Case Report

**Pan-Echinocandin-Resistant *Candida glabrata***

Bloodstream Infection Complicating COVID-19: A Fatal Case Report

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Abstract: Coinfections with bacteria or fungi may be a frequent complication of COVID-19, although coinfections with *Candida* species in COVID-19 patients remain rare. We report the 53-day clinical course of a complicated type-2 diabetes patient diagnosed with COVID-19, who developed bloodstream infections initially due to methicillin-resistant *Staphylococcus aureus*, secondly to multidrug-resistant Gram-negative bacteria, and lastly to a possibly fatal *Candida glabrata*. Development of FKS-associated pan-echinocandin resistance in the *C. glabrata* isolated from the patient after 13 days of caspofungin treatment aggravated the situation. The patient died of septic shock shortly before the prospect of receiving potentially effective antifungal therapy. This case emphasizes the importance of early diagnosis and monitoring for antimicrobial drug-resistant coinfections to reduce their unfavorable outcomes in COVID-19 patients.

Keywords: SARS-CoV-2; coinfection; diabetes; bloodstream infection; *Candida glabrata*; echinocandin resistance; FKS mutation

1. Introduction

Since the beginning of the respiratory tract infection epidemic in China [1] caused by the 2019 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), known as coronavirus disease 2019 (COVID-19), a substantial number of COVID-19 associated deaths have been reported worldwide [2]. While sepsis may be a fatal complication of COVID-19 [3], coinfection (also named superinfection) with bacteria or fungi may occur, albeit confined to the respiratory tract [4,5]. In two independent studies from Chinese hospitals, 27 (96.4%) of 28 [6] and 11 (16%) of 68 [7] COVID-19 patients who died had secondary infections. This is consistent with a failed homeostasis between innate and adaptive responses [8] or a pronounced immune suppression [9], which is partly dependent on the loss of lymphocytes, following SARS-CoV-2 infection [10]. Diabetes is the most common comorbidity in COVID-19, with its late complications (e.g., ischemic heart disease) contributing to further increase COVID-19 severity [11]. Additionally, diabetes increases not only the risk of infections [11] but also of infection-related deaths [12]. In this context, diabetes seems to alter the intestinal barrier function allowing gut microbiota members (e.g., *Enterobacteriales* or *Candida* species) to reach the bloodstream and, then, to spread systemically [13]. We describe the case of a COVID-19 patient with complicated type-2 diabetes who developed bloodstream infection...
due to a Candida glabrata isolate that acquired pan-echinocandin resistance after 13 days of caspofungin treatment. The patient died of septic shock in the intensive care unit (ICU), shortly before the prospect of receiving potentially effective antifungal therapy.

2. Case Report and Results

A 79-year-old male presented to the emergency department with cough and dyspnea, following a suspected COVID-19 diagnosis because of his previous contact with a SARS-CoV-2 positive patient in a rehabilitation facility. Two days prior to admission (defined as day 1), he had been suffering from fever (38.0°C). His 6-year medical history was significant for poorly controlled type 2 diabetes, ischemic heart disease, and a stage IV peripheral artery disease treated with lower extremity revascularization, which culminated into left leg amputation in 2019. On physical examination, the amputated leg stump displayed necrotic and ulcerative lesions, whereas the patient was afebrile and negative for abnormal lung sounds and had a 98% blood oxygenation. His leukocytes (× 10^9/L) were normal (4.7; normal range 4.0–10.0) whereas serum creatinine (mg/dL) (1.3; normal range 0.7–1.2), C-reactive protein (CRP, mg/L) (37.8; normal range 0.0–5.0), and interleukin 6 (IL6, ng/L) (13.6; normal range 0.0–4.4) were altered. The patient’s chest X-ray and computed tomography findings were consistent with pneumonia, and positive SARS-CoV-2 RNA detection results (Ct 30.3; E gene [14]) on nasal/pharyngeal swabs obtained in the emergency department allowed to confirm COVID-19 diagnosis [15]. Subsequent nasal/pharyngeal swabs taken from the patient at different times from admission will test positive for SARS-CoV-2 RNA.

