

Coexistence of *Malassezia* Species and *Microsporum canis* in the Lesions of Adult with Tinea Capitis

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Abstract: Tinea capitis, primarily caused by dermatophytes such as *Trichophyton* and *Microsporum* species, is a superficial fungal infection affecting the scalp and hair, commonly observed in prepubertal children but rare in adults. Here we report a unique case of an adult female with tinea capitis presenting as diffused alopecia and erythema inflammation on the scalp's apex, mimicking seborrheic dermatitis. Examination of the hair and scalp using fluorescence microscopy and fungal culture identified the presence of hyphae from *Malassezia globosa*, *Malassezia furfur* and *Microsporum canis*. The patient underwent with oral antifungal treatment for 3 months, resulting in the resolution of the rash and subsequent hair regrowth, with no recurrence during 6-month follow-up. In vitro co-culture experiments of *Microsporum canis* and *Malassezia* (both *Malassezia globosa* and *Malassezia furfur*) revealed that *Malassezia* appears to facilitate *Microsporum canis* growth, while the reverse was not observed. This data suggests that *Malassezia*'s use of long-chain fatty acids by might reduce its antibacterial effect, potentially aiding adult tinea capitis development caused by *Microsporum canis*.

Keywords: *Microsporum canis*, *Malassezia*, tinea capitis, coinfection

Introduction

Tinea capitis is a superficial fungal infection that affected the scalp and hair, predominately occurring in children, particularly those between the ages of 2 and in 8. Infection is rare in adults.¹ In children with tinea capitis, there is usually no significant difference in the proportion of affected boys and girls in. However, in adult cases of tinea capitis, female are more commonly affected.^{2,3} The main causative fungi for both children and adults are dermatophytes, which include anthropophilic, zoophilic and geophilic fungi.

In recent years, several studies have shown an increasing incidence of tinea capitis in healthy adults, which emphasizes the necessity for greater attention to this condition in future research.^{2,4} Adult tinea capitis often presents with mild inflammatory symptoms, making a challenge in differentiation from psoriasis capitis, seborrheic dermatitis and atopic dermatitis of the scalp.⁵ This might be attributed to the development of sebaceous glands during puberty, which secrete long-chain fatty acids known for their antifungal properties.² It has been suggested that differences in scalp microorganisms between children and adults due to gradual maturation of the sebaceous gland during puberty, leading to increased sebum production and the lipophilic microorganisms such as *Malassezia* and *Cutibacterium*.⁶⁻⁸ After menopause, the incidence of tinea capitis tends to increase due to decreased sebaceous gland secretion and scalp oil.⁹

We encountered a 60-year-old female in our clinical setting with tinea capitis, exhibiting three types of fungal growth: *Microsporum canis*, *Malassezia globosa*, and *Malassezia furfur*. Interestingly, we observed that *Malassezia* and *Microsporum*

canis can coexist on the scalp, especially in regions with heightened sebum production. To investigate the interaction of *Microsporum canis* and *Malassezia*, we conducted in vitro co-cultures of isolated *Microsporum canis* with two species of *Malassezia*. Our finding indicated that *Malassezia* may promote the growth and activity of *Microsporum canis*. These findings hint at a potential association between the atypical manifestations of adult tinea capitis and specific *Malassezia* species.

Case Presentation

On May 1, 2022, a 60-year-old female presented to our department with complaints of rapid diffuse hair loss on the top of her scalp, accompanied by mild pain for a month. She had then been previously diagnosed with folliculitis of the scalp and received treatment with various antibiotics at a local hospital, but her symptoms did not improve. The patient has a history of type 2 diabetes, which has been well-managed with oral medications, including metformin, acarbose and glimepiride, with her blood glucose levels maintained within the normal range. There is no history of autoimmune diseases or tumors. Upon further investigation, it was revealed that the patient had been in contact with a dog before the onset of disease. However, no areas of shedding or rash were observed on the dog.

During the physical examination, patchily greasy erythema was observed on the top of the scalp, with fine flakes that were easily removable. The hair on the top of the head appeared sparse, with some strands being dry and tapered (Figure 1A). Wood lamp examination revealed bright green fluorescence in the area of hair loss on the top of the head (Figure 1B).

