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Two Cases of Skin Mycosis Due to *Hanseniaspora Opuntiae* and *Cutaneotrichosporon Mucoides* at Basrah Southern of Iraq

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ABSTRACT

Mycoses of the skin are a group of infections that affect the skin and its appendages, mainly caused by dermatophytic fungi, and may be caused by yeasts or rearly by non-dermatophytic molds. Skin swabs collected from 60 patients attending the Dermatology Clinics at Madinah Central Hospital and Al-Fayhaa General Hospital in Basrah province from October 2021 to March 2022 were surveyed for the presence of dermatomycoses. Direct microscopical examination was carried out with 15% KOH, and repeated cultures were performed on Sabouraud dextrose agar with chloramphenicol showed the same colonies. In this paper,we present two interesting cases in which *Hanseniaspora opuntiae* HAM17 and *Cutaneotrichosporon mucoides* HAM14 can be distinctly identified as causative agent of cutaneous mycoses. In the first case, we describe a new etiologic agent, *Hanseniaspora opuntiae* HAM17, which was implicated in a cutaneous infection in a 45-year-old woman with a history of diabetes mellitus, and to the best of our knowledege, it represents the fourth clinical case due to this fungus in the world. The second case involved cutaneous mycosis due to *Cutaneotrichosporon mucoides* HAM14 in a 23-year-old woman showed inflammatory lesions similar to acne on the back, and she was suffering from hormonal disorders. This case and the etiologic agent are reported for the first time in Iraq. The isolated yeast species were examined and purified for phenotypic identification and genetical analysis using the primers ITS1-ITS4. Sequences were deposited into Japanese Genbank as new strains under accession numbers LC722487 and LC722484.

1. Introduction

Skin mycosis in humans raise great concern all over the world, and constitute a psychological and economic burden for those infected. They are infections caused by fungal invasion and colonization of the keratinous structures of the skin, hair and nails (Lopes et al. 2017). In general, people have a high level of innate immunity against fungi, as the skin and mucous surfaces act as essential barriers to any infection caused by fungi that primarily colonize the superficial layers of the skin, cutaneous and subcutaneous layers (Gnat et al., 2021). It also contributes to hydration

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and fatty acid content the host's body temperature, pH, skin epithelial cells change, as well as the natural microbiota group in the resistance of the host (Theelen et al., 2018; Chen et al., 2020). Humoral factors such as Transferrin restrict the growth of many fungi by limiting the amount of iron available to them. These factors may restrict the growth of dermatophytes to the outer layers of the skin (Lee et al., 2011; Murray et al., 2020).

There are many classification systems for fungal infections, including the following classification depending to the site of infection (Vallabhaneni et al., 2016). Superficial mycoses are characterized by fungal invasion of the stratum corneum of the skin, hair and nails (Vallabhaneni et al., 2016; Weeks et al., 2003). Cutaneous mycoses: the infections extend deeper into the epidermis and are caused by a group of transparent fungi known as Keratinophilic fungi (Gnat et al., 2021; Lakshmanan et al., 2015). Subcutaneous mycoses: Infections include the dermis, subcutaneous tissue, and muscles, its clinical forms include chromoblastomycosis and eumycetoma (Kauffman, 2000) Systemic mycoses: It is an infection that originates primarily in the lung but may spread to many other organs in the body, including the skin. It is caused either by endemic dimorphic fungi such as Histoplasmosis which causes *Histoplasma capsulatum* and Blastomycosis caused by Blastomyces dermatitidis , or by opportunistic fungi such as Candidiasis, Aspergillosis, Mucormycosis and Cryptomycosis which is caused by some species of *Candida sp* and *Aspergillus sp* and *mucor sp* (Miron et al., 2014; Hasan, 2014).

