First recovery of Candida africana from the oral cavity of children with leukaemia receiving chemotherapy in Basrah, Iraq

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Abstract

Twenty-nine Candida isolates were assessed with green-coloured colonies using CHROMagar Candida medium with a positive germ-tube test. Based on morphological and biochemical tests, 29 strains were isolated from the oral cavity of children with leukaemia who were undergoing chemotherapy in Basrah, Iraq. Three (10.3%) of them were identified as Candida africana. Identification of the isolated yeasts was further confirmed at the molecular level by amplifying the internal transcribed spacer sequencing (ITS) region of rDNA comprising the ITS1-5.85-ITS2 regions of fungal rRNA genes. This study represented the first recovery of C. africana from the oral cavity for immunocompromised patients with leukaemia and considered the first record of species in Iraq.

Introduction

Candida africana was originally isolated from patients in Africa and Germany in 1993 as atypical *Candida albicans* strains [1]. Later, Tietz *et al.* [2] proposed it as a new species. Since 2001, the new species was reported in several countries in the Middle East [3-5], Europe [6-9], East Asia [10-14], South America [10,15,16], Africa [17-19], and North America [20, 21]. In addition, *C. africana* was primarily isolated from the genital specimens but rarely from the skin [2], from blood cultures (in Chile [10]), and from systemic infection with renal involvement in preterm newborn babies (in the United States [21]). In Turkey, re-examining 195 vaginal *C. albicans* strains using hyphal wall protein 1 (HWP1) gene polymorphism proved that none of them were*Candida dubliniensis* or *C. africana* [22]. Similarly, in Malaysia, 98 *C. albicans* isolates were recovered from the vagina, but none of them were found to be C. *africana* [23].

Candida albicans possesses a highly dynamic genome that is subjected to a wide range of changing, resulting in the evolution of new variants or strains. As an example of such evolution, *C. albicans*shaped the emergence of *C. africana* as a new species that is characterised by lower virulence and the inability to produce chlamydospores. Extensive molecular analysis of a panel of *C. albicans* that were recovered from vaginal swabs revealed several characteristics that distinguished those re-examined strains from the*C. albicans,* such as lacking N-acetylglucosamine assimilation due to a typical polymorphism in HXK1, which compromised the utilisation of this amino acid. In addition, multi-locus sequencing analysis showed significant differences between those strains and *C. albicans* and demonstrated a high probability of variation, which suggests that this is an adequate type to study evolution. As a result, Chowdhary et al. [24] and Giosa et al. [25] positioned them in a separate species called *C. africana* .

The *Candida* species were extensively studied in Iraq, focusing on the clinically related ones, which were isolated from different parts of the human body [26-36]. Despite that, this work is considered the first record of this species in Iraq from the oral cavities of leukaemia patients.

Materials and Methods

Ethics statement

Committees from the following institutes approved the sampling that is required to achieve this study: Research and Knowledge Unit, Training and Development of Personnel Division in Planning, the Basrah Children's Specialty Hospital, and the Resource Development Department of the Basra Health Department in Basrah Governorate, Iraq. We gained written agreement from the parents or guardians of the children after a detailed explanation of the goals of our research. We are ethically committed not to breach the privacy of the patient's family or physically harm the patients, as all sampling was conducted under the care of a physician.

Oral swabs were collected from 25 children with leukaemia (age range 2-16 years old) who were hospitalised in the Basrah Children's Specialty Hospital, Basrah Province, southern Iraq, during the period from August 2016 to June 2017. The samples were immediately spread on a Sabouraud dextrose agar supplemented with 50 mg/L of chloramphenicol. The petri dishes were incubated at 37^{o}C for 48 h. Identification of the growth strains was performed through several traditional tests, including the germ-tube test [37], a test of the ability to produce chlamydospores on corn-meal agar (HiMedia, India) with 1% Tween 80 [38] with growth at 30^{o}C, 37^{o}C, and 42^{o}C [15], a test of the colony appearance and colour on CHROMagar (Paris, France), [39] and the detection of carbohydrate assimilation using the API 20c system (bio Merieux Marcy IE toile, France) [15].