The patient's blood routine and erythrocyte sedimentation rate examination were within normal limits. Fluoroscopy of the patient's dandruff and hair showed a significant presence of mycelium and spores. The fungal culture results and molecular sequencing confirmed the coexistence of *Microsporum canis*, *Malassezia globosa* and *Malassezia furfur*.

The patient was diagnosed with tinea capitis based on the presence of a rash and findings from fluorescence microscopic. The patient was informed about the necessary precautions for managing conditions, including cutting her hair and disinfecting bedding sheets and other items. The patient was treated with oral itraconazole at a dose of 200 mg per day and topical ketoconazole ointment twice daily for a duration of 2 weeks. Prior to starting treatment, the patient had her hair cut short (Figure 1C). Three weeks later, the erythema on the top of the head had disappeared, desquamation was significantly reduced, the hair became thicker compared to before, and the fluorescence microscopy test showed a negative finding (Figure 1D). During the 2-month follow-up, there was no signs of erythema or desquamation, and no recurrence was observed at the 6-month- follow-up.

Mycological Study on the Causative Pathogen from Scalp Lesions

The Direct Observation of Causative Pathogen Under Fluorescent Microscopy and Light Microscopy

Specimens were collected for direct mycological examination. The scalp scales and hair were stained with calcofluor white stain (CFW) and examined under a fluorescence microscope. The results revealed the presence of antler-like,

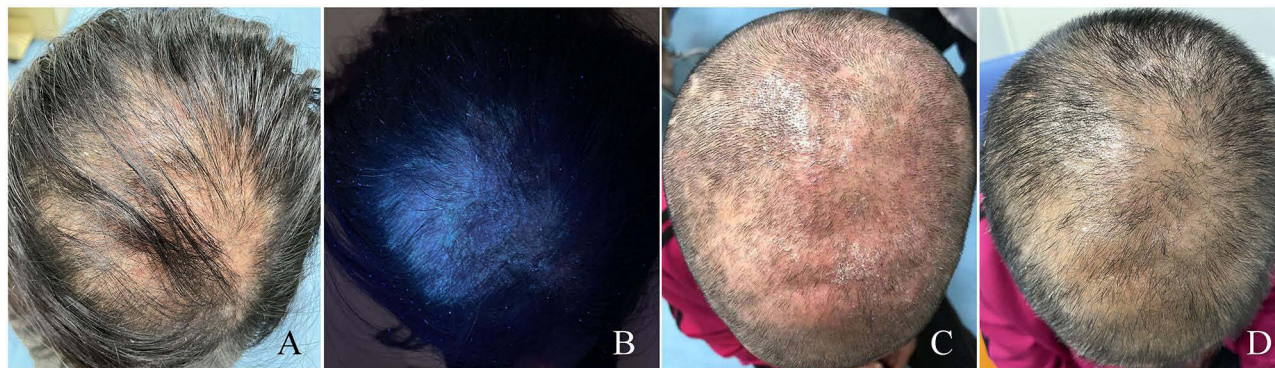


Figure 1 The clinical manifestations of the patient. (A) image showing the seborrheic dermatitis-like rash and hair loss on the scalp of patients with initially diagnosed tinea capitis. (B) image showing the lesions represents the same field as A under the Wood lamp. (C and D) images showing the lesions on the scalp of patients with tinea capitis before and after treatment.

separated hyphae and globose spores (Figure 2A). Additionally, large spindle-shaped conidia were observed in the same field of view when examined under a light microscope (See Figure 2B and Supplementary Material 1). Further examination of scalp scrapings and hair from the affected area on the top of scalp showed a significant presence of antler branch hyphae and spherical spores both under a fluorescence microscope (Figure 2C) and a light microscope (Figure 2D).

