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Penicillium citrinum: Opportunistic pathogen or idle bystander? A case analysis with demonstration of galactomannan cross-reactivity

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ABSTRACT

We present a case of an immunocompromised woman with fever, pulmonary infiltrates and multiple bronchoalveolar lavage (BAL) cultures positive for *Penicillium citrinum* with a concomitant high BAL galactomannan level. We report the results of *Aspergillus* galactomannan testing performed on culture supernatants from her *P. citrinum* strain that confirmed the suspected cross-reactivity. Finally, we discuss the clinical significance and antifungal susceptibility of *P. citrinum* in our case and review the literature.

1. Introduction

Non-marneffei *Penicillium* species are among the most common fungi worldwide [1]. They inhabit diverse indoor and outdoor environments causing the occasional nuisance (moldly bread) in addition to having played spoiler in the perhaps the greatest failed experiment of all time (Alexander Fleming's discovery of penicillin). Despite their ubiquity, non-marneffei *Penicillium* species very rarely cause human disease.

We present a case of an immunocompromised stem cell transplant recipient with acute respiratory symptoms and a bronchoalveolar lavage (BAL) culture that grew *Penicillium citrinum* on multiple plates. The BAL galactomannan level was very high despite negative fungal smears and no isolation of *Aspergillus* sp. in culture. A number of non-Aspergillus fungi have been reported to have galactomannan cross-reactivity including some strains of Fusarium sp., Paecilomyces sp. and Penicillium sp. (not *P. citrinum*) [2–5]. We present the first data confirming galactomannan cross-reactivity specifically for *Penicillium citrinum* as measured by a validated clinical assay.

We performed an extensive literature search identifying a total of four reports of *P. citrinum* infection in humans, three of which occurred in patients with acute leukemia receiving intensive chemotherapy [6–8] and one which occurred in the setting of direct inoculation into an immune-privileged space [9]. Based on our patient's significantly milder degree of immunocompromise relative to that induced by acute leukemia plus chemotherapy, we concluded that *P. citrinum* was unlikely to have been a tissue-invasive cause of pneumonia in her case. However, given growth of P. citrinum from multiple BAL cultures, we suspected the organism's legitimate presence in her tracheobronchial tree.

Finally we reviewed reported anti-fungal sensitivity data for other clinical *P. citrinum* isolates in conjunction with the data obtained from our patient's isolate. It became apparent that the mean inhibitory concentration (MIC) for voriconazole was consistently high (> 16 μg/ml), while other triazoles and other classes of anti-fungals displayed less skewed MIC distributions.

2. Case

A 25-year-old woman with history of aplastic anemia treated with haplo-cord stem cell transplant 7 months prior presented to the hospital with acute neurologic symptoms. Brain magnetic resonance imaging (MRI) revealed large, enhancing bilateral basal ganglia lesions with vasogenic edema producing mass effect. The patient was started on IV steroids to decrease cerebral edema and rituximab based on high suspicion for Epstein-Barr virus (EBV)-related post-transplant lymphoproliferative disorder (PTLD). A plan for multi-viral T cell therapy led to a preparative steroid taper. One week into the steroid taper the patient was suddenly found unresponsive. Repeat MRI showed acute bilateral occipital lobe infarctions, presumably secondary to herniation syndrome given the lack of vascular obstruction or stenosis detected by magnetic resonance angiography (MRA) or magnetic resonance venography (MRV). The patient was restarted on high dose steroids with a peak dose of intravenous (IV) dexamethasone 6 mg (mg) every 6 h. Brain biopsy was performed which confirmed the diagnosis of EBV PTLD.

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One month into her hospitalization the patient developed low-grade fever and a low-flow oxygen requirement associated with symptoms of dry cough and mild shortness of breath (day 0 for reference). Same-day computed tomography angiography (CTA) of the chest showed new diffuse, bilateral pulmonary infiltrates without evidence of pulmonary embolism. At this point her steroid dose had been tapered down to dexamethasone 2 mg IV every 12 h and she was on Day 2 of piperacillin-tazobactam for a possible Citrobacter urinary tract infection (UTI). She remained on prophylactic micafungin 100 mg daily (started on day −12) and prophylactic trimethoprim sulfamethoxazole in addition to pre-emptive ganciclovir therapy (started on day −14). She had completed a 5-day course of oseltamivir three weeks prior following a positive nasal wash test for Influenza A. Mild symptoms of nasal congestion without fever, shortness of breath or myalgias had prompted respiratory viral testing at that time.

