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Antifungal susceptibility profile of *Trichosporon inkin*: About three cases of White Piedra

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ABSTRACT

Trichosporon spp. usually cause systemic or superficial infections. Three cases of White Piedra produced by *Trichosporon inkin* are described. The *in vitro* antifungal activity to fluconazole, amphotericin B, ketoconazole and caspofungin against the three clinical isolates were evaluated. Sensitivity to fluconazole and ketoconazole was evidenced. However, the treatment of this mycosis is still a challenge.

1. Introduction

The genus *Trichosporon* includes species that are usually found in water sources, wet wood, and organic substrates [1] or are part of the gastrointestinal microbiota, the oral cavity, the respiratory tract, and the skin [2]. However, some species like *T. inkin, T. cutaneum*, *T. ovoides T. asahii, T. asteroides*, and *T. mucoides* are associated with superficial infections, such as White Piedra, or with systemic infections, particularly in immunocompromised patients [3–5].

White Piedra is an asymptomatic superficial fungal infection characterized by the presence of whitish nodules on the hair or vellum shaft [2]. Treatment of this infection is mainly based on haircut or shaving, and the use of topical products based on ketoconazole, ciclopiroxolamine, and selenium sulfide [2,6]. However, due to the increase in species resistant to available antifungals, including *T. inkin* [7], there is currently growing interest in the search for new therapeutic alternatives. Three cases of White Piedra are described as well as the identification of the etiological agent and the *in vitro* antifungal susceptibility.

2. Case presentation

2.1. Case 1

A 9-year-old woman, living in the city of Medellín (Colombia), with no significant medical history. After bathing in a river in Distracción (La Guajira, Colombia), whitish nodules were observed in the middle zone of the hair shaft (day 0) (Fig. 1A); direct examination with 10% KOH revealed blastoconidia and arthroconidia (Fig. 1D) for which some affected hairs were cultured on Sabouraud dextrose agar (SDA; Merck®, Darmstadt, Germany) at 35 °C and after 48 h of incubation, white, wrinkled colonies with a cerebriform appearance were evidenced (Fig. 2A). Microscopically, fungal structures compatible with *Trichosporon* spp. were observed (day +8), which were identified as *T. inkin* by Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) (Bruker Daltonik Bremen Germany). In Fig. 3A, the protein profile of the isolate is shown, confirming the diagnosis of White Piedra. The minor was treated with a ketoconazole (KTC)-based shampoo (day +15) without improvement. Intensive hair washing was used with a commercial shampoo based on natural products and constant drying of the hair was recommended, thus achieving the visible disappearance of the nodules (day +45).

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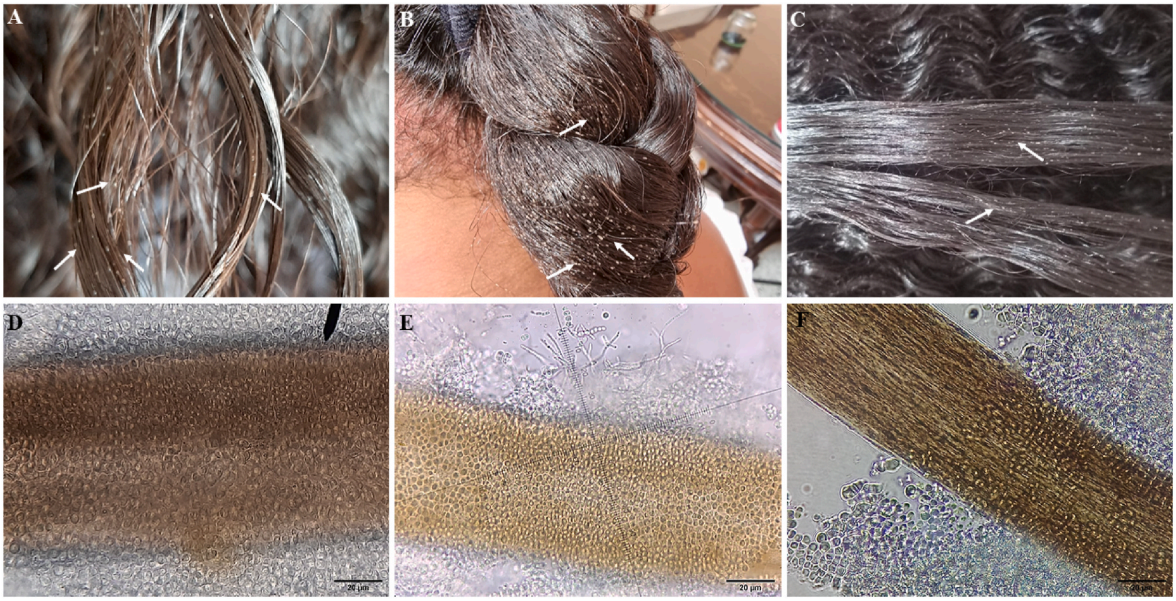