In the occipital area, spherical spores of varying sizes were observed in the scalp debris under fluorescence microscope (Figure 2E), as well as in corresponding light microscope images (Figure 2F). Hair samples from this area showed the presence of attached spores but no hyphae when examined under a fluorescence microscope (Figure 2G) and a light microscope (Figure 2H). Generally, under direct microscopic examination, fungal load in dander and hair samples from the top of the scalp is greater than that observed in samples from the occipital area, as evidenced by more severe damage in the samples from the top of the scalp.

The Isolation and Identification of Causative Pathogen

Dander samples were scraped from the affected scalp area and the periphery, and then inoculated into a medium for a period of 7 days. The results revealed fast-growing colonies with villous-shaped mycelium on Sabouraud dextrose agar (SDA) from both the parietal region (Figure 3A) and occipital region (Figure 3B). Initially, the colonies appeared white at the center and gradually changed to grey towards the periphery. The cultured colonies from the top of the scalp samples grew faster compared to the occipital samples.

In addition, the colonies on mDixon agar displayed light yellow cheesy colonies and plush filamentous colonies, both from the parietal region (Figure 3C) and occipital region (Figure 3D). However, the filamentous colonies were more pronounced in the scalp samples, while the cheesy colonies were more pronounced in the occipital samples.

The primary cultured colonies from the parietal region were further purified on SDA and mDixon agar. Three colonies with different textures, shapes, and sizes were isolated. To identify the fungal strains, DNA sequencing was performed on the isolated strains using the ITS1/ITS4 region of ribosomal DNA (rDNA). The isolated fungi were identified as *Microsporum canis* (Figure 3E), *Malassezia globosa* (Figure 3F), and *Malassezia furfur* (Figure 3G).

To study the morphology and characteristics of *Microsporum canis*, the small-culture method was employed to cultivate and observe the characteristics. After 6 days of cultivation in the rice medium, a fluorescent dye was added to a slide. The cover slide was then removed from the petri dish and placed on the slide, allowing it to sit for 5 minutes before microscopic observation. Under both a light microscope (Figure 4A) and a fluorescence microscope (Figure 4B),

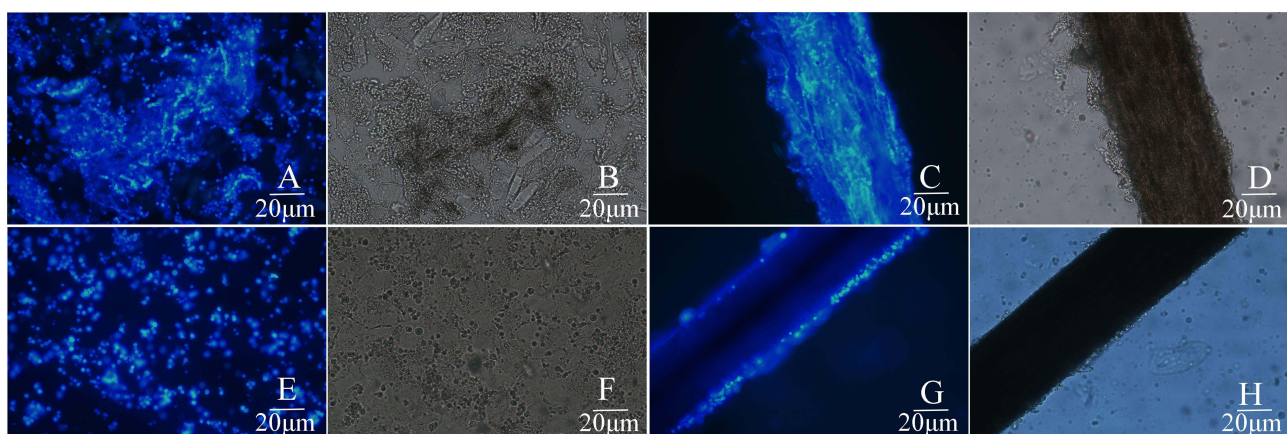


Figure 2 Specimens from the skin lesion were subjected to direct microscopic examination. (A) a representative image of fungal hyphae and spores from the scalp scales on the top of head staining with calcofluor white under fluorescence microscope (original magnification, $\times 400$); (B) represents the same field as A under the light microscope. (C) a representative image for fungal hyphae and spores from hair in the rash area on the top of the head staining with calcofluor white under fluorescence microscope (original magnification, $\times 400$); (D) represents the same field as C under the light microscope. (E) a representative image of fungal spores from the scalp scales on the occipital staining with calcofluor white under fluorescence microscope (original magnification, $\times 400$); (F) represents the same field as (E) under the light microscope. (G) a representative image for fungal spores from hair in the rash area on the occipital staining with calcofluor white under fluorescence microscope (original magnification, $\times 400$); (H) represents the same field as G under the light microscope.