On exam (day 0) the patient was febrile to 38.4 C, tachycardic with rate 110–120 beats minute^{-1} and normotensive. She was slightly tachypneic while saturating 91–93% on room air. Oxygen saturation improved to > 95% with 2 l of oxygen via nasal cannula. Laboratory data were notable for stable lymphopenia (absolute lymphocyte count 380 cells dl^{-1}) and lack of neutropenia (absolute neutrophil count 1670 cells dl^{-1}). Computed tomography (CT) of the chest revealed diffuse bilateral peri-hilar nodules and ground-glass opacities.

Bronchoalveolar lavage was performed (day +1) which did not reveal any endobronchial lesions nor any thick or bloody secretions. Gram stain, wet mount and acid-fast stains were negative. Qualitative cytomegalovirus (CMV) PCR was positive as well as PCR for Influenza A (the same subtype as was previously detected in her nasal wash). Of note, serum CMV levels measured concurrently were low. The BAL galactomannan level registered above the limits of quantification (index ≥ 3.750) although no *Aspergillus* species were detected by wet mount or culture. By day 4 *Penicillium citrinum* had grown from three separate cultures (fungal, *Legionella*, and *Nocardia*) inoculated with the same BAL specimen. The isolate displayed a characteristic grey-green colony with yellow diffusing pigment as classically described (Fig. 1). Organism identification was initially performed by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (duplicate scores > 2.00) and later confirmed by genetic sequencing of the internal transcribed spacer (ITS) region (100% match to type strain CBS 117.65 (GenBank accession number GU944562)). Routine bacterial and acid-fast bacilli (AFB) cultures were negative. Additional microbiologic assays for *Pneumocystis*, *Herpes simplex* virus (HSV), *Histoplasma*, *Toxoplasma*, human herpesvirus 6 (HHV-6) and common respiratory viruses also returned negative.

Fig. 1. *P. citrinum* colony growth after 4 days of incubation on Sabouraud dextrose agar at 27 °C.

Table 1
Results of Platelia™ Aspergillus Enzyme Immunoassay Testing.

Culture sample	Index value	Interpretation
Sterile media	0.39	Negative
Penicillium citrinum	6.85	Positive
Aspergillus fumigatus	3.75	Positive

To assess whether *P. citrinum* could account for the positive BAL galactomannan, supernatant from a pure *P. citrinum* broth culture was submitted for testing by the Platelia™ Aspergillus Enzyme Immunoassay (MiraVista Diagnostics). *Aspergillus fumigatus* broth culture supernatant (positive control) and sterile fungal broth culture media (negative control) were submitted in parallel. Results are summarized in Table 1. Relative differences in index values between *Penicillium citrinum* and *Aspergillus fumigatus* culture supernatants cannot be ascribed any definite significance (as may be growth rate and strain dependent), although it is worth noting that both molds were inoculated at the same time with the aim of producing approximately equal culture densities.

The *P. citrinum* BAL isolate was sent to the University of Texas Health Science Center (San Antonio, TX) for sensitivity testing by a clinical mycology reference laboratory. The following MICs (μg ml^{-1}) were reported (results obtained on day +30): 1 for amphotericin B, 0.125 for caspofungin, 0.06 for micafungin, 4 for itraconazole, 2 for posaconazole and > 16 for voriconazole. There are no clinical breakpoints for filamentous molds set by the Clinical and Laboratory Standards Institute (CLSI), so these MICs represent unofficial benchmarks only and have no established clinical interpretation.

A broad differential diagnosis was entertained for this presentation of fever plus an acute pulmonary process. After assimilation of all clinical, radiographic and microbiologic data, Influenza pneumonia, pulmonary EBV-PTLD and post-transplant pulmonary complications such as cryptogenic organizing pneumonia (COP) were considered reasonably likely etiologies. Considered less likely but still possible was pneumonia secondary to other respiratory viruses, *Aspergillus* or other commonly invasive fungi, *Penicillium citrinum,* hospital-acquired bacterial organisms, CMV, Nocardia, *Pneumocystis jirovecii* or *Toxoplasma gondii*.The patient was covered broadly for the potential infectious etiologies described above. Oseltamivir and posaconazole were started (on day +1 and +4 respectively), micafungin, ganciclovir, and trimethoprim-sulfamethoxazole were continued, and piperacillin-tazobactam was exchanged briefly for meropenem before broad-spectrum anti-bacterial agents were discontinued altogether. Dexamethasone tapering continued but the patient did not receive EBV-PTLD-specific treatment (rituximab or cytotoxic T cells) in this acute time period.