Molecular Identification

The DNA was extracted from the isolated yeasts using the genomic DNA extraction mini kit (Favor Prep^{TM} Fungi/Yeast Genomic DNA-Canada). The extracted DNA was migrated using agarose gel electrophoresis. The PCR amplification was performed in a final volume of 25 µl. Each reaction consists of 12.5 µl of Green Master Mix, 0.5 µl of $MgCl_2$, 0.5 µl of each forward (ITS1,F´5-TCC GTA GGT GAA CCT GCG G-´3) and reverse (ITS4, R-5´ TCC GCT TAT TGA TAT GC-´3) primer, and 1.5 µl of the template DNA. The reaction volume was completed to the final volume with 9.5 µl. The thermal cycles were programmed as follows: an initial denaturation step at 94^{o}C for 5 min, a second step of 25 denaturation cycles at 94^{o}C for 30 s of annealing at 56^{o}C for 45 s, and extraction at 72^{o}C for 1 min with a last step at 72^{o}C for 7 min. The amplified products were visualised using 0.8% agarose gel electrophoresis in the TBE buffer and then stained with 0.2 µl of ethidium bromide and photographed [40]. The PCR products of the isolated strains were sent to Macrogen Company (hhtt://dna. Macrogen. Com, South Korea) for sequencing.

Results and Discussion

Out of the 29 *Candida* strains that were recovered from the oral cavity of male and female children with leukaemia before and after chemotherapy, three isolates (10.3%) were identified as *C. africana* based on morphological, biochemical, and molecular features.

As shown in Table 1, the ICL21 isolate was obtained from the oral cavity of a 5.5-year-old female with acute lymphoblastic leukaemia (ALL) after receiving chemotherapy. Before induction, there was no signs of infection with candidiasis, as the culture of the concerned sample was negative. The ICL26 was isolated from oral swabs taken from a 10-year-old male afflicted with acute myeloid leukaemia (AML) after chemotherapy. The patient showed the typical oral signs and symptoms, including pseudomembranous candidiasis on the tongue and diffused painful erythematous mucositis. Unfortunately, this patient died after 9 months of chemotherapy. The third isolate ICL27 was recovered from the oral *Candida* growth of a 2.8-year-old male suffering from ALL before having any course of chemotherapy.

All patients were examined thoroughly by the physicians and subjected to a specific protocol of chemotherapy including methotrexate (MTX), 6-mercaptopurine (6MP), and vincristine (VCR).

Developing such diverse and frequent oral yeast infections and mucositis is attributed to the chemotherapy courses, which increases the number and diversity of such infections [41, 42]. Various prophylactic sched-

ules, including oral hygiene, Mycostatin droplets, and chlorohexidine, may be considered to reduce oral opportunistic pathogens [43-45].

To our knowledge, the present findings represent the first recovered *C. africana* isolates from the oral cavity of immunocompromised patients with leukaemia, and the first report of the species in Iraq. To date, the majority of *C. africana* isolates have been registered from female genital samples. However, it has also been reported in other sites of the human body [2, 10, 21, 12].

Similar to Romeo and Criseo [46], our data showed that *C. albicans* was the most frequent species that comprised 89.6% of the strains, followed by *C. africana* at 10.3%. This study did not recover any *C. dubliniensis* strains, which is a clear contradiction with the findings by Romeo and Criseo [46].

The present isolates of *C. africana* possess several diagnostic features that distinguish them from other typical strains of *C. albicans* . These phenotypic characteristics are shown in Table 2, including negative results for chlamydospore on corn-meal agar with 1% Tween 80, no growth at 42^{o}C, the inability to assimilate the amino sugars N-acetylglucosamine and glucosamine as well as trehalose and DL-lactate, and small, bluish-green colonies on the CHROMagar (Fig. 1A).

