Candida quercitrusa candidemia investigation of three clustered cases and mycological characteristics of this novel species

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INTRODUCTION

Candidemia in hospitalized patients has substantial mortality with high costs. Some studies suggested that up to one-third of cases may occur as nosocomial clusters. Further, uncommon or novel *Candida* species are increasingly recognized to cause candidemia.

We describe the clinical characteristics of three candidemia cases caused by the novel species, Candida quercitrusa, which occurred within a 2-month period in a single ICU in China.

MATERIAL AND METHODS

Case. Patient 1 was admitted to the hospital's ICU with acute upper gastrointestinal bleeding in May 2010. On day 7, peripheral blood cultures grew a "*Candida pulcherrima*" (strain 10H1064) as identified by the VITEK 2 YST system (Fig. 1A). Patient 2 presented to the same ICU in April 2010 with major trauma. On days 50 and 59 of admission, blood cultures from a central line grew a "*C. pulcherrima*" (strain 10H1067, initially identified as for Patient 1) (Fig. 1B). Patient 3 was admitted to ICU on July 2010 with fever, seizures and obtundation. A "*C. lusitaniae*" (strain 10H1076) as identified by API 20C AUX was recovered from peripheral blood cultures at day 10 (Fig. 1C).

Lab studies. DNA sequencing was performed by amplifying the ITS region and D1/D2 domain of the rRNA gene. Comparison of all available *C. quercitrusa* sequences was then performed using the Maximum Parsimony (MP) method. Isolates were also analyzed by Bruker MALDI-TOF MS, and protein profiles were further studied by MSP analysis. Primers RAPD24 and RAPD1283 were used for random amplified polymorphic DNA (RAPD) analysis. *In vitro* susceptibility to amphotericin B, fluconazole, voriconazole, itraconazole and caspofungin was determined by CLSI M27-A3 methodology.

RESULTS

Phenotype. On Sabouraud dextrose agar and chromogenic media, the isolates grew well at 25°C/30°C. They grew slowly at 37°C over five days (cf. with *C. quercitrusa* type strain CBS 4412, not grow at 37°C) but did not grow at 42°C. Colonies were dark blue on CHROMagar *Candida* (as *C. tropicalis*) and dark green on Brilliance *Candida* (as *C. albicans*) (Fig. 2) .

Molecular ID and typing. The ITS and D1/D2 sequences of all studied isolates were identical and shared 98.9% and 99.8% sequence similarity to those of *C. quercitrusa* type strain CBS 4412, respectively. MP analysis of the ITS region revealed genetic heterogeneity amongst *C. quercitrusa* strains, and there was clustering of the three patient isolates 10H1064, 10H1967 and 10H1076 which were clearly separated from the non-clinical strains (Fig. 3). MALDI-TOF MS assigned "no identification" to the patient isolates. Their RAPD profiles were identical as were their MALDI-TOF MS spectra.

Antifungal susceptibilities. All isolates had fluconazole MICs of 16-32 µg/ml. The MIC range for itraconazole and voriconazole was 0.25-0.5 µg/ml and 0.125-0.25 µg/ml, respectively. MICs for both caspofungin and amphotericin B were low (MIC range 0.5 to 1 and ≤0.5 µg/ml, respectively).

Discussion

This report details, for the first time, candidemia due to the novel species *C. quercitrusa*. Although *C. quercitrusa* has been recovered from plant, water and insect material in a number of countries, the species has not been reported to cause human infection. The cases herein are also notable for their clustering in the same hospital within only a 2-month period.

Taken together with their identical RAPD and MALDI-TOF MS protein profiles, the findings suggest that the isolates may originated from a common source. Further, MP analysis of their ITS sequences indicated they are genetically distinct from previously isolated strains from non-clinical sources. A limitation of the present study is that it was performed retrospectively. Consequently, we were unable to investigate for a possible environmental source or the potential for human-to-human.

Transmission of infection through contamination of blood culture vials, or of intravenous preparations such as TPN bags remains another possibility. However, as not all patients received TPN, and the blood culture bottles were of two different batches, these were unlikely to represent the source.

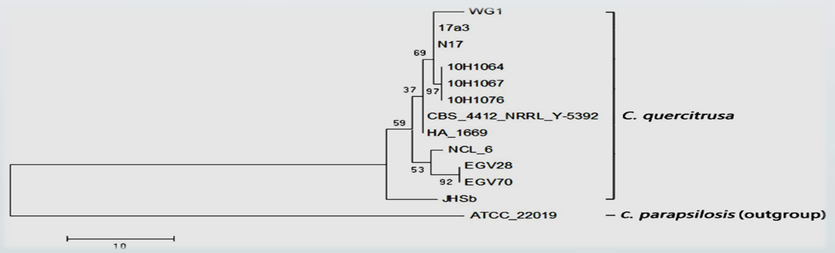

FIG 3. The MP tree generated from *C. quercitrusa* ITS sequences.

FIG 1. Clinical features of patients with *C. quercitrusa* candidemia. Lines with solid squares, antifungal treatment; inverted solid black arrows, positive *C. quercitrusa* blood culture.

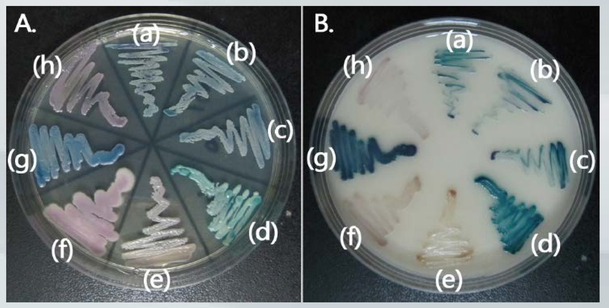

FIG 2. *C. quercitrusa* isolates on CHROMagar *Candida* (A) and Brilliance *Candida* agar (B): (a)-(c) *C. quercitrusa* (d) *C. albicans* (e) *C. parapsilosis*; (f) *C. krusei*; (g) *C. tropicalis*; (h) *C. glabrata*.

CONCLUSION

We detail *C. quercitrusa* as a human pathogen for the first time with clustering of three C. quercitrusa candidemia cases in a single ICU. Molecular methods are needed for accurate identification of this species. The species is less susceptible to fluconazole.

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