

Occurrence and *In Vitro* Antifungal Susceptibility of *Candida Spp.* Isolated from Decayed Tree Parts in Green Urban Areas from Pisa (Central Italy)

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ABSTRACT

Trees and grass are proven sources of potentially pathogenic yeasts. Among fungi regarded as human/animal pathogens, several *Candida Spp.* are involved. Since wooded areas are frequented by people and their pets, aim of the present study was to investigate the occurrence of potentially pathogenic yeasts on decaying vegetal material from trees in 2 city parks in Pisa, Italy. A total of 186 samples was collected. The antimycotic drug sensitivity pattern of cultured *Candida spp.* was evaluated versus fluconazole, voriconazole, caspofungin and amphotericin B, by Etest. Sixteen trees out of 31 (51.6%) scored positive for yeasts, and twenty-six fungal isolates were cultured. Thermotolerant strains, such as *Candida Famata* (6/6), *Candida Guilliermondii* (4/4), *Candida Membranaefaciens* (2/3) and *Candida Parapsilosis* (1/1), were obtained. All the yeasts were sensitive to amphotericin B, 2 *C. Famata* were resistant to assayed azoles, and this fungal species was the only one susceptible to caspofungin, while fluconazole was effective versus all *C. Guilliermondii* isolates. Although the relatively small sample size, decaying vegetal material from trees growing in sampled recreational green areas, would seem to be a natural site harboring potentially pathogenic yeasts, characterized by low sensitivity against some important antimycotic drugs.

Introduction

Yeasts can act as important pathogens both in animals and humans and it is well known that most of them occur as saprophytes in the environment, being able to grow on not viable organic matter. Moreover, these organisms are frequently able to form biofilms [1] and some among them are characterized by intrinsic and/or acquired resistance to one or more commonly administered antifungal drugs [2]. Among fungi regarded as human and animal pathogens, several *Candida spp.* are reported. *Candida Albicans* represents the main causative agent for human candidiasis, although the numerous non-*albicans* species have increased their occurrence in candidiasis in the last decades to >50% of bloodstream infections throughout the world [3]. Droppings of domestic as well as free ranging birds are proven sources of potentially pathogenic yeasts [4-7], such as decomposing plant material, trees and grass [8]. For instance, *Cryptococcus neoformans* and *Cryptococcus gattii* have been cultured from decaying vegetal materials [9-11], while several other yeasts would dwell onto different trees.

In particular *Candida tropicalis*, *Candida parapsilosis* and *C. albicans* have been recovered from trees [12-14] as well as woodland are considered a privileged site for *Saccharomyces spp.* [15]. Furthermore, wooded and green areas are visited by large numbers of people and their pets, and in the last years reports of mycoses caused by yeasts, mostly referring to fungi belonging to *Candida* genus, are more frequently encountered than in the past [2,3,16,17]. For these reasons, considered also the increased occurrence of immunocompromised patients, the knowledge of the ecology of these fungal species may be of interest. To the best of our knowledge, there are no reports about the presence of yeast species, in particular *Candida spp.*, associated with trees in Italy. Therefore, aim of the present study was to investigate the occurrence of potentially pathogenic yeasts on decaying vegetal material from trees in 2 city parks in Pisa, Italy, evaluating also the drug sensitivity pattern of *Candida spp.* isolated versus some among the most common used antifungals in human medicine.

Materials and Methods

Specimens were drawn from trees growing in 2 green recreational areas, within the urban area of Pisa (43°42'30" N10° - 24'12" E), Central Italy, visited by both people and pet dogs. Thirty-one trees were sampled. In detail 6 *Pinus pinea*, 4 *Tilia platyphyllos*, 1 *Platanus acerifolia*, 1 *Fagus sylvatica*, 1 *Chicas revoluta* and 1 *Taxus baccata* from site 1, and 4 *P. pinea*, 2 *Salix babylonica*, 1 *P. acerifolia*, 3 *Quercus ilex*, 1 *Chamaciparis lawsoniana*, 1 *Morus alba*, 1 *Laurus nobilis*, 1 *Nerium oleander*, 1 *Carpinus betulus* and 1 *Gleditsia triachantos* from site 2, respectively, were selected. Only decaying parts of trees were selected and for each tree roots, tree hollows and leaves were sampled by swabbing technique, as reported by Maganti et al. [12]. For each sampled site 2 swabs were drawn, obtaining 186 total specimens. All samples were immediately transferred to the laboratory and therefore seeded onto Malt Extract Agar (MEA) supplemented with 0.1% byphenil. Subsequently, they were incubated at both 25°C and 37°C, until a noticeable fungal growth was ascertained. The latter temperature was selected in order to evaluate the thermotolerance of isolates. Yeast identification was achieved by microscopic analysis, as well as auxanographic profile using ID32C galleries (BioMérieux, Marcy l'Etoile, France). All strains presumptively referring to *Candida* genus were subsequently subjected to PCR analysis, performed by amplification and partial sequencing of the D1/D2 domain of 26S rRNA encoding gene, as described by Lopandic et al. [18], as confirmatory identification test. DNA partial sequences obtained were then compared with those present in the NCBI database, by matching the BLAST program from GenBank. Sequences with a 98 to 100% identity with GenBank sequences were retained as significant for the molecular identification of yeasts. *Candida* spp. sensitivity to most frequently employed antimycotic drugs (amphotericin B, fluconazole, voriconazole, caspofungin) was evaluated by Etest (BioMérieux, Marcy l'Etoile, France). Minimal inhibition concentrations (MIC) breakpoints, when available, were calculated following the CLSI recommendations for *Candida* spp. [19]. For amphotericin B this value is not available, so the antifungal clinical breakpoint provided by EUCAST was considered [20].