These results agree with those obtained by previous studies [2, 3, 5, 7-9]. However, our *C. africana* isolates are associated with *C. albicans* in the positive germ-tube formation, growing well at 30^{o}C and 37^{o}C. These features are identical to those described by Tietz et al. [2], Romeo and Criseo [8, 47], and Borman et al. [9]. Colonies of *C. africana* grow well on Sabouraud dextrose agar at 28^{o}C, as small cream-coloured (Fig. 1B). pseudohyphae are rare.

Specific universal primers for *Candida* strains were employed to amplify the ITS1-5.8S-ITS2 regions of rDNA genes, giving the PCR product a molecular size of 572 bp (Fig. 2). Nonetheless, comparing all sequences that were obtained in this study with the database of GenBank and CBS revealed that three (10.34%) out of 29 isolates were identified as *C. africana,* and they showed >99% sequence similarity with that of the *C. africana* strain (CBS 8781), while the rest of the isolates (89.65%) belonged to *C. albicans,* which were mostly analogous to the strain *C. albicans* (ATCC 18804).

In addition to the phynotypic similarities between *C. africana*and other species of *C. albicans* complex, there was a high percentage of molecular proximity among them. Therefore, based on several reports and the sequencing of the D1-D2 regions, the *C. africana* was considered a variant and was not discriminated from the *C. albicans* species complex because the level of dissimilarity was too small to be a distinct species [7, 8, 47, 49]. However, several investigators showed that molecular diagnostic methods, such as sequencing the internal transcribed spacer region 2 or the PCR amplicon length of the HWP1 could be used for definitive identification of *C. africana* [9, 12, 14, 16, 48, 50], while Rodriguez-Leguizmon et al. [15] indicated that the identified isolates based on sequencing the D1-D2 region of the rDNA and the HWP1 gene were in agreement with those for *C. albicans* . Therefore, their study recommended using the matrix-assisted laser desorption-ionisation time of flight mass spectrophotometry (MALDI-TOF MS) and phenotypic and molecular identification to separate the three species of *C. albicans, C. africana,* and *C. dubliniensis.*

In our study, the isolation of *C. africana* in the oral cavity of the patients with leukaemia was not expected because it has been documented that the species is common in human genital organs, particularly in vaginal samples [1-3,7,9,10,18,20, 21,47,49]. However, it was possible to use genes and more to separate this species from *C. albicans.* In contrast to local and international studies regarding *C. dubliniensis,* which primarily occurs in the oral cavity [8, 51, 52], the techniques employed in this work did not show the presence of this species. Abdul-Raheem [32] and Aldossary et al. [36] recovered this species from the oral cavity of diabetic and cancer patients, respectively. Therefore, isolating *C. africana* from the oral cavity is a surprising finding, as it is the first study reporting the presence of *C. africana* in the oral cavities of immunocompromised patients. *Candida africana* has been reported from Saudi Arabia (west border of Iraq) [3] and recently from Iran (east border of Iraq) [4, 5] as an etiological agent of vulvovaginal candidiasis, while it was absent in all re-examined vaginal specimens in Turkey [22]. Hence, the present findings represent the first report of *C. africana* from the oral cavity of immunocompromised patients with leukaemia receiving chemotherapy and

the first record of the species in Iraq. However, further epidemiological, clinical, phenotypical, and molecular inspections based on a larger sample size are strongly recommended to uncover the actual recrudescence of *C. africana* in clinical specimens.

Disclosure statement: The authors declare that they have no conflicts of interest

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Figures captions

1- Fig. 1 Culture of *Candida africana* (1) and *Candida albicans* (2) on CHROMagar (A) and Sabouraud dextrose agar (B). 2- Fig. 2 Gel electrophoresis of the amplified rDNA internal transcribed sequence region of Candida species. Amplified PCR products were migrated on 0.8 agarose at 50 volts for 40 min. Lane M: ladder (100 bp DNA marker). Lane 1: Candida albicans ATCC 18804 and lanes 2-4 C. africana strains.

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