It is interesting that skin infections are prevalent in tropical and subtropical countries, especially in developing countries such as India, where the hot climate and humid weather are suitable for the acquisition, recurrence and spread of infection, as the fungus appears endemic (Pierre et al., 2016) and there is currently no country that is free of Dermatophytosis infection . Research shows that fungal infections of the skin and/or nails affect up to 20-25% of the world's population and can affect anyone of any age (Ebert et al., 2020; Gupta & Cooper, 2008).

Fungal skin infections have become a major health problem affecting children, adolescents and adults, and they are on the rise over the past few decades, significantly among patients, including immunocompromised patients, for example, patients who have undergone organ transplantation, HIV patients, acquired immunodeficiency syndrome patients and cancer patients (Hindy & Abiess, 2019; Kannan et al., 2006). Besides the high rate of infection among different communities, the difficulty of applying effective control measures, the duration of the disease, the high cost of treatment and the negative effect of some antifungals make it difficult to overcome these infections (Seebacher et al., 2008; Lopes et al., 2017). We report two cases of skin mycosis caused by *Hanseniaspora opuntiae* and *Cutaneotrichosporon mucoides* from Basrah Governorate ,southern Iraq.

2. Materials and Methods

Samples Collection

From October 2021 to March 2022, 60 specimens were taken by swab from skin lesions of patients who were attending the outpatient units of Dermatology Department at Madinah General Hospital and Al-Fayhaa General Hospital in Madinah and Hartha district, Basrah, Iraq. Specimens were transferred to the mycology Laboratory University of Basrah College of Science Department of Biology.

Direct Examination and Culture

A part from each swab sample was placed on a slide and an aqueous solution of 15% potassium hydroxide (KOH) was added , then heated gently over a flame and examined under the light microscope for the presence of fungal elements and their diagnostic morphology (Ellis, 1994). All swab samples were then cultured on Sabouraud Dextrose Agar (SDA) supplemented with 250 mg/l Chlormphenicol and incubated at 37° for two to four days, Then the cultures were purified and examined for their phenotypic and genetic diagnosis.

Genetic Diagnosis

Presto Mini gDNA Yeast Kit supplied from Geneaid/ Korea was used for the DNA extraction from the isolated cultures. ITS region is amplified using universal primers ITS1 (5`-TCCGTAGGTGAACCTGCGG-3`), ITS4 (5`TCCTCCGCTTATTGATAT GC-3`) using thermal Cycler from Bioneer Corporation / Korea. In a total volume of 25µl consisting of 3µl DNA Form, 1µl F. Primer, 1µl, R. Primer, Mastermix 12.5 µl, Nuclease free water 7.5 µl. The PCR program was as follows : 94° C for 3 min, followed by 35 cycles at 94° C for 45 s and 52° C for 1 min and 72° C for 1 min, with a delay at 72° C for 10 min (White et al., 1990). The PCR products were analyzed after RedSafe™ staining by 2% agarose gel electrophoresis alongside DNA Ladder100 bp (Sambrook et al., 2012; Nishiura, et al., 1997). Sanger sequencing of gene performed at Macrogen Inc. (South Korea). A homology search was conducted using the Basic Local Alignment Search Tool (BLAST) program that is available at NCBI and the sequences saved at Genes bank.

3. Results and Discussion

Based on the phenotypic and genetic diagnosis of yeast isolates, it was found that the isolated species belong to four genera, *Candida*, spp., *Malassezia* spp. , *Cutaneotrichosporon* spp. and *Hanseniaspora sp.*

Hanseniaspora Opuntiae

Colonies appear cream color, butyrous, glossy, flat, and slightly raised in the center when grown on SDA medium at 28°C (Cadez, et al., 2003). Yeast cells are ovate to elongated, 3-12 x 2-4 µm, found singly or in pairs. Budding from the bipolar poles (Fig. 1).

Specimen Examined

The strain *Hanseniaspora opuntiae* HAM17 was recorded from a swab taken from the underarm infection of a 45-years-old female, who had diabetes and had contact with animals, on 7/25/2022, Hartha, Basrah, Iraq. Living culture was deposited at Mycology Laboratory, College of Science, University of Basrah. Genbank accession number LC722487.