Results

Sixteen trees out of 31 (51.6%) scored positive for yeasts in at least one sampled site. Twenty-six fungal isolates were cultured and identified as *Candida* spp., *Debaryomyces polymorphus*, *Rhodotorula* sp. and *Pichia etchelsii*. In particular, thermotolerant *Candida* isolates were obtained for *Candida famata* (6/6), *Candida guilliermondii* (4/4), *Candida membranaefaciens* (2/3) and *Candida parapsilosis* (1/1). Eight *P. pinea* out of 10 scored positive, with *Candida* spp. as the most prevalent genus, recovered from at least 1 site. *Candida* spp. were isolated from 1 *T. platyphyllos*, 2 *S. babylonica*, 1 *C. lawsoniana* and 1 *L. nobilis*. Different fungal species from the same tree were detected in 3 *P. pinea*, 1 *T. platyphyllos* and 1 *P. acerifolia*. PCR analysis confirmed in all cases the presumptive *Candida* species identification based on phenotypic features, showing a high

similarity (>99%) with the deposit species sequences described in the GenBank database determined by BLAST. *Cryptococcus neoformans/gattii* was never been isolated during the study. All *Candida* isolates were sensitive to amphotericin B, 2 *C. famata* were resistant to all azoles assayed, while fluconazole was effective versus all *C. guilliermondii* isolates. Interestingly, *C. famata* was the sole yeast species fully sensitive to caspofungin. More detailed data about the source of yeasts and drug sensitivity pattern are reported.

Discussion

The fungal species isolated are in agreement with the findings of Maganti et al. [12], who report the occurrence of *Candida*, *Rhodotorula* and *Pichia* Genera from tree hollows in urban areas. A limitation of this study is the low number of sampled trees, indeed. Unfortunately, it was not possible to overpass this problem, since the study design was focused on decaying trees only, that were present in low numbers in sampled areas. Among *Candida* spp., *C. parapsilosis* is an important etiological agent of invasive candidiasis [21], representing the second or third most common species isolated in human candidemia worldwide. The isolate obtained in our study showed resistance to fluconazole and caspofungin. These findings agree with literature data, and the fluconazole resistance of some strains within this yeast species has been related to an overexpression of MRR1 [22]. *C. famata* was the most prevalent recovered yeast species and it is considered as a rare, but emergent [23] cause of candidiasis, with a prevalence ranging from 0.2% to 2% [24]. These yeasts have been reported as agents associated to urinary tract infection in pets, characterized by similar antimycotic drugs sensitivity patterns [25]. In the present study, 5 out of 6 isolates were resistant to fluconazole, while the other one had a dose dependent sensitivity. Moreover, 2 isolates resulted resistant to voriconazole. This finding would appear of interest, showing that isolates characterized by high rates of resistance would be widely spread in urban environment. *C. guilliermondii* has been reported as a cause of candidemia in neoplastic patients [26], being involved in up to 2% of human cases [27], and in a joint infection in a dog [16]. Three out of 4 strains scored resistant to caspofungin, while the other one showed intermediate sensitivity, in agreement with Marcos-Zambrano et al. [27]. *C. membranaefaciens* is a yeast infrequently reported in intravenous catheter-associated invasive infections [28,29], whose sensitivity to fluconazole is a matter of debate [28]. The isolates obtained in our work seem to confirm the resistance to fluconazole. The intermediate sensitivity of all the three isolates to caspofungin appears to be an interesting finding. The results obtained from this study would indicate that decaying vegetal material from trees occurring in investigated recreational green areas, seems to harbor potentially pathogen yeasts, characterized by low sensitivity against some currently used antimycotic drugs.

Disclosure of Interest

The authors declare no conflict of interest.

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