** were less common, with ***Malassezia spp.*** being more closely linked to dandruff, particularly in younger age groups. The total prevalence was highest in the **21–30 years** group, with 35% of cases, followed by the **11–20years** group at 30%. The chi-square analysis indicated that there was no statistically significant difference in the distribution of fungal species across different age groups ($\chi^2 = 5.929$, $p > 0.05$, critical value = 15.507 at df = 8), suggesting that age does not significantly influence the prevalence of these fungi in individuals with dandruff.

Table 4. Prevalence of Fungi Across Different Age Groups with dandruff.

Fungal species	11–20 Years	21–30 Years	31–40 Years	41–50 Years	51–62 Years	Total
A. niger	6 (15%)	12 (30%)	5 (12.5%)	4 (10%)	2 (5%)	**29**
A. flavus	2 (5%)	1 (2.5%)	0 (0%)	0 (0%)	0 (0%)	**3**
Malassezia spp	2 (5%)	1 (2.5%)	0 (0%)	1 (2.5%)	1 (2.5%)	**5**
Total	10 (30%)	14 (35%)	5 (12.5%)	5 (12.5%)	3 (7.5%)	**37**

Chi-square analysis indicated no statistically significant difference in the distribution of fungal species across different age groups ($\chi^2 = 5.929$, $p > 0.05$, critical value = 15.507 at df = 8).

The data in table (5) shows the relationship between bacterial type prevalence and skin/non-skin disease. *S. aureus* was identified in **36 cases**, with **13 cases (36.1%)** in skin disease patients and **23 cases (63.9%)** in non-skin disease patients. *S. epidermidis* was identified in **24 cases**, with **7 cases (29.2%)** in skin disease patients and **17 cases (70.8%)** in non-skin disease patients. The total number of bacterial cases was **60**, with **20 cases (33.3%)** in skin disease patients and **40 cases (66.7%)** in non-skin disease patients. These findings indicate that *S. aureus* and *S. epidermidis* were more frequently found in non-skin disease patients compared to skin disease patients, with *S. aureus* showing a higher overall prevalence in non-skin disease cases.

Table (5): The Relationship Between Bacterial Type Prevalence and Patients with skin diseases(n=14) / without skin diseases(n=28.).

Type of Bacteria	Total Number of Affected	Patients with skin diseases(n=14)			Patients without skin diseases(n=28)		
		N	%	Total % of Affected	N	%	Total % of Affected
S. aureus	**36**	13	(92.85%)	**36.1%**	23	(82.15%)	**63.9%**
S. epiderdimidis	**24**	7	(50%)	**29.2%**	17	(60.71%)	**70.8%**
Total	**60**	20		**33.3%**	40		**66.7%**

Chi-square analysis indicated no statistically significant difference in the distribution of bacterial species between patients with skin diseases and Patients without skin diseases ($\chi^2 = 0.3125$, $p > 0.05$, critical value = 3.841 at df = 1).*p value> 0.05.*

As shown in Table 6, the relationship between fungal species prevalence and patients with skin diseases versus patients without skin diseases is presented. The study includes 14 patients with skin diseases and 28 patients without skin diseases, with a total of 37 affected individuals across the fungal species analyzed. Among the affected patients, *Aspergillus niger* (As. niger) was the most prevalent species, affecting a total of 29 individuals. Of these, 10 patients (34.48%) were from the skin disease group, and 19 patients (65.52%) were from the non-skin disease group. This shows that As. niger is more common among patients without skin diseases, but it also has a significant presence in patients with skin diseases. *Aspergillus flavus* (As. flavus) affected a total of 3 individuals, with 1 patient (33.34%) from the skin disease group and 2 patients (66.67%) from the non-skin disease group. The prevalence of As. flavus was relatively low in both groups, but it was slightly more frequent in patients without skin diseases. *Malassezia spp.*, another fungal species, affected 5 individuals in total. All 5 patients (100%) were from the non-skin disease group, representing 100% of the affected patients in that category, and none of the patients with skin diseases were affected. This indicates that *Malassezia spp.* exclusively affects patients without skin diseases in this sample. Our results demonstrate that *Aspergillus niger* showed the highest overall prevalence and was more common among patients without skin diseases. *Aspergillus flavus* and *Malassezia spp.* had a lower prevalence, with *Malassezia spp.* exclusively affecting patients without skin diseases.

Table (6): The Relationship Between Fungal Type Prevalence and Patients with skin diseases / Patients without skin diseases.