The presence of *H. opuntiae* was initially associated with members of the Cactaceae family. It was the first record of the species from Cactus rot (Opuntia ficus - indica) in the Hawaiian Islands, USA (Cadez et al., 2003). It was later isolated from Grape berries from Australia, Greece, South Wales and China (Luan et al., 2018; Nisiotou & Nychas, 2007). Clinical significance of this species was not known,

but the fungus can grow at a body temperature of 37 °C. The species was found to be dominant in post-harvest fermentation of Cocoa beans on a farm in Malaysia (Papalexandratou et al., 2013). The species *H. opuntiae* is a rare fungus obtained from clinical samples, and the first record of the species was from clinical samples of patients in Tunisian hospitals, as three isolates of the fungus were obtained and constituted less than 1.7% in the study (Eddouzi et al., 2013).

Recently, the species *H. opuntiae* was recorded from blood cultures of an immunosuppressed patient in Spain (González-Abad, 2018). It was also isolated at a low rate (1.2%) compared to *Candida* yeast species from the oral cavity of Iranian patients suffering from hematological malignancies (Arastehfar et al., 2019). From follow-up research and studies around the world, it appears that the current record represents the first case of isolation of *H. opuntiae* from a superficial mycoses infection of a diabetic patient and a fourth case from clinical specimens.

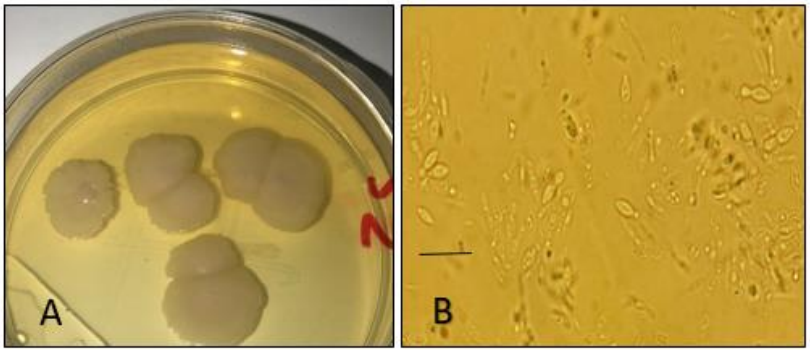

Fig. 1. A. Colonies of the yeast *Hanseniaspora opuntiae*, B. yeast cells. B, scale bar. = 10 µm

Cutaneotrichosporon Mucoides

Fungal colonies appear on SDA medium at 28 °C, pale to creamy in color, smooth, shiny, buttery-butyrous, with a smooth edge, later transforming into radial fissures with friged extensions and hyphae (Guého et. al., 1992). Yeast cells are white, elliptical or elongated, 3-6 x 3-10 µm, singly or in pairs. Fungal hyphae, as well as the arthroconidia were formed (Fig. 2).

Specimen Examined

The strain *Cutaneotrichosporon mucoides* HAM14 was recorded from a swab taken from the back of a 23-years-old female, on 25/7/2022, Al-Hartha, Basrah. Living culture was deposited at Mycology Laboratory, College of Science, University of Basrah. Accession number in Genbank LC722484.1.

The first description of this species was under the genus *Trichosporon* from a strain isolated from Meningitis of a patient with lymphoma in Belgium (Guého et al., 1994), and this species was transferred to a new genus *Cutaneotrichosporon* in a new formulation *Cutaneotrichosporon mucoides* com.nov. Depending on the phenotypic and molecular characteristics (Liu et al., 2015). No environmental information is available about the fungus. It can be an opportunistic human pathogen and produce systemic infections that may be fatal in immunocompromised hosts.