The patient was transferred to the COVID-19 care unit where he started on antiviral therapy (that will be continued for next five days) with darunavir/ritonavir (800/100 mg q48h) combined with hydroxychloroquine (200 mg q12h), which was our national policy at that time. On days 4 and 5, the patient’s clinical conditions worsened, and his serum creatinine, CRP, and leukocytes increased to 3.5 mg/dL, 155.4 mg/L, or 6.9 × 10^9/L, respectively. The patient developed fever (38.2°C), productive cough, and his blood oxygenation decreased to 92% demanding oxygen administration through a Venturi mask (fraction of inspired oxygen, 24%). Due to highly suspected bacterial superinfection, he received empirical treatment with piperacillin/tazobactam (2.25 g q6h).

On day 8, the patient was still febrile (38.5°C), his serum creatinine (3.9 mg/dL), CRP (177.2 mg/L), and leukocytes (9.4 × 10^9/L) increased further, and blood cultures from day 5 grew a methicillin-resistant Staphylococcus aureus organism. Consequently, piperacillin/tazobactam was discontinued and teicoplanin (200 mg q24h) was started. He apparently improved and subsequent blood cultures, a transthoracic echocardiogram, and ultrasound studies to evaluate deep vein thrombosis were all negative. On day 25, teicoplanin was discontinued. The next day, both orthopedic and vascular surgeons who evaluated the patient decided for a new, more proximal amputation of his left leg. On day 27, the patient became febrile (38.5°C). His leukocytes increased to 10.8 × 10^9/L and infection indexes, including procalcitonin (PCT; normal range, 0.0–0.5 ng/mL), were elevated (CRP, 275 mg/L; PCT, 1.65 ng/mL). While his kidney injury seemed to recover (serum creatinine, 1.5 mg/dL), the patient became stably anemic (hemoglobin, g/dL; 7.4; normal range 13.0–17.0) requiring regular blood transfusions (until two days before death). On day 28, blood cultures from day 27 grew Morganella morganii (found to be resistant to cephalosporins and piperacillin/tazobactam but susceptible to carbapenems), which prompted initiation of antibiotic therapy with ertapenem (1 g q24h). Concomitantly, cultures from a progressively enlarging ulcer on the patient’s leg stump revealed growth of Proteus mirabilis, Klebsiella pneumoniae, and Escherichia coli (all found to be susceptible to carbapenems).

On day 35, the patient again became febrile (38.2°C) but CRP decreased (177.2 mg/L) and leukocytes remained unvaried (9.3 × 10^9/L). Blood cultures yielded a yeast organism, later identified as C. glabrata using a previously described matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry based method [16]. The isolate (defined as isolate 1) was
susceptible to anidulafungin, micafungin, and caspofungin with MICs of 0.03, 0.03, and 0.06 μg/ml (SensititreYeastOne® method; Thermo Fisher Scientific, Cleveland, OH, USA), according to the Clinical and Laboratory Standards (CLSI) clinical breakpoints [17]. On day 37, the patient started to take caspofungin (70 mg loading dose, day 1; 50 mg q24h, subsequent days). Blood cultures from day 39 were negative. After 13 days of antifungal therapy, the patient became again febrile (38.3°C) and his blood parameters (creatinine, 2.71 mg/dL; leukocytes, 12.48 × 10⁹/L) or infection indexes (CRP, 278.4 mg/L; PCT, 20.58 ng/ml) were abnormal. On day 49, blood cultures were positive for Acinetobacter baumannii (found to be only susceptible to colistin) and again for C. glabrata. While ertapenem was discontinued and colistin (2.25 mU/q12h) was started, the patient continued to receive caspofungin. Shortly after (day 51), antifungal susceptibility testing was repeated on two morphologically different C. glabrata isolates that grew from blood cultures. One of the isolates (defined isolate 2) revealed increased MICs of anidulafungin, micafungin, and caspofungin, indicating resistance to all echinocandins (as discussed below).