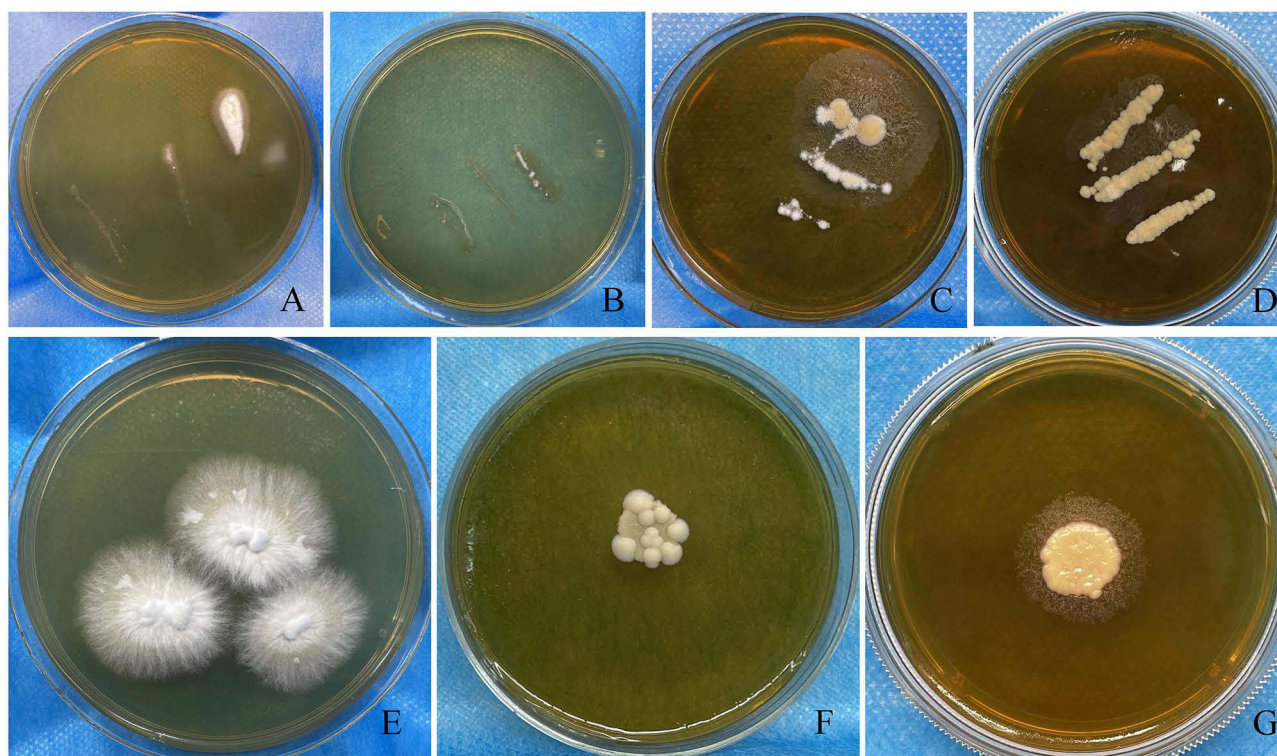


Figure 3 The culture results of patients with tinea capitis. (A and B) the image for direct culture of colonies on Sabouraud's Dextrose agar (SDA) after sampling from the scales on the top of the head and occipital, respectively. (C and D) the image for direct culture of colonies on Modified Dixon agar after sampling from the scales on the top of the head and occipital, respectively. (E) a representative image for purification of colony of *Microsporidium canis* after culture on Sabouraud's Dextrose agar at 37 °C for 1 week. (F) a representative image for purification of colony of *Malassezia globosa* after culture on Modified Dixon agar at 37°C for 1 week. (G) a representative image for purification of colony of *Malassezia furfur* after culture on Modified Dixon agar at 37 °C for 1 week.

