Trichosporon mycotoxinivorans Infection in Patients with Cystic Fibrosis

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We describe the clinical course of four patients who had *Trichosporon mycotoxivorans* recovered from multiple sputum cultures over time, with varying clinical consequences but no fatalities. We also report successful rapid identification of this organism using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.
In a single pediatric cystic fibrosis (CF) program, we found 4 pediatric patients in whom *Trichosporon mycotoxinivorans* was isolated. All had had persistently positive cultures for *Trichosporon* spp. at the time *T. mycotoxinivorans* was confirmed. All patients had severe CFTR gene mutations and pancreatic insufficiency, and were treated with pancrelipase and appropriate vitamins, as well as inhaled beta agonists and airway clearance. Further clinical characteristics of the patients are summarized in the table. All were infected with common bacteria associated with CF, took maintenance inhaled and oral antibiotics, and were intermittently treated with additional oral and/or intravenous antibiotics for pulmonary exacerbations of cystic fibrosis.

Cases 1 and 2 both experienced episodes of severe pulmonary exacerbation requiring prolonged hospital stays, which occurred at least 2 years after initial isolation of the organism and resolved without anti-fungal treatment, though pulmonary function has not returned to baseline. Case 3 showed consistently poor response to intravenous antibiotics after several consecutive courses of therapy for pulmonary exacerbation and was treated with antifungal therapy. Voriconazole was associated with intolerable photosensitivity, and he was subsequently treated with inhaled amphotericin B, with a reduction in pulmonary exacerbation frequency but not improvement in pulmonary function, which had declined over time. Case 4 had one episode of pulmonary exacerbation 3 years after initial isolation, and had recovered completely.

**Microbiology laboratory methods and results:** For culturing bacteria from specimens from patients with cystic fibrosis, respiratory samples were inoculated on standard solid media (1-3), or on Sabouraud-Dextrose and Mycobiotic agars when a fungal culture was ordered.
Yeast like organisms that grew as predominant organism or in pure growth from bacterial culture, or any quantity from fungal culture were identified biochemically using the Microscan Rapid Yeast Identification Panel (Siemens Healthcare Diagnostics Inc., West Sacramento, CA).

Prior to this study, respiratory specimens from these patients had multiple positive growth of organisms identified as *Trichosporon* spp. based on phenotypic characteristics (4). Since none of these previously obtained isolates had been saved, their true identification cannot be confirmed.

From January of 2013, isolates were also tested using the MALDI-TOF system (Bruker Daltonics Inc., Billerica, MA) with the RUO software and database (Biotyper 3.1) and the API 20 C AUX system (bioMerieux, Inc., Durham, NC). When *T. mycotoxinivorans* species was definitively identified the first time from each patient, organisms from cases 1, 3, and 4 grew moderately on the primary plates of Tryptocase Soy Agar with 5% sheep blood, and organism from case 2 grew on Sabouraud dextrose agar, all after two day incubation from the time direct samples were inoculated. The organisms were recognized as yeast like organisms based on colony morphology and Gram stain, and were all identified to the species level by MALDI-TOF as *T. mycotoxinivorans* with no ambiguity (scores were greater than 2.0). Interestingly, the MicroScan system identified all four isolates as *Trichosporon beigelii* with very high probability of 99.99%. The API 20 C AUX identification systems suggested *T. mucoides* with low probability ranging from 67.2% to 86.0%. However, species name of *T. mycotoxinivorans* is not included in databases of these two phenotypic identification systems. All these isolates were referred to The Fungus Testing Laboratory (San Antonio, TX), and the identification was confirmed by using a combination of phenotypic characterization and DNA sequencing of the ITS and D1/D2 targets (100% identity for all four isolates). Subsequently, more isolates from these patients continued to be identified as *T. mycotoxinivorans* using the MALDI-TOF.
Discussion. Trichosporon species are uncommon pathogens but can cause life-threatening infections in immunocompromised patients. Yeast-like Trichosporon spp. are found throughout the environment, predominantly in tropical and temperate areas. Members of this species are able to colonize and proliferate in the gastrointestinal tract, respiratory tract, skin and vagina. Trichosporon can cause deep seated, mucosa-associated or superficial infections. Invasive trichosporonosis is mostly seen in patients with malignancies while superficial infections are relatively more common in immunocompetent hosts. (5)

Trichosporon mycotoxinivorans was initially described in 2004 (6). The first case of human infection was reported in 2009 (7) and documented a fatal case of pneumonia caused by the organism in an adult CF patient. In a second report, T. mycotoxinivorans was one of the organisms associated with a disseminated fatal infection in a CF patient who had received lung and liver transplantation (8). The final case was reported in a cohort of CF patients who had Trichosporon infection; among 8 cases, only one was reported to have T. mycotoxinivorans. The organism persisted during a 6 year follow up period and was thought to play a role in a less severe clinical course (9). Our small case series contributes further to the understanding of the significance of T. mycotoxinivorans in CF pulmonary infection.

None of our patients had fulminant disease associated with initial isolation of T. mycotoxinivorans. While two had significant pulmonary exacerbations following isolation of the organism, the time frame between isolation and exacerbation makes it unlikely that this was the
cause of exacerbation, and both had resolution of exacerbation without specific antifungal
therapy. In a third case, ant-fungal treatment was initiated due to poor response to intravenous
antibiotics targeted towards bacteria, with possible improvement. The final case has had an
unremarkable course since the organism was first isolated. Thus, isolation of this organism is
not necessarily associated with significant changes in clinical status. Because *T. mycotoxinivorans*
has only recently been described and because identification requires specialized DNA testing that
is not readily available in most clinical laboratories, it may be some time before its clinical
implications are completely understood.