On day 52, the patient underwent surgery for previously planned left leg re-amputation. Unfortunately, the same day of surgery and before the patient could eventually benefit from antifungal therapy change (i.e., amphotericin B instead of caspofungin) based on available antifungal susceptibility results, his clinical conditions worsened. The patient was immediately transferred to the ICU due to refractory septic shock, as identified by the receipt of vasopressor therapy and the elevated lactate (mEq/L) level (4.2; normal range 0.0–2.0) despite adequate fluid resuscitation. On day 53, the patient died.

Table 1 summarizes the results of both antifungal susceptibility testing and FKS2 gene sequencing for C. glabrata isolates 1 and 2. Only for echinocandin antifungal agents, MIC values obtained with the SensititreYeastOne® method were confirmed by the CLSI M27-A3 reference method [17]. As noted, except for all three echinocandins, the antifungal susceptibility profile of isolate 2 did not change compared to that of isolate 1. According to the echinocandin-resistant breakpoint values established by the CLSI [18], isolate 2 showed resistance to anidulafungin (MIC, 2 mg/L), caspofungin (MIC, 8 mg/L), and micafungin (MIC, 8 mg/L). Conversely, isolate 1 had echinocandin MICs (anidulafungin and micafungin, 0.03 mg/L; caspofungin, 0.06 mg/L) below the CLSI echinocandin-resistant breakpoint values [18]. Interestingly, both the isolates showed an intermediate susceptibility to fluconazole (MIC, 8 mg/L) and, according to the epidemiological cutoff values established by the CLSI [19], a wild-type susceptibility to the amphotericin B and the other azole (itraconazole, posaconazole, and voriconazole) antifungal agents tested. Sequence analysis of the FKS1/FKS2 genes allowed us to identify T1976A (hot spot 1) and A3997T (hot spot 2) mutations in the FKS2 gene that resulted in an F659Y or I1333F amino acid change, respectively, with the former being already known [20–22] and the latter probably responsible for the observed echinocandin resistance. Furthermore, MALDI-TOF MS-based analysis of profiles from
Table 1. Antifungal susceptibility testing and FKS2 gene sequencing results of two sequential candidemia isolates.

| Species   | Isolate   | MIC (mg/L) for polypene antifungal class | MIC (mg/L) for echinocandin antifungal class | MIC (mg/L) for azole antifungal class | FKS2 gene hot spots 1 and 2 |
|-----------|-----------|------------------------------------------|---------------------------------------------|--------------------------------------|-----------------------------|
|           |           | AMB | AFG | CAS | MFG | FLZ | ITC | POS | VRC | Nucleotide change | Amino acid change |
| C. glabrata | Isolate 1 | 0.5  | 0.03 | 0.06 | 0.03 | 8   | 0.5 | 1   | 0.25 | Wild type | T1976A |
| C. glabrata | Isolate 2 | 0.5  | 2   | 8   | 8   | 8   | 0.5 | 1   | 0.25 | F659Y | A3997T |

Abbreviations: MIC, minimum inhibitory concentration; AMB, amphotericin B; AFG, anidulafungin; CAS, caspofungin; MFG, micafungin; FLZ, fluconazole; ITC, itraconazole; POS, posaconazole; VRC, voriconazole.

Antifungal-resistant breakpoint values established by the CLSI for C. glabrata are ≥0.5 mg/L for anidulafungin and caspofungin, ≥0.25 mg/L for micafungin, and ≥64 mg/L for fluconazole. Because no resistance breakpoints were available for other listed antifungal agents, we used epidemiological cutoff values (ECVs) established by the CLSI for C. glabrata, according to which non-wild-type MIC values (>ECVs) of amphotericin B, itraconazole, posaconazole, and voriconazole are >2 mg/L, >4 mg/L, >1 mg/L, and >0.25 mg/L, respectively.
C. glabrata isolates 1 and 2 allowed to compare with each other and with profiles from clinical collection isolates (C. glabrata UCSC1–12, UCSC17–21). As shown in Figure 1, the dendrogram resulting from the MALDI-TOF MS cluster analysis strongly suggested identity for C. glabrata isolates 1 and 2.