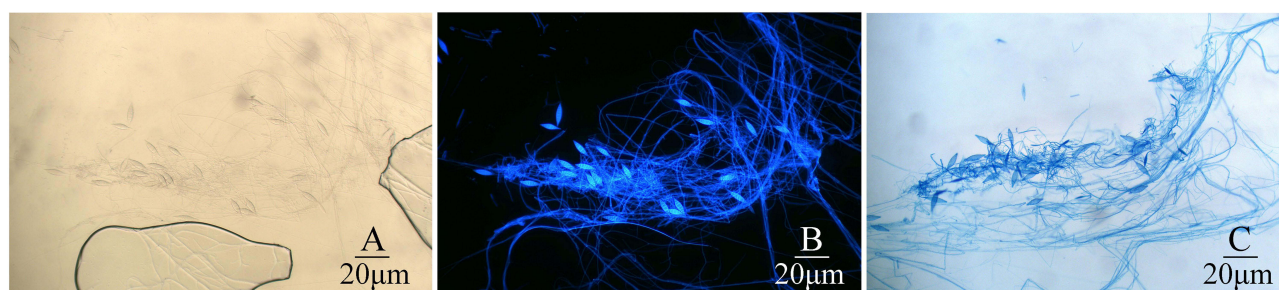


Figure 4 Image showing microscopic observation of *Microsporidium canis* in the same part of the paddle after 7 days of rice medium culture. (A) Image showing *Microsporidium canis* under light microscope (original magnification, ×400); (B) Image showing *Microsporidium canis* staining with calcofluor white under fluorescence microscope (original magnification, ×400); (C) Image showing *Microsporidium canis* staining with lactophenol cotton blue under light microscope (original magnification, ×400).

the morphology of the microorganism was observed in the same field of view. The majority of the large conidia appeared spindle-shaped, with thick, rough, and prickly walls, as well as septal hyphae. However, the separation of mycelium and the morphology of the conidia were clearer and more accurate under the fluorescence microscope.

Subsequently, lactophenol cotton blue dye was added from the edge of the same slide, and any excess dye was absorbed using filter paper on the other side. Once the entire slide was filled with lactophenol cotton blue dye, the morphology of *Microsporidium canis* was observed under a light microscope in the same field of view as shown in Figure 4A and B. The results revealed clear peripheral contours of the mycelia and conidial morphology (Figure 4C).

The Co-Culture of *Microsporum canis* and *Malassezia* in vitro

To investigate whether *Malassezia* inhibits the growth of *Microsporum canis*, we conducted co-culture experiments using the isolated *Microsporum canis* and two species of *Malassezia* (*Malassezia globosa* and *Malassezia furfur*) from this patient.

First, both *Malassezia globosa* and *Malassezia furfur* were suspended in normal saline and diluted to a concentration of 1×10^4 /mL. Then, 200 μ L of each *Malassezia* suspension was added to individual plate containing SDA or mDixonA medium and subsequently spread evenly on the surface of the agar. Next, a 2×2 mm² colony of *Microsporum canis* was picked up using a sterile pick stick and placed in the center of the medium. The growth of the *Microsporum canis* colony was observed. Control culture plates containing *Microsporum canis* or *Malassezia* only were also set up to monitor the colony growth.

The combination of these three fungal species led to the formation of five experimental groups: (1) *Malassezia globosa* group, (2) *Malassezia globosa* and *Microsporum canis* co-culture group, (3) *Microsporum canis* group, (4) *Malassezia furfur* and *Microsporum canis* co-culture group, and (5) *Malassezia furfur* group. All groups were cultured simultaneously in SDA and mDixonA media at temperatures of 27°C, 30°C, and 37°C.