Type of Bacteria	**Total Number of Affected**	**Patients with skin diseases(n=14)**			**Patients without skin diseases(n=28)**		
		N	%	Total % of Affected	N	%	Total % of Affected
As. niger	**29**	**10**	71.43	**34.48**	19	67.86	**65.52**
As. flavus	**3**	**1**	7.14	**33.34**	2	7.14	**66.67**
Malassezia spp.	**5**	**0**	0.00	**0.00**	5	17.85	**100**
Total	**37**	11		**29.73**	26		**70.27**

Chi-square analysis indicated no statistically significant difference in the distribution of fungal species between skin disease and non-skin disease patients ($\chi^2 = 2.4884$, $p > 0.05$, critical value = 5.991 at df = 2).

This current study, as shown in Table (7), illustrates a notable variation in the distribution of fungal species (*Aspergillus niger*, *Aspergillus flavus*, and *Malassezia spp.*) in relation to dandruff type (dry vs. oily) on the scalp. Regarding *A. niger*, 12 samples were observed with dry dandruff, representing 30% of the total sample, while 17 samples were found with oily dandruff, accounting for 42.5% of the total. Thus, *A. niger* accounted for 29 samples, representing 48.3% of the overall sample size. As for *A. flavus*, the distribution was considerably lower. Only 2 samples were identified with dry dandruff, corresponding to 5% of the total, while 1 sample was found with oily dandruff, representing 2.5%. Therefore, the total number of *A. flavus* samples was 3, accounting for 5% of the overall sample. Concerning *Malassezia spp.*,1 sample was found with dry dandruff, corresponding to 2.5% of the total, while 4 samples were identified with oily dandruff, representing 10%. In total, *Malassezia spp.* accounted for 5 samples, representing 8.3% of the overall sample size. The total sample size was 60, with 15 samples exhibiting dry dandruff (37.5%) and 22 samples showing oily dandruff (55%). Chi-square analysis indicated a statistically significant difference in the distribution of fungal species between dry dandruff and oily dandruff ($\chi^2 = 8.99$, $p < 0.05$, critical value = 5.991 at df = 2). This suggests that the type of dandruff has a significant impact on the distribution of specific fungal species on the scalp.

Table (7): Fungal Spread and its Relationship to Dandruff Type (Dry/Oily).

Fungal Species	Dry Dandruff		Oily Dandruff		Total
	Number (N)	Percentage (%)	Number (N)	Percentage (%)	
A. niger	12	30%	17	42.5%	**29**
A. flavus	2	5%	1	2.5%	**3**
Malassezia spp	1	2.5%	4	10%	**5**
Total	**15**	**37.5%**	**22**	**55%**	**37**

Chi-square analysis indicated a statistically significant difference in the distribution of fungal species between dry dandruff and oily dandruff ($\chi^2 = 8.99$, $p < 0.05$, critical value = 5.991 at df = 2)

As shown in Table 8, the study presents the antibiotic susceptibility profiles of ***Staphylococcus spp.*** strains isolated from dandruff samples, evaluated against six antimicrobial agents: Chloramphenicol (CHL), sulfamethoxazole/Trimethoprim (SXT), Streptomycin (ST), Penicillin (PG), Tetracycline (TE), and Ampicillin (AMP). The activity of ***S. aureus*** and ***S. epidermidis*** was categorized as susceptible (S), intermediate (I), or resistant (R) based on the inhibition zones observed for each antibiotic. For ***S. aureus*** (n=3), the results showed full susceptibility to Chloramphenicol (100%), while the strain was resistant to Penicillin (PG), Tetracycline (TE), and Ampicillin (AMP), each exhibiting 100% resistance. In response to sulfamethoxazole/Trimethoprim (SXT), the strain displayed intermediate susceptibility (33.3%), with the remaining isolates being resistant (66.7%). Streptomycin (ST) also showed an intermediate response, with 33.3% of the isolates displaying intermediate susceptibility and 66.7% being resistant. For ***S. epidermidis*** (n=3), the strain showed full susceptibility to Chloramphenicol (100%) and Streptomycin (100%). However, it was resistant to Penicillin (PG) (100%) and Ampicillin (AMP) (100%). For Tetracycline (TE), ***S. epidermidis*** exhibited 33.3% susceptibility and 66.7% resistance, while it showed an intermediate response to sulfamethoxazole/Trimethoprim (SXT) (100% intermediate).

Table (8): Percentage antibiotic activity of *staphylococcus. Spp.* isolated from dandruff.