Figure 1. Cluster analysis of MALDI-TOF mass spectra obtained for 19 C. glabrata isolates, including the patients’ isolates 1 and 2. Shown is a dendrogram in which the distance between isolates is indicated as relative units. Zero means complete similarity and 1000 means complete dissimilarity. An arbitrary distance level of 500 was chosen to assess clustering among isolates.

3. Discussion

This case illustrates the 53-day clinical course of a COVID-19 patient with persistent SARS-CoV-2 infection (repeated nasal/pharyngeal swabs tested positive for SARS-CoV-2 RNA) who needed protracted hospitalization, probably attributed to his major comorbidity (diabetes with its vascular complications). The patient met the clinical (fever, cough, and dyspnea), laboratory (high CRP), and imaging (unilateral pneumonia) features recently recognized as COVID-19 hallmarks [10]. Yet, this case emphasizes the current uncertainty about the clinical disease evolution, partly linked to the presence of risk factors for either admission to the ICU or fatal outcome of hospitalized patients [10]. In our patient, a succession of bloodstream infections, initially due to methicillin-resistant S. aureus, secondly to multidrug-resistant Gram-negative bacteria, and lastly to a possibly fatal echinocandin-resistant C. glabrata outlined the COVID-19 associated clinical course (Figure 2).
Figure 2. Timeline of major microbiological events during the patient’s clinical course and relative antimicrobial treatments. Fever (dashed line) or procalcitonin (solid line) patterns are shown.

DRV/RTV, darunavir/ritonavir; HCQ, hydroxychloroquine; TZP, piperacillin/tazobactam; TEC, teicoplanin; ETP, ertapenem; CAS, caspofungin; CST, colistin.

Therefore, at least three relevant causes might have contributed to determine fatal illness in the present case. First, COVID-19, which has significantly been associated with complications and deaths. A recent systematic review and meta-analysis [10] reported a case fatality rate of 13.9% (95% CI, 6.2 to 21.5%) in seven studies, a ICU requirement rate of 20.3% (95% CI, 10.0 to 30.6%) in six studies, an acute kidney injury rate of 7.9% (95% CI, 1.8 to 14.0%) in four studies, and a shock rate of 6.2% (95% CI, 3.1 to 9.3%) in three studies. Second, the diabetes, which remains a major comorbidity for severe COVID-19. It was the second (after hypertension) or third (after hypertension and cardiovascular disease) most prevalent underlying disease (11.9% [95% CI, 9.1–14.6%], and 9.7% [95% CI, 7.2–12.2%]) in two large, independent meta-analysis studies [10,23]. Furthermore, while chronic disease, such as diabetes, may increase the risk of COVID-19 severity [23] and mortality [1], type-2 diabetes individuals with poorly controlled blood glucose are likely to die at a higher rate than those with better-controlled blood glucose [24]. Third, superinfection, which represents a new albeit scarcely studied condition in COVID-19 [5], particularly for invasive fungal infections [25]. The peculiar pathophysiology of either diabetes [11] or COVID-19 [26] may
account for the occurrence of bacterial and fungal coinfections in our as in other cases [3,27]. The diabetes-induced immune dysregulation may exacerbate the virus-activated hyper-inflammatory “cytokine storm”, which in turn leads to complications (e.g., acute respiratory distress syndrome, shock, multiorgan failure, and death) seen in severe COVID-19 phases [10]. However, diabetes (and other comorbidity) and COVID-19, as risk factors for bacterial or fungal infection, are in an undiscernible balance, particularly during the ICU stay [25].

In our patient’s disease phase upon his admission to the hospital, COVID-19 together with diabetes might have created a milieu that favored microorganisms (e.g., *C. glabrata*, the last in temporal sequence), including those resistant to antimicrobial agents, to thrive (likely in the gastrointestinal tract) and, hence, reach the bloodstream [28]. Immunosuppression and mucosal barrier disruption are, among others, well-recognized factors for isolation of *C. glabrata* from patient blood cultures [29] and, to some extent, bloodstream isolates are in vitro resistant to echinocandins [20,22,30]. This poses a great challenge for patient management [31] because echinocandins represent the first line of treatment in cases of invasive *C. glabrata* infections, including candidemia [32], due to the intrinsic low *C. glabrata* level of susceptibility to azoles (which was not the case of our patient’s isolates) [18].

