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Case Report

Co-isolation of *Trichosporon inkin* and *Candida parapsilosis* from a scalp white piedra case

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White piedra is a rare fungal infection of the hair shaft characterized by small, firm, irregular white-brown nodules. The infection is caused by basidiomycetous yeasts in the genus *Trichosporon*. We report a case of a 28-year-old female patient who acquired the infection in Qatar. In this case, the scalp was the only site affected, but infection at that site was extensive. The hair had a *Saccharomyces*-like yeast odor and appeared to be beaded, with light-brown nodules of varying sizes up to 2 mm long. *Trichosporon* sp. accompanied by *Candida parapsilosis* grew out along hair shafts planted in primary isolation media. Molecular identification of the *Trichosporon* carried out by analyzing the 26S ribosomal gene gave a 100% match with *Trichosporon inkin,* a major cause of pubic white piedra. The patient was treated with daily applications of ketoconazole shampoo followed by econazole shampoo and cream, and was considered clinically and mycologically cured after 2 months. Novel findings in the present case are the first identification of *T. inkin* as an agent of scalp white piedra, and the heavy outgrowth of *C. parapsilosis* from the concretions, although in the latter case it is not clear if the co-occurring yeast was etiologically contributory to the pathogenesis of the white piedra.

Keywords *Candida parapsilosis*, scalp hair, *Trichosporon inkin*, white piedra

Introduction

Piedra is a fungal infection of the hair shaft, and it is characterized by the formation of small, firm, irregular nodules. If the nodules are dark, the infection is classed as black piedra and the nodule consists of the ascomycetous fruiting bodies (ascostromata) of the fungus *Piedraia hortae*. If the nodule is whitish or brownish off-white, the infection is called white piedra. White piedra was considered for a long time to be produced by the basidiomycetous yeast *Trichosporon beigelii* [1]. The name *T. beigelii* referred to a heterogeneous group of organisms that were later subdivided using molecular data into distinct species with different ecological niches. Recent molecular taxonomy indicates that six of these species have been associated with human disease: *Trichosporon asahii*, *T. asteroides*, *T. cutaneum*, *T. inkin*, *T. mucoides,* and *T. ovoides* [2–5]. It has been suggested that the major etiologic agents of *Trichosporon* infection differ in the types of disease they commonly cause. *T. asahii* and *T. mucoides* are involved in deep-seated infection, while *T. asteroides* and *T. cutaneum* are associated with superficial infection. Capital white piedra is caused by *T. ovoides*, whereas *T. inkin* causes pubic piedra.

White piedra is a rare fungal infection of the scalp hair, but it occurs rather commonly on hairs of the beard, moustache and genital areas [6–9]. There is evidence that cases may sometimes be transmitted

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sexually [10]. The disease occurs worldwide in both tropical and temperate climates. It has recently been reported in Saudi Arabia [11,12] and in Kuwait [13,14], The present case describes a patient in Qatar, in which the infection seen was due to *T. inkin* accompanied by *Candida parapsilosis*.

Case report

A 28-year-old Qatari woman presented to the department of Dermatology, Hamad Medical Corporation, with brown soft nodules on the scalp hair. In this case the scalp was the only site affected, but the affected area was extensive and was progressively increasing in size. Clinical examination revealed that the hair had a characteristic *Saccharomyces*-like yeast odor and appeared beaded, bearing light brownish, loosely adherent nodules up to 2 mm in length surrounding the hair shafts. Eyelashes, eyebrows, axillary, and pubic hairs were unaffected. Examination of hair under Wood's lamp was negative for fluorescence. Direct microscopic examination of the hair with 10% KOH showed that the soft nodules, which ensheathed the hair shaft in a sleeve-like manner, were so compact that their anatomy in terms of discrete fungal structures could not readily be discerned (Figs. 1 and 2). Many infected hairs were cultured onto two sets of three media, Sabouraud dextrose agar plus 40 U/ml streptomycin and 20 U/ml penicillin (SDA + SP), Sabouraud dextrose agar lacking antibiotics, and Brain–heart infusion plus 40 U/ml streptomycin and 20 U/ml penicillin (BHI). One set of plates was incubated at room temperature and the other at 37°C. Two types of yeast colonies grew along the hair shaft on agar media within 3 days at 37°C and at room temperature. Colonies of the first organism were soft and creamy whitish. The organism was purified using SDA + SP and identified using a Vitek II instrument (BioMerieux, Marcy l'Etoile, France) with its corresponding yeast ID card. The result was compatible with an 'excellent' identification for *C. parapsilosis.* The mycological identification of the second type of yeast-like colonies was at first based mainly upon the unique macroscopic and microscopic features seen. The colonies were initially creamy but developed irregular folds upon aging. They finally became wrinkled and increasingly adherent to the agar; thereafter, the center became heaped and the color darkened to waxy yellowish (Fig. 3). Microscopic examination of the culture in lactophenol cotton blue (Fig. 4) showed blastoconidia with granular contents, many pseudohyphae, and hyphae disintegrating into rectangular arthroconidia.

