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# Molecular Epidemiology of *Candida albicans* and Its Closely Related Yeasts *Candida dubliniensis* and *Candida africana*[▽]

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**We performed a molecular study to determine the occurrence of *Candida albicans*, *Candida africana*, and *Candida dubliniensis* in different clinical samples. The study provides new insights into the epidemiology of candidiasis in hospitalized patients in three hospitals in southern Italy. It also reports the first detailed epidemiological data concerning the occurrence of *C. africana* in clinical samples.**

The incidence of infection caused by *Candida* spp. has increased steadily over the last two decades, and *Candida albicans* remains the most common fungal pathogen isolated from clinical samples (14, 15, 19, 24). In 1995, *Candida dubliniensis* was previously described as being an opportunistic pathogen linked to oral candidiasis in human immunodeficiency virus-infected patients (21), although it has also been recovered from other anatomical sites (7, 11, 12, 25).

Recently, atypical *C. albicans* strains such as *Candida africana* have been reported as cause of vaginitis from African, German, Spanish, and Italian patients (2, 5, 17, 23).

*Candida africana* has been considered to be an atypical *C. albicans* strain, but in 2001, it was proposed to be a new *Candida* species that produces a germ tube but not chlamydospores (23). However, comparative studies based on genetic techniques have clearly shown that *C. africana* cannot be considered as a new species of *Candida* (2, 5, 17). Therefore, *Candida albicans* var. *africana* was suggested to be a more suitable name for these atypical *C. albicans* strains (17).

More recently, we described the first specific molecular method for discriminating between *C. albicans*, *C. africana*, and *C. dubliniensis* by using a single pair of primers targeting the Hwp1 gene and its homologues in a PCR-based assay (16). Because *C. albicans*, *C. africana*, and *C. dubliniensis* can be identified and differentiated easily and quickly by using this method, we decided to perform a molecular study in order to determine the incidence and distribution of these three important pathogenic yeasts in different clinical samples.

This study provides accurate insights into the epidemiology of candidiasis in hospitalized patients in three hospitals in southern Italy. Moreover, we report here the first detailed epidemiological data concerning the occurrence of *C. africana* in clinical samples.

Four hundred ninety-eight isolates of *Candida* spp. obtained from several anatomical sites and clinical samples were examined (Table 1). The strains were obtained from three different hospitals in southern Italy: Riuniti Hospitals, Reggio Calabria (276 strains); Civil Hospital, Vibo Valentia (138 strains); and the University of Messina Medical School (84 strains). In addition, we conducted a retrospective analysis of 52 *C. albicans* isolates stored in the yeast culture collection at the Department of Life Sciences, University of Messina, Messina, Italy.

The germ tube test was the initial screening test. Biochemical identifications were also performed on all isolates by using the Vitek 2 ID-YST system (bioMerieux) in accordance with the manufacturer's instructions. Only germ tube-positive isolates (324 of 498 strains plus 52 strains from a retrospective study) were selected for molecular analysis and were included in this study. In total, 376 isolates were viable and were thus analyzed by PCR.

Molecular identification was performed according to methods described previously by Romeo and Criseo (16).

*C. albicans* ATCC 10231, *C. dubliniensis* CD36 (a kind gift from Derek Sullivan, University of Dublin, Republic of Ireland), *C. dubliniensis* CBS 7987, and *C. africana* CBS 11016, including strains A1605, A1622, A1653, and M8684 (kind gifts from Hans-Jürgen Tietz, Universitätsklinikum Charité, Berlin, Germany), were used as reference strains.

We obtained 324 isolates of germ tube-positive *Candida* species, which represented 65% of the *Candida* species found among 498 examined yeast isolates (Table 1). The remaining 174 isolates were identified as being *C. glabrata* (32.2%), which was the most prevalent one, followed by *C. parapsilosis* (22%), *C. tropicalis* (16.6%), *C. krusei* (12.6%), *C. kefyr* (10.9%), *C. lusitaniae* (4%), and *C. guilliermondii* (1.7%).

Of 376 germ tube-positive isolates, 366 were identified as being *C. albicans* isolates, whereas 10 isolates were unequivocally identified as being *C. dubliniensis* isolates by using the Vitek 2 system and the ID-YST card.

Three hundred thirty-eight isolates of *Candida* spp. produced a DNA fragment of 941 bp that was identical to that of the reference strain *C. albicans* ATCC 10231. Twenty-seven isolates produced a DNA fragment of 700 bp, indicating that these strains belong to *C. africana*, whereas only 11 isolates showed a DNA fragment of 569 bp and were identified as being *C. dubliniensis*. An overview of the discriminatory power of the PCR primers used is given in Fig. 1.