Ultimately, appearance of echinocandin resistance in our patient’s *C. glabrata* isolate aggravated the feared adverse prognosis of candidemia [33]. Consistent with previous case reports [21,34,35], we provided the evidence of an *in vivo* development of FKS-associated echinocandin resistance during the patient’s treatment with caspofungin. In two reports [21,34], echinocandin-resistant isolates were recovered from blood cultures of patients who had recurrent or persistent *C. glabrata* infections, thus implying micafungin treatments for 86 days in one case [21] and 30 days in the other case [34]. In another report [35], echinocandin resistance emerged within 8 days of the patient’s treatment with micafungin, and surprisingly the patient had not previous or prolonged echinocandin exposure [36] but only uncontrolled diabetes as a potential risk factor for microbiological failure. Abdominal cavity and mucosal surfaces are reservoirs for *Candida* species and a potential source for antifungal resistance due to uneven drug penetration [37,38]. Considering the high *C. glabrata* propensity to acquire *in vitro* resistance following echinocandin exposure [39], it is possible that underlying gastrointestinal disorder or dysbiosis had acted as selectors of FKS mutant *C. glabrata* subpopulations in our as in other [35] case patients. Notably, a study assessing the emergence of *in vitro* resistance for the three echinocandins showed that 82 of 247 *C. glabrata* breakthrough isolates (i.e., bloodstream isolates exposed to each echinocandin agent) harbored FKS hot spot mutations, of which 6 in *FKS1* and 76 in *FKS2* [40]. Of the three echinocandins, caspofungin seemed to be the most sensitive indicator of FKS mutations, whereas only four breakthrough isolates did not develop an FKS hot spot mutation despite showing >4-fold increases in echinocandin MICs relative to the parental isolates [40].

Although non-FKS-mediated echinocandin resistance has been reported [41,42], phenotypic resistance (MICs above CLSI breakpoints) to all three echinocandins is uniquely attributable to the presence of mutations in hot spots of both *FKS1* and its paralog *FKS2* [43], which results in attenuated echinocandin activity [44]. As recommended by the current Infectious Diseases Society of America (IDSA) guidelines [32], we performed echinocandin susceptibility testing on the *C. glabrata* isolates causing candidemia in our patient. Thus, we documented that isolate 2 ("breakthrough" isolate), compared to isolate 1 ("parental" isolate), had increased MIC values to anidulafungin, caspofungin, and micafungin, and all values were higher than CLSI resistance breakpoints [18]. As specifically shown for *C. glabrata* and echinocandins [45], the automated blood culture systems currently used to detect bloodstream infections allow to reliably recover isolate populations composed of echinocandin-resistant and echinocandin-susceptible cells. However, in cases with a low proportion of resistant cells, picking up single colonies to perform standard antifungal susceptibility testing may result in missed detection of echinocandin resistance [45]. In our case, taking advantage of morphologically different *C. glabrata* colonies from the patient’s blood
culture that yielded isolate 2, we were able to detect echinocandin resistance testing more than one colony. Consistent with recent studies [21,22], we found that isolate 2 harbored the FKS2 HS1 F659Y. In a two-year antifungal resistance surveillance study [22], eight (15.7%) of 51 C. glabrata isolates with FKS HS alterations harbored the FKS2 HS1 F659S/V/Y [20,46], which was the second found after the FKS2 HS1 S663P (16 isolates). It is noteworthy that mutations at positions S663 and F659 tended to be associated with breakthrough infections in patients receiving echinocandin therapy [20,47]. In our case, MIC results (later confirmed by FKS mutation results) were promptly available to clinicians but, given the critical patient’s condition, the ensuing change of antifungal therapy was unsuccessful.

In conclusion, this case highlights that bacterial and fungal coinfections, including those associated with antimicrobial resistance, in COVID-19 may be a further challenge for both clinicians and microbiologists. In waiting for epidemiological studies to evaluate their frequency and impact, it is imperative to be vigilant for these coinfections being complicating the outcome of COVID-19.
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