The organism grew at 37°C, was sensitive to cycloheximide and was positive for urease activity. Assimilation profiles, as determined by the Vitek II yeast ID card, included positive responses to dextrose, sucrose, maltose, cellobiose, lactose and xylose, and negative growth for melibiose, sorbitol, raffinose, dulcitol, galactose, melizitose and nitrate. Results of assimilation profiles gave confidence level of low discriminatory value, but consistent with the identification of *Trichosporon* with a T-index of 0.68 for *T. inkin* and 0.51 for *T.*

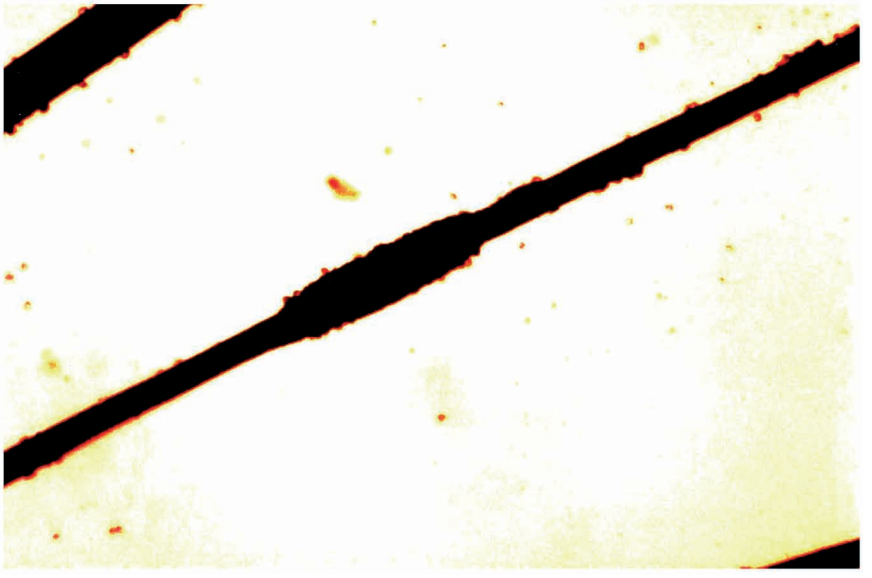

Fig. 1 Spindle-shaped *Trichosporon inkin* nodule ensheathing the hair in a case of co-isolation of *Trichosporon inkin* and *Candida parapsilosis* from scalp white piedra in Qatar (× 100).

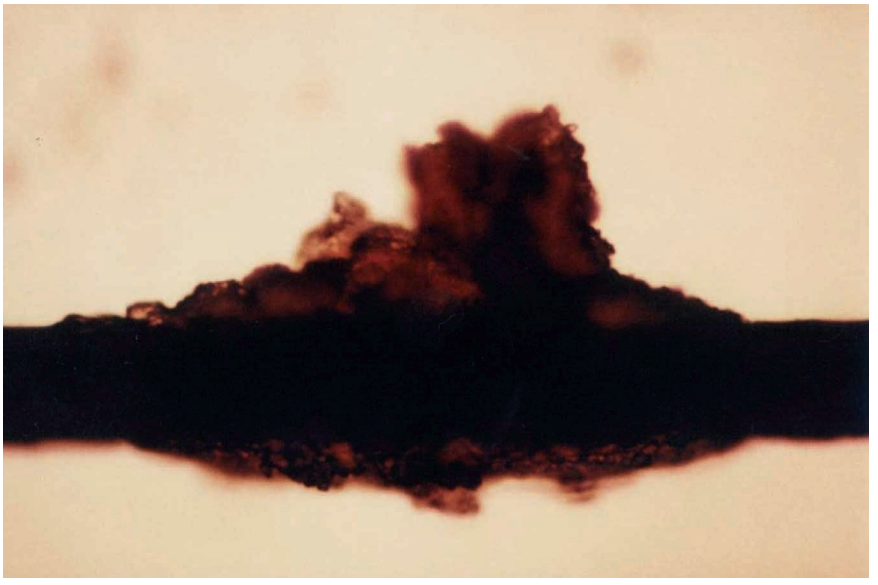

Fig. 2 Nodule showing outgrowth of fungal elements arranged perpendicularly to the hair shaft in a case of co-isolation of *Trichosporon inkin* and *Candida parapsilosis* from scalp white piedra in Qatar (× 400).