The patient continued to spike intermittent low-grade fevers for one week with negative blood cultures. Serial CT chest scans showed gradual improvement over the course of several weeks. By day 31, almost complete radiographic resolution had been achieved and supplemental oxygen was unnecessary. The patient had completed a 7 day course of oseltamivir by this point and had received 27 days of IV posaconazole. Serum posaconazole levels were serially checked and consistently therapeutic (1750 ng/ml on day +10, 939 ng/ml on day +37). Posaconazole 300 mg IV daily was thereafter transitioned to oral posaconazole at the same dose before its eventual discontinuation on day 97.

3. Discussion

The growth of *P. citrinum* in three separate BAL cultures from a symptomatic, immunocompromised patient prompted careful consid-

eration of a *P. citrinum* pneumonia diagnosis. After review of the literature and consultation with a clinical mycology expert, we concluded that *P. citrinum* pneumonia was an unlikely diagnosis in our patient but that the organism was likely to have been present in her tracheobronchial tree.

This case highlights several important aspects related to interpretation and management of *P. citrinum*-positive clinical isolates. We summarize the three most salient below:

1. *Infection with P. citrinum is exceedingly rare despite its environmental ubiquity*
   A thorough literature search yielded only four reports of *P. citrinum* infections in humans [6–9]. One report described *P. citrinum* keratitis in immunocompetent farmers after corneal trauma from vegetable matter debris. Two reports were of cavitary *P. citrinum* pneumonia and the fourth report documented an ulcerative *P. citrinum* skin lesion. In the latter three cases the diagnosis was supported by tissue biopsy demonstrating septate hyphae plus a positive culture of *P. citrinum* (and no other molds) from the site of infection. Excluding keratitis, we did not encounter any reports of *P. citrinum* infection based on positive culture results alone. We favor preservation of a strict case definition seeing as how more often than not, microbiologic recovery of *Penicillium* species reflects laboratory contamination or its non-pathogenic presence, rather than true infection. The isolation of *P. citrinum* from multiple cultures combined with its being a relatively uncommon species in our institution makes laboratory contamination less likely in our case.
   It is striking that all three reports of non-ophthalmic infection occurred in patients with acute leukemia receiving intensive chemotherapy. An immunocompromised state would seem to be a hard and fast prerequisite for systemic *P. citrinum* infection, but these cases suggest that the bar may be set even higher. Extrapolating from our clinical experience, acute leukemia plus chemotherapy imposes a far greater degree of immunocompromise than that expected from a moderate amount of steroids and mild leukopenia in an engrafted 7-month post-transplant patient. This lack of appropriate pre-disposition, in addition to uncharacteristic radiographic features and a plausible alternate explanation for the positive BAL culture, ultimately led us reject *P. citrinum* pneumonia as a likely diagnosis in this case.
2. *P. citrinum demonstrates cross-reactivity in clinical Aspergillus antigen assays*
   To the best of our knowledge this is the first report to confirm *P. citrinum* cross-reactivity with a clinically-validated Aspergillus antigen (galactomannan) assay. This result is consistent with reports of galactomannan cross-reactivity exhibited by few other *Penicillium* species [3–5]. This information may have clinical utility in cases such as the one described here, but it is worth underscoring that antigen cross-reactivity, even if wide, does not necessarily confer poor test specificity. Illustratively, a positive BAL galactomannan level has shown good specificity for invasive Aspergillosis when applied to a high pre-test probability transplant population [10]. It is also important to note that our patient received piperacillin-tazobactam, an antibiotic to which false-positive galactomannan test results have been attributed. However, a recent report has shown that this no longer occurs with either generic or brand name formulations used in the United States [12].
3. *Voriconazole should not be considered first-line for the empiric treatment of P. citrinum*
   The voriconazole MIC for our patient's isolate was $> 16\ \mu g\ ml^{-1}$, well out of accepted susceptibility range if the isolate had been *Aspergillus fumigatus*. Only one of the 4 published case reports of *P. citrinum* infections reported a voriconazole MIC. That MIC was also above the limits of quantification, albeit at $> 256\ \mu g\ ml^{-1}$ [8]. The only other report of *P. citrinum* sensitivities encountered after a broad literature search was authored by a group at the Fungal Testing Laboratory at the University of Texas Health Science Center [11]. Their report included MIC data from a bank of fungal isolates sent from around the United States and primarily derived from patients. Of the 10 *P. citrinum* isolates tested, all 10 had a measured voriconazole MIC $> 16\ \mu g\ ml^{-1}$. No other anti-fungal drug tested in the panel, including other triazoles, demonstrated this degree of MIC laterality.

P. citrinum-positive cultures require careful clinical interpretation. Although *P. citrinum* was unlikely to have been the etiologic agent of pneumonia in this case, it is our aim that the analysis presented here will provide helpful guidance for clinicians considering or managing *P. citrinum* infection.

Conflict of interest

The authors have no conflicts of interest.

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