Bacterial species	State	Antimicrobial agents					
		CHL	SXT	ST	PG	TE	AMP
S. aurous **(n=3)**	S % I % R %	(100%) (0%) (0%)	(0%) (33.3%) (66.7%)	(100%) (0%) (0%)	(0%) (0%) (100%)	(0%) (0%) (100%)	(0%) (0%) (100%)
S. epidermis**(n=3)**	S % I % R %	(100%) (0%) (0%)	(0%) (100%) (0%)	(100%) (0%) (0%)	(33.3%) (0%) (66.7%)	(100%) (0%) (0%)	(0%) (0%) (100%)

S: susceptible, I: Intermediate, R: resistant. C30: Chloramphenicol 30 µg: sulfamethoxazole/trimethoprim (Sxt) 25, Streptomycin (ST)10 µg, Penicillin(P)10 µg, Tetracycline (TE)10 µg, Ampicillin (AMP)10 µg.

As shown in Table 9 the percentage of antifungal activity exhibited by different fungal species isolated from dandruff samples, evaluated against three antifungal agents: Ketoconazole (KT), Nystatin (NY), and Miconazole (MCL). The activity of each fungal strain was classified as susceptible (S), intermediate (I), or resistant (R), based on the response to each agent. The results show that *Aspergillus niger* species was fully resistant to Ketoconazole (100%) and Nystatin (0%), while it exhibited complete susceptibility to Miconazole (100%). In contrast, *Aspergillus flavus* demonstrated resistance to Ketoconazole (100%) and Nystatin (0%) but was partially susceptible to Miconazole (66.7%). *Malassezia spp.*, another common fungus found in dandruff, exhibited complete resistance to Ketoconazole (100%) and Nystatin (0%), with partial susceptibility to Miconazole (33.3%). These findings indicate a significant variability in the antifungal activity of the agents tested, with Ketoconazole showing the highest level of resistance among the fungal species studied, followed by Nystatin. Miconazole, on the other hand, displayed better efficacy, especially against *Aspergillus niger* and *Aspergillus flavus*. The results highlight the potential limitations of certain antifungal agents, such as Ketoconazole in treating dandruff-associated fungal infections, and underscore the need for alternative therapeutic options for fungal species that exhibit high resistance to conventional treatments.

Table (9): Percentage antifungal activity of different fungi isolated from dandruff.

Fungal species	State	Antimicrobial agents		
		KT	NY	MCL
A. niger	S %	(0%)	(100%)	(0%)
	I %	(0%)	(0%)	(100%)
	R %	(100%)	(0%)	(0%)
A. flavus	S %	(0%)	(100%)	(66.7%)
	I %	(0%)	(0%)	(33.6%)
	R %	(100%)	(0%)	(0%)
Malassezia spp.	S %	(0%)	(100%)	(33.3%)
	I %	(0%)	(0%)	(33.3%)
	R %	(100%)	(0%)	(33.3%)

S: susceptible, I: Intermediate, R: resistant. Ketoconazole (KT). Nystatin (NY). Miconazole (MCL).

Discussion

Dandruff is a prevalent dermatological condition affecting approximately 50% of the global population. Its management primarily involves antifungal treatments; however, the exact role of microbes in exacerbating the condition remains poorly understood [20]. Bacteria were identified as causative agents in 50 cases (96.15% of total cases), while fungi were responsible for 35 cases (67.30%). These findings align with previous studies indicating that both bacteria and fungi are significant pathogenic factors in dandruff [8,20,21].

In the current study, the most abundant bacterial genera on the scalp were ***Staphylococcus aureus*** and ***Staphylococcus epidermidis***, consistent with studies from France [8] and China [22]. The data indicate that ***Staphylococcus epidermidis*** is a major bacterial species on the scalp, and its abundance is associated with dandruff. This aligns with [13], who reported that ***Staphylococcus epidermidis*** accounted for 41.3% of cases. In contrast, ***Propionibacterium acnes*** was the most common bacterial isolate (54.8%), followed by ***Staphylococcus aureus*** (2.33%) and ***Staphylococcus capitis*** (1.5%) [13].

Among fungi, ***Aspergillus niger*** was the most common isolate (29.89%), followed by ***Aspergillus flavus*** (3.10%). These findings are similar to Obum-Nnadi et al., [9], who reported ***Aspergillus niger*** in 25% of cases. Additionally, ***Malassezia spp.*** accounted for 5.15% of cases, differing from studies in Ethiopia where its prevalence was 51.15% [23]. This variation may be attributed to factors like climate or ethnicity. Clavaud et al. [8] found ***Malassezia spp.*** to be the predominant fungal species on the scalp, accounting for 97% and 84% of sequences in normal and dandruff subjects, respectively.