As show in Figure 5A and B, after a 10-day culture period, *Malassezia globosa* did not growth in mDixonA medium at 27°C, but its growth increased at higher culture temperatures. Similarly, *Malassezia furfur* showed increased growth with higher temperatures. Conversely, in mDixonA medium, the diameter of *Microsporum canis* colonies decreased gradually at 27°C, 30°C, and 37°C. No growth of *Malassezia* was observed in SDA medium, and the diameter of *Microsporum canis* colonies decreased with increasing temperature (Figure 5C and D).

Interestingly, was co-culturing *Microspora canis* with *Malassezia furfur* in the mDixon medium at 27°C, 30°C and 37°C resulted in larger colony diameter compared to when *Microspora Canis* was cultured alone ($P= 0.0005$, $P< 0.0005$, $P< 0.0005$). Opposite phenomenon was observed when *Malassezia globosa* and *Microspora Canis* were co-cultured at 27°C and 30°C ($P= 0.0005$, $P= 0.0251$). However, no similar findings were found on SDA media.

These findings indicate that *Malassezia* and *Microsporum canis* can coexist in areas of the scalp with high sebum secretion. Additionally, *Malassezia* may even promote the growth and activity of *Microsporum canis*.

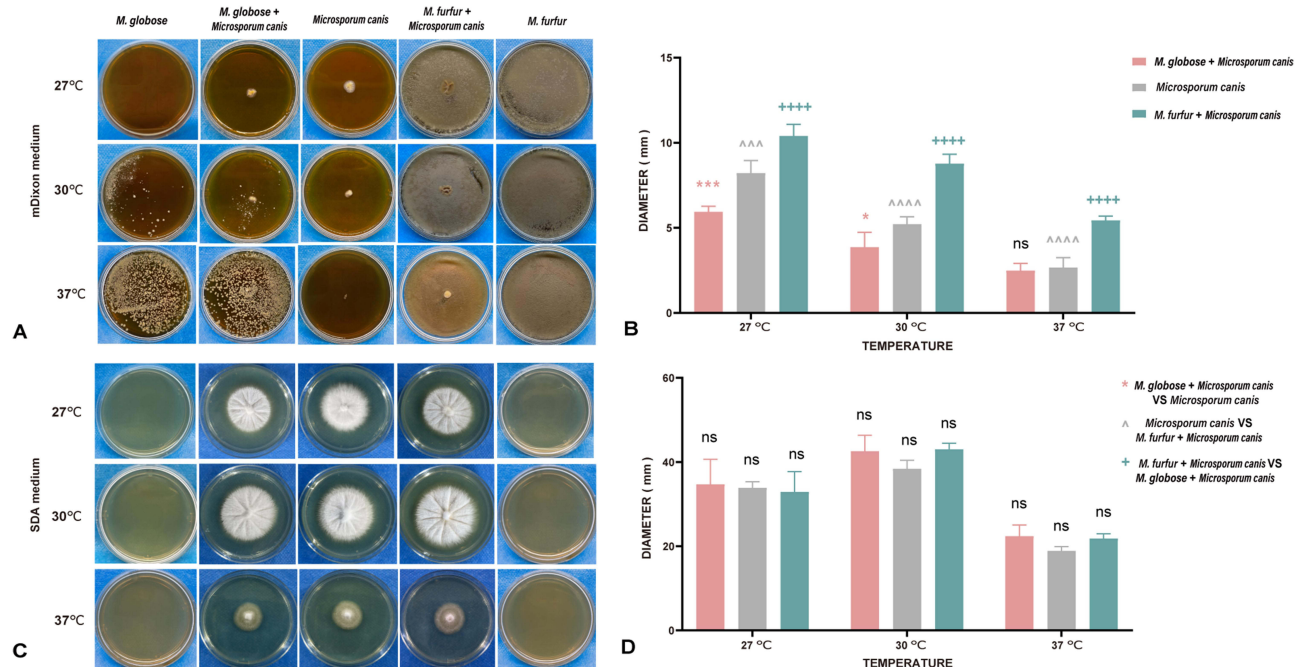


Figure 5 The effect of *Malassezia* on the growth of *Microspora canis* in vitro. The experiment included groups: *Microspora canis* cultured alone, *Microspora canis* co-cultured with *Malassezia globosa*, and *Microspora canis* co-cultured with *Malassezia furfur*. The strains were inoculated on mDixon agar or Sabouraud dextrose agar (SDA) and incubated at temperatures of 27°C, 30°C, and 37°C for a period of 10 days. The colonies on mDixon agar (A) or SDA (C) were photographed and the diameter of the colonies was measured using the ImageJ software. The diameter of the colonies was measured and recorded on the Y-axis (B and D). To ensure reliability and consistency, the experiment was repeated three times. P is considered significant when <0.05 ; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$ (**Malassezia globosa* and *Microsporum canis* co-culture group VS *Microsporum canis* group; ^*Microsporum canis* group VS *Malassezia furfur* and *Microsporum canis* co-culture group; +*Malassezia furfur* and *Microsporum canis* co-culture group VS *Malassezia globosa* and *Microsporum canis* co-culture group).

Discussion