asahii. Therefore, molecular identification was carried out by analyzing the partial sequences of the 26S nuclear ribosomal gene as well as the ribosomal internal transcribed spacers (ITS). Both sequences were compared to the US National Center for Biotechnology Information (NCBI) GenBank database and the *Yeasts of the World* CD-rom (Springer-Verlag, Heidelberg, Germany). The NCBI BLAST analysis showed a match at 99% similarity for *T. inkin* (GenBank accession number AF 444420.1 for isolate CBS 5585 (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands) with 498 of 502 bp identical. The second hit was at 98% for *T. faecale* (AF 444419.1/CBS 4828): 495/503 bp. The CD-rom also gave *T. inkin* as the best hit with 99% match, followed by 97% similarity for *T. asahii.* In comparison, both the CD-rom and NCBI (AF 105396) gave a 100% match with *T. inkin* to the large subunit (LSU) of the 26S ribosomal gene. We

Fig. 3 Wrinkled creamy-tan colony of *Trichosporon inkin* after 5 days growth on antibiotic-supplemented Sabouraud dextrose agar in a case of co-isolation of *T. inkin* and *Candida parapsilosis* from scalp white piedra in Qatar.

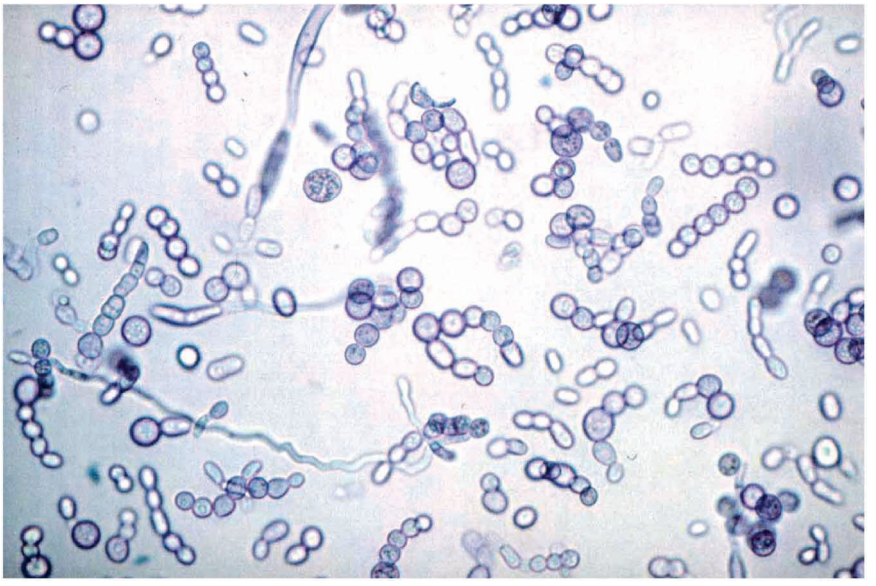

Fig. 4 Lactophenol cotton blue mount from the colony of *Trichosporon inkin* showing blastoconidia, pseudohyphae and rectangular arthroconidia in a case of co-isolation of *T. inkin* and *Candida parapsilosis* from scalp white piedra in Qatar (× 400).

therefore concluded that the present scalp white piedra organism is *T. inkin*. The organism was deposited as CBS 9554.

Treatment of the patient consisted of daily application of econazole shampoo and cream after shampooing with ketoconazole. This regimen led to complete recovery (seen as both clinical and mycological cure) after 2 months, with no recurrence of the disease.

Discussion

Certain members of the basidiomycetous yeast genus *Trichosporon* are the causative agents of white piedra, a superficial cutaneous infection that is non-life threatening and easily treated. Recently, *Trichosporon* species have been recognized as opportunistic pathogens in immunocompromized and immunocompetent hosts [15–17]. Disseminated *Trichosporon* infection is potentially life-threatening. Although uncommon, it is increasingly being reported, mostly in patients with malignant diseases [18–20]. Cases have also been reported in organ transplant patients [21–23], neonates [24–26] and HIV patients [27–29]. *Trichosporon* fungemia has also been reported [30,31]. Members of the genus *Trichosporon* have occasionally been implicated as nail pathogens [32,33]. *Trichosporon* is considered an opportunistic agent and therefore recovery from pathological specimens in the clinical microbiology laboratory of *Trichosporon* species capable of growing at 37°C should be regarded as potentially significant, especially in immunocompromized patients [34], even though members of this genus are also extremely common as contaminants, especially of the skin.