*C. albicans* was the most common species, representing

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TABLE 1. Clinical samples and *Candida* isolates examined in this study

| Source | No. of samples | No. of GTP[a] strains from clinical samples | No. of strains from culture collections | Total no. of GTP strains | No. of strains of other *Candida* species |
|---|---|---|---|---|---|
| Oral | 121 | 76 | 13 | 89 | 45 |
| Feces | 31 | 23 | 4 | 27 | 8 |
| Vagina | 162 | 136 | 27 | 163 | 26 |
| Urine | 19 | 10 | 0 | 10 | 9 |
| Blood | 16 | 9 | 1 | 10 | 7 |
| Gastric fluid | 29 | 21 | 5 | 26 | 8 |
| Drainage fluid | 67 | 35 | 2 | 37 | 32 |
| Skin lesion | 53 | 14 | 0 | 14 | 39 |
| Total | 498 | 324 | 52 | 376 | 174 |

[a] GTP, germ tube positive.

89.9% of the isolates, followed by *C. africana* strains (7.2%) and *C. dubliniensis* strains (2.9%).

*C. albicans* was recovered from all different kinds of tested samples. A higher incidence (41%) of this species was found in vaginal samples (139 isolates). Indeed, *C. dubliniensis* isolates (11 in total) were found primarily in oral specimens (8 isolates [72.7%]), followed by vaginal samples (2 isolates [18.2%]), and one isolate (9.1%) was recovered from gastric fluid.

*C. africana* was found only in vaginal specimens (27 isolates).

Only 2 of 52 *Candida* isolates used in the retrospective study were identified as being *C. africana* isolates.

The remainders of these isolates were all recognized as being *C. albicans* isolates.

The results obtained by using our molecular method showed that all 27 *C. africana* strains and 1 isolate of *C. dubliniensis* were misidentified as being *C. albicans* isolates and were not resolved by the Vitek 2 system.

The development of improved methods for identifying non-*C. albicans* species such as the well-known *C. dubliniensis* during the past years has resulted in a large volume of published data describing the epidemiology of this species (20). However, no rapid and accurate phenotypic methods are available for clinical laboratory applications, and the identification of *C. dubliniensis* still requires genotyping techniques (9, 10). *C. africana*, considered to be an atypical chlamydospore-negative *C. albicans* strain, which has recently been proposed to represent a separate species within the *Candida* genus, has been added.

Infections due to *C. africana* in Africa (22) and some European countries (2, 17, 23) have been reported. However, on the basis of some data reported in the literature, we assume that this microorganism has a worldwide distribution.

In 1991, Asakura et al. (3) reported seven atypical *C. albicans* isolates that shared an unusual assimilation pattern, serotype B, from Japan, and they failed to produce chlamydospores. One of these seven strains (strain TCH23) also showed an atypical electrophoretic karyotype and was classified as a variant of *C. albicans* with an atypical phenotype (8). Moreover, Al-Hedaithy and Fotedar (1) recovered and studied 25 atypical *C. albicans* strains from 21 specimens from female patients in Saudi Arabia. The majority of these clinical isolates showed a carbohydrate assimilation profile typical of the *C. africana* strains reported in other studies (2, 17).

Recently, Odds et al. (13) analyzed data from multilocus sequence typing for a panel of 1,391 *C. albicans* isolates. Unlike *C. africana* strains, which have so far been isolated from genital sites (1, 2, 17, 23), one strain from Chile, South America, studied by Odds et al. (13) was isolated from blood culture. Interestingly, this Chilean strain could represent the first isolation of *C. africana* from a different clinical sample, suggesting that this fungus can be associated with a more wide clinical spectrum.

Our data clearly indicate that the incidence of *C. albicans* in clinical samples could be overestimated if the variants of this species were placed into a separate group from typical *C. albicans* isolates. However, until now, none of the epidemiological studies took into account the incidence of *C. africana*. This is because most clinical laboratories use automated identification systems that do not always allow discrimination between closely related *Candida* species and/or variants (4, 6). In addition, only a few clinical laboratories perform extensive mycological investigations, including chlamydospore production on cornmeal agar at 24°C for up to 5 days. Therefore, as still happens for some *C. dubliniensis* strains, *C. africana* strains are often misidentified as being typical *C. albicans* strains. In fact, in this study, the Vitek 2 system identified all *C. africana* isolates as being typical *C. albicans* strains (with 85% probability, which is an acceptable identification), indicating that the biochemical reactions of the Vitek ID-YST card are not appropriate for distinguishing between these two closely related yeasts.

On the other hand, infections due to *C. dubliniensis* and/or *C. africana* are underestimated and not well known because although some discriminative methods for these pathogenic yeasts are described in literature, they are not routinely used in many clinical diagnostic laboratories.

To our knowledge, this is the first well-documented report regarding the occurrence of *C. africana* in clinical samples.

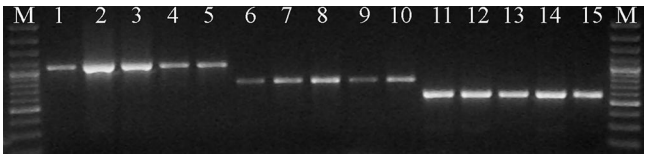


FIG. 1. Molecular discrimination between *C. albicans* (lanes 1 to 5), *C. africana* (lanes 6 to 10), and *C. dubliniensis* (lanes 11 to 15) by using a single pair of primers derived from hwp1 genes. M, molecular size markers.

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