Tinea capitis is primarily caused by tinea fungi, with *Trichophyton* and *Microsporum* species being the main culprits. In Europe and Southeastern Korea, *Microsporum canis* is still the most commonly reported causative agent of tinea capitis.^{4,10} In Portugal, the most common etiological agents are *Microsporum audouinii* (20%) and *Trichophyton rubrum* (20%).⁹ Changing in hygiene conditions and lifestyle in America and England, have resulted in a different epidemiology of dermatophytosis, with *Trichophyton tonsurans* emerging as the most prevalent species.^{11–13} A multicenter study has indicated that zoophilic agents are more likely to infect children in China, while adult patients are more susceptible to anthropophilic dermatophyte infections. Although the species spectrum of pathogenic fungi may vary across regions, *Microsporum canis* still remains the primary pathogen for both children and adult patients with tinea capitis.¹

Lipophilic yeasts of the genus *Malassezia* are important skin commensals and opportunistic skin pathogens in a variety of animals. They live almost exclusively on the skin and mucosal sites of warm-blooded vertebrates¹⁴ and birds.¹⁵ The pathogenic potential of *Malassezia* for infection and its association with other diseases, such as cancer, have been increasingly reported.^{16–20} *Malassezia* species are the most abundant fungi found in all sampled healthy human skin body sites except the feet, with *M. restricta* and *M. globosa* standing out as the most common and most numerous. Microbiome dynamics revealed that healthy skin communities dominated by *M. restricta*, *M. globosa* and *M. furfur*.²¹ Although the skin is the primary *Malassezia* reservoir, it can be found in other parts of the body, including the human and murine gut, human breast milk, and internal organs including those of the central nervous system.²² Various *Malassezia* species occur on human skin as commensals, and they are also associated with multiple skin disorders, such as pityriasis versicolor, *Malassezia* folliculitis, seborrheic dermatitis/dandruff, atopic dermatitis, and psoriasis.²³

Recently, the incidence of tinea capitis in healthy adults is increasing, accompanied by notable changes in the microbial spectrum. These changes require warrant caution in future clinical practices. The alteration of the scalp microflora greatly influences the onset and progression of the disease, and the susceptibility of patients to tinea capitis may contribute to the shift in the scalp microbial environment.^{7,8,10} A study conducted by Tao et al observed that the Shannon diversity of the bacterial community was higher in tinea capitis patients when compared to the healthy scalp samples. Non-metric multidimensional scaling (NMDS) analysis at the genus level revealed interesting results. While two specimens exhibited the presence of *Microsporum canis* and *Malassezia* in culture, the NMDS results indicated a higher abundance of *Malassezia* compared to *Microsporum canis*, especially when *Malassezia globosa* and *Malassezia restricta* were presented.⁷