Guého *et al.* [2,3] significantly revised the taxonomy of the genus *Trichosporon* on the basis of partial 26S rRNA sequences, combined with a reanalysis of morphological and biochemical properties and an analysis of the co-enzyme Q system. The genus *Trichosporon* was delineated as containing six clearly differentiated opportunistic pathogens of humans [2]. These species were distinguishable by several key characteristics including carbon assimilation patterns, cycloheximide resistance, and the ability to grow at 37°C. Results of carbohydrate assimilation tests for our case isolate were assigned a low discriminatory value by the Vitek system but suggested *T. inkin* or *T. asahii*. Both alternatives were unexpected, as *T. asahii* is involved mainly in deep-seated infections, while *T. inkin,* though it is known as an agent of white piedra, is only known from pubic white piedra cases. Although clinical yeast species identification is routinely performed using biochemical profiles, nucleotide sequencing of the rRNA gene opens up new possibilities for accurate identification. As detailed above, our sequencing results gave a 100% match with *T. inkin.*

White piedra is a disease of worldwide occurrence, but it appears to be favored in temperate and subtropical climates. It occurs more commonly in the orient and South America than in Europe and North America [1]. The source of the infection in the present patient was not traced. The organism is a natural

inhabitant of soil and occasionally constitutes a part of the normal flora of human skin, throat and lower gastrointestinal tract [17,34]. Pubic white piedra is more frequently reported in the literature [6,8,9,35,36] than is capital white piedra [11–14,37].

Our patient, examined in Qatar, had very long hair and used a traditional type of hair covering that requires tight enclosure of the scalp with a veil. Low air exchange levels and elevated humidity are important factors in the pathogenicity of this disorder. Because shaving of the hair, which is commonly used as an effective therapeutic procedure in men, could not be performed, a therapeutic regimen based on therapy with topical azoles was used, with complete success.

It is worth mentioning that disseminated *Trichosporon* infection often has an unfavorable response to treatment because of the resistance of *Trichosporon* species to amphotericin B [17]. Previous studies, however, have suggested some antifungal activity might be found with azole therapy, as miconazole and itraconazole had higher *in-vitro* activity than was found with amphotericin B [38,39]. However, clinical response does not always correlate with the results of *in-vitro* studies. Anaissie *et al.* [40] suggested that azoles were an effective therapy for *Trichosporon* infection. In the present case, the success of azole (ketoconazole and econazole) treatment may have been partly or largely due to the superficial nature of white piedra.

White piedra has been described in horses, monkeys, and dogs, and the etiologic agents have been isolated from soil and water [3,41]. Factors such as humidity and temperature [13,42], or poor hygienic habits such as bathing in stagnant water [6], may act as predisposing factors for development of scalp white piedra. Sexual and familiar transmission are also suggested as predisposing factors, particularly in the cases of pubic white piedra [8,43].

In this study, *C. parapsilosis* was found to occur in association with *T. inkin* along the hair shaft in the primary isolation media. Quantitative maceration and dilution analysis was not done, but, gauging growth levels on a scale ranging from minimal (1+) to maximal (4+), and taking the (4+) value as equal to the growth level seen with *T. inkin*, *C. parapsilosis* was observed to grow at a relatively high density (3+). This explains the *Saccharomyces*-like yeast odor of the infected hair, since *T. inkin* does not produce this characteristic odor. It is not clear if *C. parapsilosis* was etiologically contributory to the white piedra described in this study or whether it is growing as a secondary invader. A previous study [44] described a bacterium, *Brevibacterium mcbrellneri* that was found to accompany the concretions of *Trichosporon* on hair, and it was suggested that this bacterium might play a synergistic role in the infection. Thérizol-Ferley *et al.* [43] suggested that trichomycosis (referred to as trichobacteriosis) might play an important role in the genesis of pubic white piedra in Africa. Similarly, Pontes *et al.* [42] reported eight cases of scalp white piedra in which association with bacteria was found. However, Figueras and Guarro [45] reported that the bacteria were always observed at the periphery of white piedra nodules, which suggests that they may not be the primary colonizers. Although *C. parapsilosis* is well known to cause cutaneous infections, it has not been reported to occur in the unusual habitat of white piedra concretions formed by *T. inkin* or other species.

In regard to the overall clinical significance of *T. inkin*, abscess in the lung due to this species has been reported [46] as has as a case of pneumonia secondary to chronic granulomatous disease [47]. Isolation from scalp white piedra in the present case further suggests that the species-specific patterns of infection previously delineated in *Trichosporon* infection [2] need reconsideration.

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