The study found no significant difference in dandruff prevalence between males and females, consistent with [9,13]. However, Basak et al., (2019)[24] reported higher prevalence rates in males (20.7%) compared to females (12.8%). Most affected individuals were aged 20–30 years, with prevalence rates of 46.67% for bacteria and 35% for fungi. The lowest prevalence was observed in individuals over 41 years (4% for bacteria and 12.5% for fungi). Similar findings were reported by [13,24,25]. The higher prevalence in younger age groups may be due to factors like excessive sweating, exposure to contaminated environments, and lack of disease awareness. A study by Ghosh et al., [26] supports these findings, indicating that such activities contribute to higher infection rates in these populations. *A. niger* was the most prevalent fungal species across all age groups, while *A. flavus* and *Malassezia spp*. were less common, with Malassezia spp. being more closely linked to dandruff, particularly in younger age groups. The total prevalence was highest in the 21–30 years group, followed by the 11–20 years group. The chi-square analysis indicated that there was no statistically significant difference in the distribution of fungal species across different age groups, suggesting that age does not significantly influence the prevalence of these fungi in individuals with dandruff. These results are consistent with a study by Gupta et al., [27] that reported a high prevalence of Malassezia species in older individuals (15-25 years). The results obtained in this study revealed that the highest prevalence of Malassezia was observed in the 10 to 20 years age group. This finding is consistent with a study conducted in Japan, which indicated that the highest prevalence of Malassezia occurred in males aged 16-18 years and in females aged 10-12 years [28]. The findings in this study indicate that *Staphylococcus aureus* and *Aspergillus niger* are more frequently found in patients with skin diseases compared to those without. ***Staphylococcus aureus*** and ***Aspergillus niger*** were more frequently found in patients with skin diseases, with prevalence rates of 92.85% and 71.43%, respectively. This aligns with Ong et al., [29], who attributed this susceptibility to deficiencies in antimicrobial peptides like β-defensins and cathelicidins [30,31]. Additionally, a previous study reported a predominance of ***Aspergillus spp.*** isolates (82.67%) as the main cause of fungal ear infections, with ***Aspergillus niger*** accounting for 43.5% and ***Aspergillus flavus*** for 38.63% in otomycosis cases [32]. These results reflect the distribution of this fungus in the study area. The results show significant variability in antibiotic resistance profiles and antifungal activity among ***Staphylococcusspp.*** strains isolated from dandruff

samples. **Staphylococcus aureus** exhibited complete resistance to Penicillin, Tetracycline, and Ampicillin but was fully susceptible to Chloramphenicol (100%). An intermediate response to sulfamethoxazole /Trimethoprim (33.3%) and resistance in the remaining isolates (66.7%) indicate emerging resistance to commonly used antibiotics. This finding is consistent with [33,34,35], who reported high resistance rates to Oxacillin, Penicillin, and Ampicillin. In contrast, **Staphylococcus epidermidis** showed full susceptibility to Chloramphenicol and Streptomycin but complete resistance to Penicillin and Ampicillin. Multi-antibiotic-resistant strains were widespread, highlighting the complexity of antimicrobial resistance.

The common dandruff-associated fungus **Malassezia spp.** showed complete resistance to Ketoconazole (100%) but was fully susceptible to Nystatin (100%) and partially susceptible to Miconazole (33.3%). Ketoconazole and Itraconazole demonstrated potent in vitro antifungal activity against **Malassezia spp.**, but resistance to Ketoconazole is consistent with recent studies [24]. These findings suggest that Nystatin and Miconazole may be more effective therapeutic options for dandruff-associated fungal infections, although alternative treatments may still be needed in some cases.

Conclusion:

This study identified *Staphylococcus aureus* (37.11%) and *Aspergillus niger* (29.89%) as the most prevalent bacterial and fungal species in dandruff. No significant differences were found in microbial distribution by gender or age, though the 21–30 age group showed the highest prevalence .*S. aureus* and *A. niger* were more common in skin disease patients. Antibiotic resistance was notable, with *S. aureus* resistant to Penicillin, Tetracycline, and Ampicillin but fully susceptible to Chloramphenicol. Among fungi, *Malassezia spp.* was resistant to Ketoconazole but susceptible to Nystatin and Miconazole. The findings highlight the need for alternative treatments and further research into antimicrobial resistance mechanisms.

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