The previously classified *C. mucoides* (*Trichosporon*) was isolated from a patient with meningitis, from the cerebrospinal fluid in humans, and from the white hair in humans. Pubic white Piedra in an HIV-infected patient and from the home of a summer-type hypersensitivity pneumonitis patient (SHP) in Japan. *C. mucoides* is usually isolated from superficial and sometimes disseminated infections with poor innate immunity (De Hoog et al, 2000). This species was known as the causative agent of superficial infections such as onychomycosis and white hair (Guého et al., 1994). It was isolated from a woman who works as a hairdresser and suffers from severe infection and necrosis of the fingernails, although this species was previously recorded as a cause of nail infections.

Nevertheless, not as severe as the case recorded in this research. The fungus was isolated from the urine of a diabetic patient in India, this is the first record of *C. mucoides* from a urinary tract infection, and this type has not been isolated from any clinical or environmental sources in India before this date (Basu et al., 2003). Trichosporonosis is usually associated with a number of clinical syndromes. Immunosuppression invasive infections have been recorded in both immunocompromised families and even those with integrated immunity, including fungemia, urinary tract infections, peritonitis, and prosthetic valve endocarditis (Kendirli et al., 2006; Colombo et al., 2011; Chen et al., 2013; Padhi et al., 2014). Recently, a case of Prosthetic valve endocarditis was reported (Oh et al., 2020) and a case of aortic root abscess Prosthetic valve endocarditis (Tse et al., 2022).

The treatment of Trichosporonosis is a challenge, because the species of the genus *Trichosporon* and the species transmitted and registered as a new combination under the genus *Cutaneotrichosporon*, which are 15 species that are relatively resistant to antifungals from the group of Triazoles (Colombo et al., 2011; Basu et al., 2015; de Almeida Junior & Hennequin, 2016; Oliveira dos santos et al., 2016). There is no real clinical data to safely recommend the use of the antifungal 5-fluorocytosine for the treatment of invasive Trichosporonosis (Colombo et al., 2011). Therefore, improving knowledge of the Biology and pathogenesis of fungi and the consequent identification of molecular targets is urgent (Zimbres et al., 2018). The case recorded in the

current study is the first in Iraq of *Cutaneotrichosporon mucoides* from a skin mycosis on the back of a girl who may suffer from hormonal disorders.

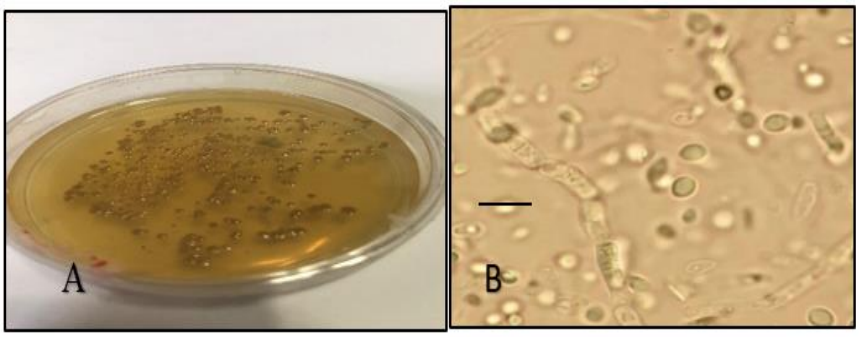

Fig. 2. A. Colonies of *Cutaneotrichosporon mucoides*, B. Arthroconidia and yeast cells. scale B = 15 µm

4. Conclusion

In this study, two species were isolated for the first time in Iraq from superficial skin infections, the first belonging to the type *Hanseniaspora opuntiae* HAM17 and the second to the type *Cutaneotrichosporon mucoides* HAM14, with lower percentages than the other yeasts like *Candida* Spp and *Malassezia* Spp. They were diagnosed phenotypically and genetically and can grow at a temperature of 35 ±2 and may possess virulence factors that enable it to cause infection.

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Competing Interests

The authors had no competing interests.

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