Fig. 1. Macroscopic and microscopic aspect (400X) of White Piedra nodules. A and D) Case 1. B and E) Case 2. C and F) Case 3. The white arrows indicate the nodules in the hair of each of the patients.

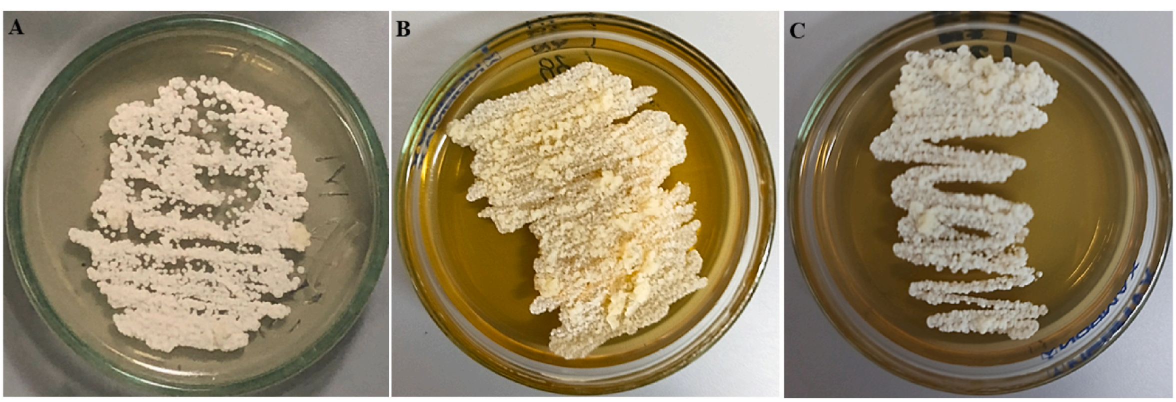

Fig. 2. Cerebriform-like colonies compatible with *Trichosporon* spp. A) Case 1. B) Case 2. C) Case 3.

2.2. Case 2

A 12-year-old woman, living in the city of Medellín (Colombia), a highly competitive swimmer, with no record of comorbidities. After swimming in a pool with poor hygiene conditions and staying with wet hair (day −7), whitish nodules were observed in the middle area of the hair (day 0) (Fig. 1B). Three weeks later (day +20) by microscopic observation of nodules made up of hyphae, blastoconidia and arthroconidia (Fig. 1E), by the growth of rough colonies in SDA (Fig. 2B), and by identification with MALDI-TOF MS of *T. inkin* (Fig. 3B), White Piedra was diagnosed. Initially, attempts were made to control the infection by cutting the hair and using a ketoconazole-based shampoo (day +30). However, after two months (day +60) of treatment, the infection had not been eradicated. Therefore, the dermatologist added 5% salicylic acid in mineral oil to the treatment, thus achieving a decrease in white nodules after one week of treatment (day +67).

2.3. Case 3

A 9-year-old woman, living in Cali (Colombia) who, after bathing in some cenotes in Cancun (Mexico) and remaining with wet hair (day −7), whitish nodules appeared in her hair (day 0) (Fig. 1C). On day +45, after microscopic observation of the nodules with 10% KOH (Fig. 1F), the growth of rough colonies on SDA (Fig. 2C) and the protein profile obtained by MALDI- TOF MS leads to the identification of *T. inkin* (Fig. 3C) and the diagnosis of White Piedra. Treatment with ketoconazole-based shampoo (day +60) was started. After ten months of treatment, the mycosis had not been eradicated.

