Direct Extraction of DNA from Positive Transparent Dressing and Swab to Identify Candida albicans Infection in Intertriginous Candidiasis in the Inguinal Region

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Abstract We report a case of a patient infected by Candida albicans which was identified by direct extraction of DNA from a positive transparent dressing and a swab. The patient was a 32-year-old male who complained of erosion in his inguinal region. Large patches of erythema and erosion were present in his inguinal and perianal region, with soya-bean like residue discharge. He was diagnosed with erythrasma and treated with antibiotics but his clinical condition did not improve. KOH examination furnished a positive result for candidiasis. Morphologic characteristics confirmed his infection was caused by Candida albicans. Sequencing of the internal transcribed spacer (ITS) 1/4 polymerase chain reaction products, amplified from positive transparent dressing and cotton swab with discharge and from primary culture isolates, established the Candida albicans lineage. The patient was cured by treatment with itraconazole 200 mg twice a day orally in combination with topical wash with 2 % ketoconazole shampoo and topical use of 1 % naftifine–0.25 % ketoconazole cream.

Keywords Candida albicans · Candidiasis · Itraconazole · 1 % Naftifine–0.25 % ketoconazole

Introduction

Cutaneous candidiasis is a common skin infection, especially in intertriginous areas, caused by Candida species [1]. Traditional diagnosis is based on direct microscopic examination and culture. A positive result from culture confirms infection by the pathogenic fungus. We describe a case of intertriginous candidiasis in a male patient. The identity of the pathogenic fungus was confirmed by two culture-independent methods, which is equivalent to confirmation by use of traditional culture methods.

Case Report

A 32-year-old male presented at our clinic with a history of erosion in his inguinal region for a month. He was diagnosed as erythrasma and was treated with fusidic acid cream, however his condition did not improve. He complained that his erosion in the inguinal region developed after topical use of halo-metasone/triclosan cream prescribed by his community doctor. A physical examination revealed large patches of erythema and erosion in his inguinal and perianal region, with soya-bean like residue discharge. Around the erosion there were large numbers of...
papules (Fig. 1a). The lesional skin temperature was elevated. A serum test for *Treponema pallidum* was negative. Besides the patient’s 10-year history of mental illness there were no signs of any immunosuppressive disorder and no significant medical history.

**Mycological Examination**

We used a transparent dressing (Chengdu Yongle Adhesive Products) to sample discharge from his inguinal region for direct mycological examination. KOH (10 %) mounts revealed extremely large numbers of pseudohyphae and yeast cells (Fig. 2). The transparent dressing was placed in a 1.5-ml centrifuge tube for direct DNA extraction. The discharge was sampled by use of a sterile swab (Chengdu City Health Material Factory) and then spread on Sabouraud glucose agar (SDA; Oxoid, Hampshire, UK) containing chloramphenicol and cycloheximide anide [2]. The cotton swab was placed in a 1.5-ml centrifuge tube for direct DNA extraction. The SDA tube was incubated at 28 °C for 7 days, resulting in smooth ivory cream-colored colonies. When incubated in CHROMagar at 28 °C for 3 days dark green colonies were observed. Microscopic examination of slide culture revealed fertile pseudomycelium and spherical budding cells. After incubation in Rice–Tween 80 agar at 28 °C for six days chlamydospores were observed.

**Analysis of DNA Sequences**

Sterile distilled water (approx. 1 ml) was added to the 1.5-ml centrifuge tubes containing the transparent dressing and swab. After incubation at 65 °C for 20 min, the transparent dressing and swab were discarded and the remaining tube contents were centrifuged at 10,000 r/min for 1 min and, finally, the supernatant fluid was discarded [3]. The two samples of sediment and primary culture isolate were identified by rRNA gene sequence analysis. Fungal DNA in the internal transcribed spacer (ITS) regions was amplified with the primers ITS1 (5'-TCCGT AGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCC GCTTATTGATATGC-3'). The amplicons were sequenced with the same primers (Invitrogen Life Sciences, Carlsbad, CA, USA).
Technologies, Shanghai, China) [3–5]. The three sequences were the same, and were deposited in GenBank with the accession number JQ364949. These DNA sequences of the nuclear ribosomal ITS region of the isolated fungus were all in accordance with C. albicans (DDBJ/EMBL/GenBank accession no. JN882316.1) with homology of 100 and 99 % by use of the Blast 2 Sequences Tool (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi).

Treatment and Follow Up

Intertriginous candidiasis caused by Candida albicans was diagnosed. The patient was started on oral therapy with itraconazole capsules (Sporanox; Xian–Janssen Pharmaceutical) 200 mg twice a day, with topical wash with 2 % ketoconazole shampoo (Triatop; Xian–Janssen Pharmaceutical) once a day followed by 1 % naftifine–0.25 % ketoconazole cream (Chongqing Huapont Pharmaceutical) once a day. The discharge, pustules, and most of the erythema disappeared by the 7th, 14th, and 21st days, respectively, after the start of treatment. The treatment protocol is 21 days. The inguinal region was clear at two weeks follow up (Fig. 1b).

Discussion

Diagnosis of candidiasis is confirmed by observation of mycelial forms on microscopic examination. Because Candida yeasts (especially C. albicans) are normal inhabitants of the skin and oral mucosa, it must always be noted that positive culture does not always indicate the presence of candidal infection [6]. Use of the positive transparent dressing to extract DNA, then PCR to validate the pathological agent that was observed under the microscope, can avoid the problem of false positive cases. Confirmation by use of a swab spread on SDA medium is more useful for infants and children, especially for the mouth and vaginal areas. For direct extraction of DNA from culture-independent samples, we should first incubate to sediment the fungi. Because the concentration of DNA is reduced (the volume is increased from 4 μl in the culture reaction mixture to 21 μl in the culture-independent reaction mixture), the reaction mixture should contain the entire DNA sample to furnish the highest DNA concentration for PCR-based sequencing [3].

Therapy for cutaneous candidiasis is dominated by use of topical antifungal agents. Azole antifungal creams (e.g., bifonazole, ketoconazole, neticonazole hydrochloride, lanconazole, and luliconazole) are most effective [6], terbinafine hydrochloride less so [7]. It is, therefore, very important to confirm the identity of the pathological agent to enable choice of the most appropriate antifungal drug as quickly as possible. Traditional confirmation of the identity of culture colonies often takes 7–14 days. Molecular biological confirmation by use of the positive transparent dressing and swab can be achieved in 48 h. Subsequent culture confirmation can be used of verify the culture-independent identification.

Our purpose in writing this paper was to report the use of three different methods for diagnosis of cutaneous candidiasis. Each of the three different methods has a specific advantage. Use of these advantages can make diagnosis more reliable and less painful to the patient.

References
