

Correspondence

Regarding "Trichosporon pullulans infection in 2 patients with chronic granulomatous disease"

To the Editor:

We would like to bring to your attention concerns regarding the recent report of invasive pulmonary infection by *Trichosporon pullulans* in patients with chronic granulomatous disease published in the Journal.¹ We were skeptical of the identification of this pathogen for several reasons: *Trichosporon pullulans* is a psychrophilic fungus that thrives at low temperatures (optimum temperature, 17°C) and fails to grow at temperatures >25°C²; *T pullulans* has never been reliably documented as a human pathogen, and previous case reports were thought to be based on misidentification³; the "fragmented hyphal forms" depicted in the photomicrographs of the histologic sections in the article are not persuasive for *Trichosporon* infection; and the lung biopsy specimens were sterile, and growth of the organism was only from the bronchoalveolar lavage at 6 weeks after the specimen was obtained, a setting most likely to yield contaminants rather than the etiologic agent. Finally, the identification of *T pullulans* in this article relied only on the Vitek Yeast Biochemical card (bioMérieux, Durham, NC), which has limited diagnostic value for identification of *Trichosporon* species. The Vitek Yeast Biochemical card does not include the taxonomically updated database for *Trichosporon* species. It lists only *Trichosporon beigelii* (outdated name of *Trichosporon ovoides*) and *T pullulans*, although there are 6 clinically important *Trichosporon* species excluding *T pullulans*. Furthermore, *T pullulans* cannot be reliably identified solely by using commercial strips or automated systems, because biochemical test profiles included in these systems do not offer a definitive differentiation of *T pullulans* from certain species of the genus *Cryptococcus*. For example, sugar and nitrogen assimilation patterns of *T pullulans* are very similar to those of *Cryptococcus albidus*, and the necessary discriminating phenotypic tests are not included in these systems.

We obtained the bronchoalveolar lavage isolate from the reference laboratory with the authors' permission. The organism grew rapidly at 30°C, producing a smooth yeast colony with a tinge of light pink color. The fungus did not produce disarticulating hyphae characteristic of *Trichosporon* species but produced encapsulated yeast cells that multiply by polar budding reminiscent of the genus *Cryptococcus*. The isolate was identified as *Cryptococcus albidus* based on the combination of its sugar assimilation pattern (using API20C, bioMérieux; and Uniyeast, Remel, Lenexa, Kan), cycloheximide tolerance, urease production, nitrate assimilation, growth temperature (grows at 30°C but not at 37°C), and morphological characteristics on cornmeal agar. The identification was further con-

firmed by agglutination tests using the Crypto-Check Iatron Kit (Iatron Laboratories, Tokyo, Japan), in which *C albidus*, but not *T pullulans*, is known to yield positive results with Factor sera #1 and #7. Because of the similarity between *Cryptococcus neoformans* and *C albidus* in the chemical structure of their extracellular glucuronoxylomannan, *C neoformans* serotype A and *C albidus* both have been proven to agglutinate with the Crypto-Check Factor sera #1 and #7. The 2 *Cryptococcus* species, however, can be readily differentiated by their growth temperature and nitrate assimilation pattern in addition to the presence or absence of melanin on birdseed agar. *Cryptococcus albidus* is often seen as a laboratory contaminant that grows well at 30°C, consistent with being a contaminant grown after a prolonged period, as was reported for 1 of these cases.

This case points out the very real difficulties in identifying rare pathogens in rare patients. When commercial yeast identification systems are used to identify rare pathogens, we suggest, at a minimum, that additional tests such as morphology on cornmeal agar and growth temperature should be included. A fungus grown from bronchoalveolar lavage 6 weeks after inoculation is most likely not the causative agent, especially if it is reported to have grown at temperatures incompatible with its life cycle.

This leaves us with the question of what these patients did actually have. We suspect that at least patient 1 with extensive bilateral infiltrates may in fact have had some fungal infection that, like *Trichosporon pullulans*, was not recovered on open lung biopsy. As far as case 2 is concerned, we cannot assess what agent was involved from the information reported. Finally, the reports from the literature that are listed are quite concerning because they are all subject to the same critical caveats (failure of this organism to grow at human body temperature) and the difficulties of knowing whether the laboratories that claimed to have identified *T pullulans* in fact did perform the confirmatory tests. At this point, we suggest that *T pullulans* should be considered neither to have caused invasive disease in patients with chronic granulomatous disease nor as a significant infectious agent in humans.

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Reply

To the Editor:

We appreciate the comments of Holland et al¹ in highlighting concerns regarding *Trichosporon pullulans* as a human pathogen. Our report² relied heavily on the mycology data provided to us that stated each isolate was identified as *T. pullulans* with 99% to 100% certainty as per the Vitek Yeast Biochemical card (bioMérieux, Durham, NC). We now know that the Vitek Yeast Biochemical card is of limited diagnostic value for identification of *Trichosporon* species.

To confirm the findings of Holland et al, molecular typing of the original isolate was performed at the University of Texas Health Science Center at San Antonio. The internal transcribed spacer (ITS) region that separates the 18S and 26S ribosomal DNA genes was amplified using the standard ITS1 and ITS4 primers,³ and sequenced. Sequence similarity search was performed using BLAST (Basic Local Alignment Search Tool⁴) (blastn) of the National Center for Biotechnology Information database. Search results revealed 100% identity (621/621 bases) to *Cryptococcus adeliensis* (strain CBS8351), accession #AF145328. A second strain listed as *Cryptococcus* sp (619/621 bp, accession #AF444449) also showed highly significant identity. *Cryptococcus albidus* var *kuetzingii* (accession #AF444378) was the closest non-*adeliensis* species in identity (617/621 bp) and was also the third closest related strain. Additional biochemical testing, performed according to the initial species description, matched the profile for *C. adeliensis*.⁵ Given that the species designation for *C. adeliensis* was based on only a single isolate,⁵ we believe it would be more appropriate at this time to classify this isolate as a variant of *C. albidus*.

The results of molecular typing confirm the identity of the fungal isolate that was obtained from a bronchoalveolar lavage specimen from a young patient with chronic granulomatous disease and active lung disease as a variant of *C. albidus* and not *T. pullulans* as initially reported.² *C. albidus* is a rare cause of human disease.⁶ In the patient described in our report,² fungal elements were apparent on the lung biopsy, *C. albidus* was isolated from the bronchoalveolar lavage specimen, and the patient responded to amphotericin B therapy. It is impossible to state whether this report now represents the first infection with *C. albidus* in a patient with chronic granulomatous

disease or represents a laboratory contaminant (3 additional isolates in past 4 years) given the time to isolation of the pathogen from the sample in addition to environmental growth restrictions. There are only 13 previously reported cases of systemic disease in which *C. albidus* was identified, with 2 cases of pulmonary disease.⁶ *Cryptococcus* spp are infrequently recognized pathogens in the setting of chronic granulomatous disease.⁷

The true identity of the fungal pathogen from the second patient in our report as well as the remaining patients in our review is open to interpretation, because these isolates are not available for further analysis. It is likely that additional referenced cases in our report² were misidentified and may indeed also have belonged to the genus *Cryptococcus*. In concurrence, this correction highlights the improbability of *T. pullulans* as a human pathogen as well as the limitations of biochemical testing for the identification of *Trichosporon* species. Growth characteristics, morphology, fermentation, and carbohydrate assimilation can provide additional pertinent identifying features.

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