Of the three etiological agents identified by MALDI-TOF MS, the respective protein profiles were obtained (Fig. 3). The identification score in each case was: case 1: 1.93, case 2: 1.82, case 3: 1.74.

3. Antifungal susceptibility testing

For the three *T. inkin* isolates, the Minimal Inhibitory Concentration values (MICs) of amphotericin B (AMB), fluconazole (FLC), ketoconazole (KTC) and caspofungin (CSF) (Sigma-Aldrich Company, St. Louis, MO, U.S.A) were determined following the CLSI standard M27 4th Edition [8]. The inoculum size was adjusted to 1×10^5 CFU with the Multiskan SkyHigh Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A) at $\lambda = 530$ nm. The geometric means (GM) of the MICs are shown in Table 1.

4. Discussion

White Piedra is a mainly cosmetic impact infection and, although it is

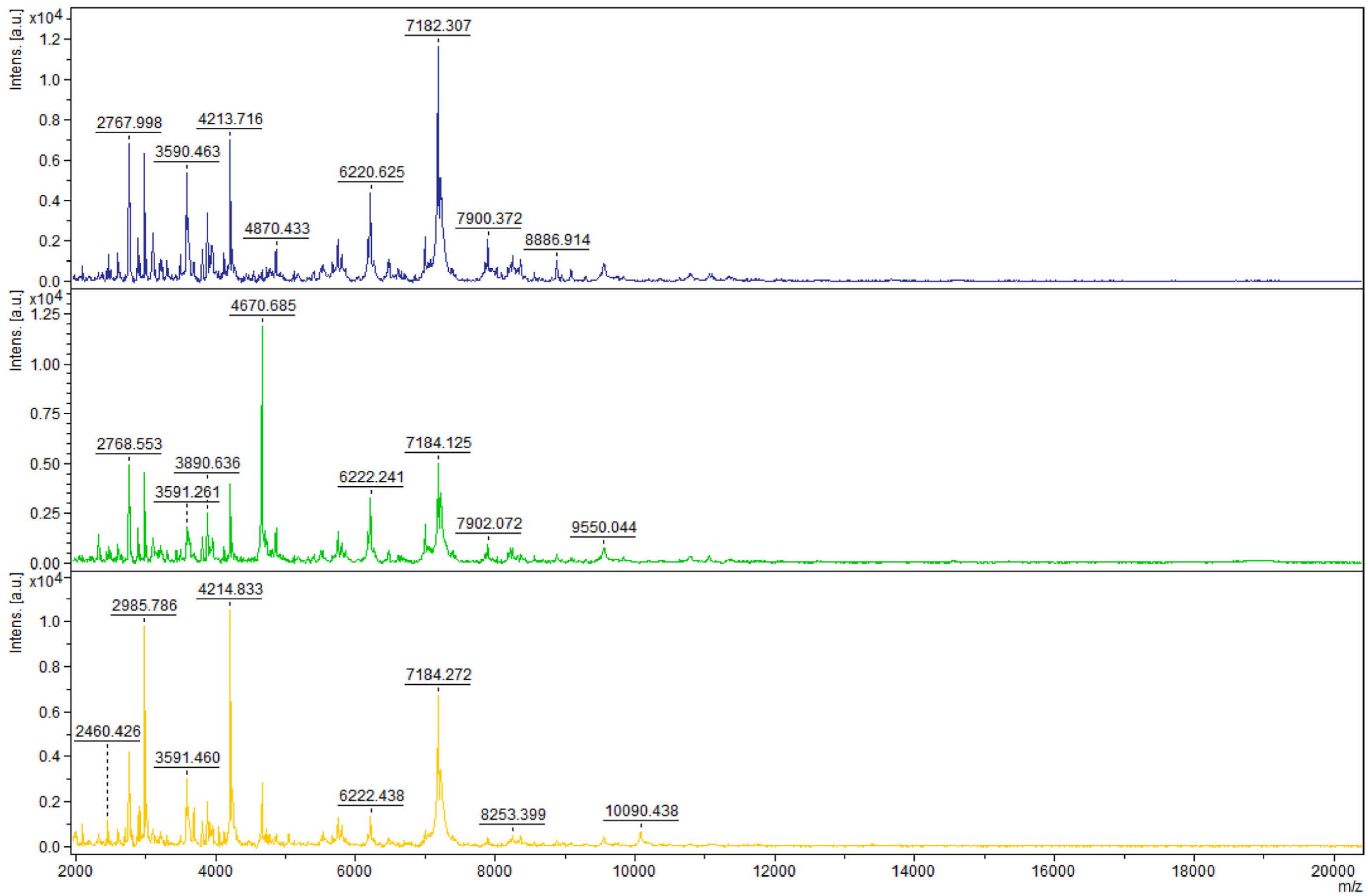

Fig. 3. Protein spectra obtained by MALDI-TOF MS corresponding to *T. inkin.* A) Case 1, B) Case 2 and C) Case 3.

Table 1
Geometric means of the minimal inhibitory concentration of antifungals against *T. inkin* clinical isolates.

Clinical isolate (case number)	GM-MIC (μg/mL)			
	AMB	FLC	KTC	CSF
T. inkin (1)	8	0.71	0.35	>8
T. inkin (2)	5.66	0.50	0.25	>8
T. inkin (3)	4	1	0.18	>8

AMB: amphotericin B; FLC: fluconazole; KTC: ketoconazole; CSF: caspofungin.

cosmopolitan, the highest incidence occurs in tropical countries, favored by humidity [9]. This factor was common in the three cases presented due to the aquatic activities carried out by the young women and possibly because of their curly hair, a condition that prevents rapid drying [3].

Different virulence factors have been identified in *Trichosporon* spp. White Piedra is possibly the result of the secretion of lytic enzymes or the formation of biofilms, which in addition to facilitating the growth of the fungus around the stem, exerting pressure and weakening the hair shaft, can confer antifungal resistance [10]. The treatment of this mycosis is based on cutting the hair and the use of topical products that contain antifungals, mainly KTC. This imidazole has been shown to be effective in eradicating the nodules that characterize the infection [2,6].

