White piedra: molecular identification of Trichosporon inkin in members of the same family

Piedade branca: identificação molecular do Trichosporon inkin em membros de mesma família

**ABSTRACT**

Introduction: White piedra is a superficial mycosis caused by the genus Trichosporon and characterized by nodules on hair shaft. Methods: The authors report a family referred to as pediculosis. Mycological culture on procedure identified the agent as Trichosporon. PCR. Family case. Piedra

**Keywords:** Trichosporon inkin. Piedra. PCR. Family case.

Results: Exame micológico revelou a infecção por Trichosporon spp. A identificação molecular demonstrou se tratar do Trichosporon inkin. Conclusions: White piedra and infection caused by T. inkin are rarely reported in Southern Brazil. The molecular tools are essentials on identifying the Trichosporon species.

**RESUMO**


Superficial mycoses are fungal infections of the skin, hair, and nail that invade only the stratum corneum and the superficial layers of the skin. Piedra is an asymptomatic superficial fungal infection of the hair shaft. It was classified by Horta in 1911 into two types: black piedra, which is caused by Piedraia hortae, and white piedra, which is caused by genus Trichosporon. The clinical characteristic of white piedra is in the form of concretions or soft, whitish nodules on hair shaft. Most cases occur in tropical and subtropical regions. Humidity, hyperhidrosis, and poor personal hygiene are important predisposing factors. The Trichosporon genus has undergone extensive taxonomic reevaluation. Traditional taxonomy based on morphological, ecological, and physiological aspects is used to group the genus Trichosporon, which usually generates inconsistent results. The reclassification based on new taxonomic concepts, including phylogenetic studies, was supported by the analysis of 26S ribosomal DNA. Six species are relevant in medical mycology: Trichosporon asahii, Trichosporon asteroides, Trichosporon cutaneum, Trichosporon inkin, Trichosporon mucoides, and Trichosporon ovoides. The etiologic agents of trichosporonosis differ according to infection: T. asahii and T. mucoides are involved in systemic infections, T. asteroides and T. cutaneum are associated with superficial infection, and T. ovoides and T. inkin are usually involved in white piedra of the scalp and the genital area, respectively.

The study included three related members: a 36-year-old woman, the mother; the 3-year-old son; and the 6-year-old daughter. The index case was the 6-year-old child referred from a small community as a case of pediculoses resistant to treatment. Clinical examination showed multiple whitish nodules firmly attached to the hair shaft. Mycological examination and culture confirmed the clinical suspicion of white piedra. It was the first of such diagnosis in 36 years of existence of the local department of Dermatology in São Paulo State, Brazil. The patients were cured within a month after being treated systemically with itraconazol and topically with ketoconazole shampoo.

The mycological observations included direct microscopic examination of hair samples and culture in Agar Mycosel®. They were incubated at room temperature (28-30°C) up to 4 weeks.

The DNA extraction from Trichosporon spp. was performed according to the methods proposed by Van Burik using the Kit Illustra Tissue & Cells genomic Prep Mini Spin® (GE Healthcare, USA). The amplification was carried out by PCR reactions, using, as primers, ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAATGTTAAGCTGTAACAAGC-3') that amplified 634 bp as described by White et al. The process was performed in 25μL containing 1X PCR buffer (10mM Tris HCl pH 8.0, 50mM KCl), 1.5mM MgCl2, 200μM dNTP, 10pmol of each primer, 10ng of purified DNA, and 0.2 U μL-1 of Platinum Taq DNA polymerase system (Invitrogen, Brazil). Thermal cycling conditions were 94°C for 5 min, followed by 25 cycles at 94°C for 1 min, at 60°C for 2 min, and 72°C for 2min and a final extension at 72°C for 7 min. PCR products were identified by agarose gel electrophoresis. Amplicons were purified by employing the commercial GFX® PCR DNA and Gel Band Purification kit (GE Healthcare). Using the sequencer MegaBace™ 1,000 (GE Healthcare), reactions were carried out in both strands according to DYE Enzymatic™ ET Dye Terminator Cycle Sequencing Kit (GE Healthcare). Sequences were analyzed through Chromas 2.3 and MEGA 4 software and compared with the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST).

Microscopic examination of hair samples presented ectothrix mass of fungal cells showing only arthrospores and blastospores...
The molecular identities of these amplicons were confirmed by sequencing, which showed 98% similarity with *Trichosporon inkin* DNA sequence deposited at Gen Bank (gb|AF 444420.1, strain CBS S585).

White *piedra* is an uncommon diagnosis in the southeast of Brazil, although some outbreak of scalp white *piedra* has been reported. Out of the 20 reported cases, 90% were observed in children under 9 years old, and all were in female patients. Most were correlated with informed behavior of routinely using emollient creams on hair. There were no familial cases in those reports. The scalp lesions of white *piedra* are usually asymptomatic and can be misdiagnosed as pediculoses.

The diagnosis of *T. inkin* was confirmed by means of morphological and molecular studies. Phenotypic methods for *Trichosporon* species identification are based on the characterization of micromorphological aspects of the colonies. It is a useful tool for triage, but because it is unable to differentiate all *Trichosporon* species, molecular techniques are considered the most reliable methods for species identification.

The identification of this microorganism using ribosomal genes represents consistent evolutionary markers, including alternating conserved and variable regions, which may be useful for species identification and phylogenetic studies. Universal primers, ITS4 and ITS5, are sufficiently discriminatory for the identification of a considerable number of species of the genus *Trichosporon*. It is worth mentioning that the genus *Trichosporon* is not only the agent of white *piedra* but also the second most commonly reported cause of disseminated yeast infection in humans, mainly in immunosuppressed patients.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**REFERENCES**