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High prevalence of *Candida dubliniensis* in lower respiratory tract secretions from cystic fibrosis patients may be related to increased adherence properties

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SUMMARY

Objectives: We identified *Candida spp* isolated from lower respiratory tract secretions obtained from cystic fibrosis (CF) patients, by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), with the aim of determining the most prevalent causative agent. We also sought to determine their adhesive properties in order to understand their biology related to CF.
Methods: Twenty-five clinical samples were collected from a cohort of 20 CF patients. Twenty-six isolates of *Candida spp* were isolated and identified by MALDI-TOF MS method. Adherence assays were performed using the Fluxion BioFlux 200, a flow apparatus that allows for the visualization of adhering cells.
Results: MALDI-TOF MS analysis revealed *C. dubliniensis* to be the most prevalent species ($n = 18$, 69%), followed by *C. albicans* ($n = 4$), *C. tropicalis* ($n = 3$), and *C. glabrata* ($n = 1$). *C. dubliniensis* showed the strongest adherence under constant flow when compared to the other species of *Candida*. In the majority of cases, *C. dubliniensis* was isolated in combination with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *C. dubliniensis* appears to be able to survive in the CF lung and coexist with bacteria.
Conclusions: The data presented here show that the presence of *C. dubliniensis* in the lower airways of CF patients may be related to increased adherence properties.

1. Introduction

Cystic fibrosis (CF) is the major genetic inherited disease found in European Caucasians, but is common worldwide.[1] CF occurs due to mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*), which results in a defective mucociliary

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clearance and, as a consequence, the production of thick bronchial mucus. This mucus facilitates the entrapment of airborne bacteria and fungal conidia, providing an environment suitable for the growth of these microorganisms.[2]

Significant risk factors for CF patients that predispose them to increased oral *Candida* colonization include steroid use, CF-related diabetes, and recurrent courses of prolonged antibiotic treatment.[3] The airways of CF patients are often colonized by bacterial species that cause chronic infections. Among these bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the predominant species, followed by *Burkholderia cepacia* and *Haemophilus influenzae*.[2,4] *Candida spp* are the most common yeasts isolated from the airways of CF patients.[5,6] In a German study, *C. albicans* was isolated with a high frequency (78.8%) from the respiratory tract of CF patients, whereas *C. dubliniensis* has been isolated with a lower frequency; however little is known about its potential to cause disease in these patients.[7]

The identification of *C. dubliniensis* in particular remains problematic due to its close relatedness and phenotypic similarity to *C. albicans*.[5] Differences between these two species are most pronounced at the genetic level and for that reason molecular methods are more accurate to distinguish *C. albicans* from *C. dubliniensis*.[8] Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is now considered as reliable as molecular diagnostics, and is applied routinely for the identification of yeast due to its potential to differentiate closely related species. The differentiation of *C. albicans* and *C. dubliniensis* is of importance in order to better understand the epidemiology and virulence of *C. dubliniensis*.[6] *C. dubliniensis* is only a minor component of the normal microbiota of oral cavities, but is distributed worldwide.[7] In recent years, however, the isolation of *C. dubliniensis* has increasingly been reported from patients with candidemia and CF patients.[9,10] The prevalence rate of this emerging pathogen among CF patients has been shown to range from 2.6% to 39%.[11,12]

In this article we report the high and frequent occurrence of *C. dubliniensis* in the respiratory secretions of CF patients from Qatar. The aim of this study was to identify *Candida* spp isolated from pediatric and adult CF patients, by MALDI-TOF MS method, to determine the most prevalent causative agent. In addition we examined their adhesive properties to understand how this aspect of their biology relates to CF.

2. Materials and methods

2.1. Patients and sampling

Over a period of 2 years, sputum samples, deep pharyngeal swabs (taken from patients who did not produce sputum), and bronchoalveolar lavage (BAL) samples were collected from pediatric and adult CF patients at Hamad Medical Corporation, Doha, Qatar. The diagnosis of CF was based on one or more clinical features consistent with CF, namely positive family history of CF in siblings and close relatives, elevated sweat chloride ($>$ 60 mmol/l) on two separate occasions, and the presence of two disease-causing mutations in the *CFTR* gene.