Even though the MG-MIC values for KTC obtained with the three clinical isolates were low (MICs-MG range 0.18–0.35), as has been reported in other studies [11,12], the efficacy of the treatments with this azole were not successful in all cases. *Trichosporon* species are susceptible *in vitro* to FLC and KTC, but resistant to AMB and CSP [13–15]. This phenotype was confirmed in this report with the three clinical isolates.

Currently, there is interest in identification of antifungal activity in essential oils (EOs) distilled from different aromatic plants or in molecules derived from these, for the development of formulations, mainly for topical use for the treatment of superficial fungal infections [7,16,17].

Various studies have demonstrated the *in vitro* activity of EOs and terpenes against yeasts and filamentous fungi [18,19]. Our group has published data on the activity of EOs from *Lippia origanoides* chemotypes and terpenes against *Candida* species with different susceptibility profiles [20]. Considering our previous results and the therapeutic failure in two of the White Piedra cases studied, the anti-*T. inkin* activity of EOs of *L. origanoides* thymol chemotype, *L. origanoides* (thymol + carvacrol) chemotypes and the commercial terpenes (thymol and carvacrol) (Sigma-Aldrich Company, St. Louis, MO, U.S.A.) was evaluates *in vitro*. Although the MICs of EOs and terpenes with *T. inkin* were not comparable to those of antifungals (GM-MIC range = 90.5–256 μg/mL), their hydrophobic nature as well as the mixture of molecules that make up EOs, they represent an advantage for the future development of products for topical use and to carry out basic studies in order to understand the mechanism of action and resistance in this fungal model.

5. Ethics statement

All patients participated voluntarily. Informed consents were signed by legal representatives of patients for the publication of any potentially identifiable images or data included in this article. The study was approved by the Ethics Committee of the School of Medicine at the University of Antioquia (Act number 042/2022).

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Conflict of interest

The authors declare no conflict of interest.

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