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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Emergence of non-*Candida albicans* species: Epidemiology, phylogeny and fluconazole susceptibility profile

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Summary
Objective. — Non-*Candida albicans* (NCA) species now account for a significant part of clinical candidiasis worldwide. In the present study, epidemiology and antifungal susceptibility profile of NCA isolated from various forms of candidiasis were studied with special focus on their phylogenetic relationship by ITS sequencing.
Patients and methods. — Seventy-nine NCA isolates were isolated from skin and nail scrapings (67.0%), vaginal discharges (8.8%), blood (8.8%), sputa (5.0%), urine (5.0%), oral swabs (2.6%), biopsy and eye tumor, each (1.4%). These isolates were identified by morphological, biochemical and molecular (ITS sequencing) techniques. In vitro antifungal susceptibility of the isolates to fluconazole (FCZ) was tested according to the CLSI method (M27-S4).
Results. — Among a total number of 79 cases of proven NCA infections, *C. parapsilosis* (36.8%) was the most prevalent species followed by *C. glabrata* (32.9%), *C. orthopsilosis* (11.4%), *C. tropicalis* (8.9%), *C. krusei* (5.0%) and *C. guilliermondii* (5.0%). The susceptibility to FCZ was assessed for *C. parapsilosis* (96.5%), *C. orthopsilosis* (88.9%), *C. tropicalis* (85.7%) and *C. guilliermondii* (50.0%). *C. glabrata* and *C. krusei* isolates were not susceptible to FCZ. NCA species were distributed in various phylogenetic clades including *C. glabrata* (1), *C. tropicalis* (3), *C. parapsilosis* (6) and C. *orthopsilosis*, *C. krusei* and *C. guilliermondii* (each 2).

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Conclusion. — *C. parapsilosis* and *C. glabrata* were the most predominant NCA species involve in the etiology of candidiasis. *C. orthopsilosis* was reported from superficial candidiasis. Taken together, our results further substantiate the increasing importance of the involvement of NCA species in the etiology of candidiasis.

Introduction

Candida species are widely distributed in nature and act as common saprophytic constituents of the normal human microflora. Some of these fungi can become opportunistic pathogens following a transition from a commensal to a pathogenic phase induced by alteration in the host environment. A number of factors have been implicated in this increased occurrence of candidiasis, but it is generally accepted that the increased and widespread use of certain medical practices, such as immunosuppressive therapies, invasive surgical procedures and use of broad-spectrum antibiotics are significant. Candidiasis can be divided into three groups: cutaneous (skin and its appendages), mucosal (oropharyngeal, esophageal, and vulvovaginal) and systemic (bloodstream infections, i.e., candidemia and other forms of invasive candidiasis). It currently represents the fourth leading cause of nosocomial infections, at 10%, and mortality due to systemic candidiasis remains high, ranging from 15 to 35% depending on the infecting *Candida* species [1]. Global surveillance programs (e.g. SENTRY and ARTEMIS) provide a tremendous amount of data regarding global trends in various aspects of NCA candidiasis including geographical variation in the frequency of species, distribution by type and age, as well as changes in the antifungal susceptibility of collected NCA isolates. Whereas, NCA species accounted for 10—40% of all systemic candidiasis from 1970 to 1990, this proportion reached 35—65% in the last two decades. A recent ten-year analysis of the worldwide distribution of these species indicated that *C. glabrata* remains the most common species and *C. parapsilosis*, *C. tropicalis*, *C. krusei* are frequently isolated. Significant geographic variation in the frequency of NCA species occurs. It has been shown that *C. glabrata* is more prominent in North America than Latin America; *C. tropicalis* was frequently isolated in Asia-Pacific, whilst *C. parapsilosis* remained more commonly recovered in North America than in Europe [1,2]. *Candida albicans* is the main cause of candidiasis. However, surveys from different institutions, cities, countries, and broad geographic regions have documented the emergence of the various NCA species as well as their potential to develop antifungal resistance [3—5]. The apparent increased involvement of NCA species in human candidiasis may partly is related to improvements in diagnostic methods, such as morphological characteristics (germ tube test, chlamydospore development, chromogenic media with the ability to differentiate *Candida* species), carbohydrate assimilation as well as the introduction of molecular techniques in the routine diagnosis of fungaemia.

Besides the conventional identification techniques, molecular-based technologies such as targeting yeast rDNA gene, operon, encoding the 18S, 5.8S, and 28S rDNA gene subunits, namely Internal Transcribed Spacer1 (ITS1), ITS2 and ITS4 is a very effective method for confirmation of identity and phylogenetic analysis of *Candida* species [6].

In vitro antifungal susceptibility testing now plays an increasingly important role in guiding therapeutic decision making as an aid in drug development studies, and as a means of tracking the change of antifungal resistance in epidemiological studies. Microdilution methods are the gold standard or reference techniques. Azole antifungals such as FCZ are often preferred treatment for many *Candida* infections as they are inexpensive, exhibit limited toxicity and are available for oral administration. Over the past 10 years, there is extensive documentation of intrinsic and developed resistance to azole antifungals, especially FCZ among several *Candida* species mainly *C. glabrata* and *C. krusei* isolates [7]. The susceptibility pattern of *Candida* species to FCZ may be changed following by the increasing use of this antifungal, so in vitro susceptibility testing is necessary to help clinicians in the selection of appropriate therapy [8].

The present study represents the distribution and molecular epidemiology of non-*Candida albicans* species isolated from various clinical samples and evaluates the in vitro antifungal susceptibility of isolated *Candida* species to fluconazole.

Patients and methods

Patients

During the year 2014, 140 cases suspected to different types of candidiasis who referred to our department were studied. Clinical samples were prepared and examined according to the standard protocols.

Isolation and conventional identification of *Candida* species

A portion of the specimens was inoculated on Sabouraud dextrose agar medium (SDA; E. Merck, Germany) supplemented by chloramphenicol (0.05 gL^{-1}) and incubated at 37 °C for 24—48 h to generate the *Candida* species. All isolates were identified by a combination of morphological and biochemical criteria by germ tube test, chlamidospore formation, carbohydrate assimilation test by the commercial system ID32 C (biomérieux Marcy l'Etoile, France) and CHROMagar Candida (CHROMagar Candida, Paris, France). Reference strain of *C. albicans* ATCC 10231 was used as control in all later experiments.

Molecular identification of *Candida* species

For DNA extraction, fresh colonies were collected upon culturing the isolates on SDA for 48 h at 37 °C. Genomic DNA was extracted using the Molecular Biology kit (Bio Basic

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