2.2. Phenotypic identification of Candida species

Samples of lower respiratory tract secretions were collected and immediately cultured on Sabouraud dextrose agar plates with chloramphenicol (SDAC) (Difco, USA) and CHROMagar *Candida* plates (Oxoid Ltd, UK) to isolate yeasts and to ensure purity of the isolates. SDAC plates were incubated at 35 °C and CHROMagar Candida plates at 30 °C for 48 h. *Candida* isolates were processed using the VITEK 2 Compact yeast identification system (bioMérieux, France). All isolates were preserved at −70 °C using cryotubes (Mast Diagnostics, UK), and were later identified by MALDI-TOF MS at the CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands.

2.3. Biochemical identification of bacterial isolates

Clinical specimens were cultured on a variety of different media (Remel, Lenexa, KS, USA) including trypticase soy blood agar with 5% sheep blood (TSA), chocolate agar (CHOC), MacConkey agar (MAC), mannitol salt agar (MS), and *B. cepacia* selective agar (BCSA). Plates were incubated in ambient air or 5% CO_2 at 35 °C and examined daily until all isolates were identified. A single colony of each isolate was identified by VITEK 2 Compact (bioMérieux) or Phoenix 100 (Franklin lakes, NJ, USA). After 2 days of incubation at 35 °C, lactose-negative and oxidase-positive colonies of *P. aeruginosa* were selected and identified, as described previously,[13] using *P. aeruginosa* reference strain ATCC 27853.

2.4. MALDI-TOF mass spectrometry

Each clinical isolate of *Candida spp* was maintained on GYPA plates (2% glucose, 0.5% yeast extract, 1% peptone, 1.5% agar) for 48 h at 30 °C. A single colony was isolated and subcultured on SDA plates for 24 h at 30 °C. Isolates were identified by MALDI-TOF MS carried out according to the Bruker Daltonics protocol, as reported previously.[14] To ensure reproducibility of the spectra, tested isolates were measured in duplicate and identified by MALDI Biotyper RTC software 3.0 (Bruker Daltonics, Germany). This research was carried out using two databases: the original, commercially available Bruker Daltonics database (BDAL) of 4110 main mass spectra (MSPs) and in addition a CBS-KNAW in-house library of 510 MSPs of reference strains of different yeasts.

2.5. Yeast cell adherence assay

Adherence assays were performed as described previously using the Fluxion BioFlux 200 (Fluxion Biosciences), a flow apparatus that allows for the visualization of adhering cells.[15] All clinical isolates were run in triplicate. For three *Candida spp*, a species-specific reference strain was assayed, namely *C. albicans* ATCC 90028, *C. dubliniensis* CBS 8500, and *C. tropicalis* ATCC 66029 as controls, and included in the experiment. The average number of adhering cells for each strain was calculated based on three replicates, and the average fold change was calculated (number of adhering cells of the clinical isolate/the number of adhering cells of *C. albicans* control reference strain). Error bars represent the standard deviation and *p*-values were calculated by *t*-test.

3. Results

3.1. Clinical results

In this study, 25 respiratory samples were collected from 20 CF patients of both genders (46% female and 54% male) aged 0.6–24 years (median 15.5 years) (Table 1). Additionally, three respiratory samples were collected from patient 1 and two from patients 9, 14, and 15. Eighteen patients of Qatari origin harbored the I1234 V mutation in the *CFTR* gene associated with pancreatic sufficiency, one patient of Bangladeshi origin had the deltaF508 mutation, and the remaining patient of Pakistani origin had an undetermined CF genotype due to an unknown *CFTR* gene mutation. Additionally, 14 patients received anti-Pseudomonas nebulized antibiotic prophylaxis with tobramycin (n = 13) and gentamicin (n = 1). The patients of Bangladeshi and Pakistani origin had pancreatic insufficiency.