Another important observation in these cases is the potential association of antimicrobial
exposure and isolation of *T. mycotoxinivorans*. Chronic antibiotic therapy is the standard of care
for CF patients who have chronic airways infection with *Pseudomonas aeruginosa*. However,
due to the propensity of a variety of bacteria and fungi to thrive in the environment of the CF
airway, other pathogens can emerge in the context of chronic suppressive antibiotics. In the
pivotal clinical trials of tobramycin inhalation solution (TIS), subjects treated with TIS had an
increased rate of isolation of *Aspergillus fumigatus*, *Candida albicans* compared to those treated
with placebo (10). All of our subjects had a history of *P. aeruginosa* infection and exposure to
TIS, and most had repeated exposures to other antibiotics for pulmonary exacerbations,
suggesting that *T. mycotoxinivorans* may be another treatment-emergent organism. The clinical
significance of treatment emergent pathogens remains unclear, possibly due to differences in
pathogen-host interaction. For example, *A. fumigatus* can be associated with allergic
bronchopulmonary aspergillosis in CF and, rarely, with severe infection, but many patients with
A. fumigatus isolated from respiratory tract culture have no evidence of disease due to this organism. We propose that T. mycotoxinivorans also has a variable effect on CF lung disease.

To assess the clinical relevance of any organism, it is essential to ensure the accuracy of species identification. Standard laboratory methods alone are not reliable for this particular organism as consistently shown in literature and in our study. Definitive identification relies on DNA testing (7,8) which is not readily available in most clinical laboratories. The identification of the disseminated case in the second report required 16 days (8). In the present study, identification of the 4 isolates at the specialized reference laboratory using combined phenotypic and DNA sequencing methods required 9-15 days. Although MALDI-TOF technology has been well studied and has been shown to be effective for identification of yeast or yeastlike clinical isolates, its utility in identifying this particular organism has not been studied (11-13). We have found that using the MALDI-TOF method, colonies of these organisms can be identified in just 1 hour. Due to the limitation of the relatively low number of clinical isolates tested in the study and limited availability of reference materials, we were unable to perform an extended method validation. Further studies focusing on the MOLDI-TOF performance specifically on this species should provide useful information to possibly allow the method to be used routinely.

In summary, we describe a clinical case series of patients with cystic fibrosis and T. mycotoxinivorans chronic infection. These cases, along with 3 previously reported in the literature, suggest that this organism has a variable effect on CF lung disease. We also demonstrate the potential utility of MALDI-TOF technology for rapid identification of the organism.
REFERENCES


Table. Clinical Characteristics of Patient Cases

| Case number | Age | Sex | CF Genotype | Best FEV1% before first isolation | Best FEV1% after first isolation | Worst FEV1% since isolation | Most recent FEV1% | Duration of *T. mycotoxinivorans* infection (years) | Percent of cultures with *T. mycotoxinivorans* | CF pulmonary therapies | No. of oral antibiotic courses | No. of IV antibiotic courses | *T. mycotoxinivorans* treatment | Other organisms |
|-------------|-----|-----|-------------|----------------------------------|---------------------------------|-----------------------------|-----------------|-----------------------------------------------|-----------------------------------------------|------------------------|-------------------------------|--------------------------|-----------------------------|------------------------|--------------------------|
| 1           | 18  | F   | F508del/p.R553X | 93                              | 102                             | 36                          | 65              | 4.5                                           | 38                             | azithromycin*            | 12                            | 1                         | No                       | MSSA                    | Pseudomonas aeruginosa | Stenotrophomonas maltophilia |
|             |     |     |              |                                  |                                 |                             |                 |                                               |                                 | TIS**                   |                                |                          |                           |                        | Serratia marcescens         | Aspergillus fumigatus |
|             |     |     |              |                                  |                                 |                             |                 |                                               |                                 | dornase alfa             |                                |                          |                           |                        | Acinetobacter pittii     |                        |
| 2           | 8   | F   | F508del/G551D  | 78                              | 121                             | 78                          | 99              | 2.5                                           | 20                             | TIS**                   | 9                             | 3                         | No                       | MSSA                    | Pseudomonas aeruginosa | Flavobacterium breve       |
|             |     |     |              |                                  |                                 |                             |                 |                                               |                                 | dornase alfa             |                                |                          |                           |                        | Stenotrophomonas maltophilia |
|             |     |     |              |                                  |                                 |                             |                 |                                               |                                 | ivacaftor                |                                |                          |                           |                        |                         |                        |
| 3           | 17  | M   | F508del/F508del| 87                              | 108                             | 57                          | 72              | 6.4                                           | 33                             | azithromycin*            | 12                            | 12                        | Yes – voriconazole, inhaled amphotericin B | MSSA                    | Pseudomonas aeruginosa | Stenotrophomonas maltophilia |
|             |     |     |              |                                  |                                 |                             |                 |                                               |                                 | TIS**                    |                                |                          |                           |                        | Haemophilus influenzae    |                        |
| 4           | 17  | M   | F508del/F508del| 102                             | 107                             | 78                          | 98              | 5.7                                           | 24                             | azithromycin*            | 28                            | 1                         | No                       | MSSA                    | Pseudomonas aeruginosa | Pseudomonas stutzeri       |
|             |     |     |              |                                  |                                 |                             |                 |                                               |                                 | TIS**                    |                                |                          |                           |                        | Pseudomonas putida/fluorescens | Haemophilus influenzae |
|             |     |     |              |                                  |                                 |                             |                 |                                               |                                 | dornase alfa             |                                |                          |                           |                        | Aspergillus fumigatus     |                        |

*azithromycin three times a week  **inhaled tobramycin 300 mg inhaled BID  ***inhaled corticosteroid
patient had grown Trichosporon prior to receiving care at this center  7% hypertonic saline inhaled BID  presumed *T. mycotoxinivorans* based on availability of data