Tinea capitis is a rare condition in adults, with the main incidence occurring among postmenopausal women. Hormonal changes in the body after menopause, characterized by decreased blood estrogen levels, can contribute to this condition. The sebaceous glands are affected by these changes, leading to a reduction in the fungistatic action of long-chain fatty acids present in sebum.^{2,3,6} Additionally, the increasing prevalence of adult tinea capitis may be associated with underlying conditions such as diabetes, increased use of steroids and immunosuppressants, as well as reduced triglycerides with antifungal properties.⁵

In this case, we observed an adult patient presenting with seborrheic dermatitis in the scalp rash area, accompanied by hair loss. A significant number of fungal spores were found in the affected area, and the sample under direct microscope observation and culture results in mDixon medium confirmed the presence of *Malassezia spp.* *Malassezia* tends to flourish and multiply in areas of the human body where sebum secretion is abundant due to the absence of fatty acid synthetase.²⁴ The skin lesions of our patient contained a significant amount of lipid components, providing an ideal environment for the growth of *Malassezia*. Interestingly, the direct observation under microscope and fungal culture results revealed the coexistence of *Microsporum canis*, *Malassezia globosa*, and *Malassezia furfur* in the lesion area, particularly in regions with high sebum secretion. It is worth noting that the clinical manifestations of tinea capitis in adults often differ significantly from those in children and may present with atypical rashes resembling seborrheic dermatitis.²⁵ Our findings suggest that *Malassezia* may have a promoting role in the development of tinea capitis, which could explain the atypical clinical manifestations observed in adult patients.⁶

Jo et al observed that prepubertal skin hosts a variety of fungi, while *Malassezia* species dominate adult skin, and the fungal community on the skin undergoes significant changes during puberty.^{8,26} During puberty, as sebaceous glands differentiate and mature, sebum secretion increases, which is associated with a decrease in tinea capitis incidence. Conversely, after menopause, sebaceous gland secretion decreases, leading to an increase in tinea capitis incidence.²⁷ Although previous studies have shown that

ethyl ester derivatives generated through the esterification of medium-chain fatty acids by *Malassezia* exhibit in vitro antimycotic activity,²⁸ and *Malassezia globosa* secretes aspartyl protease 1 (MgSAP1) with demonstrated in vitro antibacterial properties.²⁹ Our co-culture experiments yielded opposite results. We observed that *Malassezia* may play a role in promoting the growth of *Microsporum canis*. This could be attributed to the potential consumption of long-chain fatty acids by *Malassezia*, which weakens its antibacterial effect and may contribute to the occurrence of adult tinea capitis.^{27,30} These findings suggest that tinea capitis rashes may not be caused by a single strain and may be related to the balance of microorganisms in the scalp environment. *Malassezia*, as the most common commensal flora, especially on the scalp, warrants greater attention whenever addressing skin conditions.

Although the growth of *Microsporum canis* was increased by the presence of *Malassezia* in vitro, the pathogenic role of *Malassezia* in this case remains to be determined. We recognize the limitations inherent in this study, as it stands, this report represents a singular case study, necessitating additional cases to substantiate our hypothesis regarding the interaction between different fungal species.

In summary, our case report and co-culture experiment suggest a potential promoting role of *Malassezia* in the development of adult tinea capitis. While *Malassezia* is responsible for the manifestation of seborrheic dermatitis in the scalp, it may contribute to conditions like tinea capitis by consuming long-chain fatty acids. Further research is necessary to explore the complex relationship among skin microbiome and understand the mechanisms involved in interaction between different fungal species. To provide more accurate guidance for clinical diagnosis and treatment, it is crucial for future investigations and experiments to monitor the pathogenic impact of microbiome changes.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

Ethics Approval and Informed Consent

This study was approved by the Institutional Research and Ethics Committee of Jining No. 1 People's Hospital to publish the case details (Ethical approval no. 2022-029). The patient provided written informed consent for publication of this case report and any accompanying images. The study was carried out in accordance with the principles of the Declaration of Helsinki. The first author vouches for the completeness and accuracy of the data and for the adherence of the study to the protocol.

Consent for Publication

Written informed consent was obtained from the patient described in this report. A copy of the written consent is available by request.

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Disclosure

The authors report no conflicts of interest in this work.

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