Echinocandin resistance due to simultaneous FKS mutation and increased cell wall chitin in a *Candida albicans* bloodstream isolate following brief exposure to caspofungin

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Echinocandins are first-line agents for treating severe invasive candidiasis. Glucan synthase gene (*FKS1*) mutations lead to echinocandin resistance but the role of enhanced chitin expression is not well recognized in clinical isolates. We report a case of bloodstream *C. albicans* infection with both Fks1 hotspot mutation and elevated cell wall chitin.

**Introduction**

Echinocandins are widely used in the treatment of candidaemia. An overwhelming majority of strains of *Candida albicans* are susceptible to echinocandins. We report a rare bloodstream infection with a caspofungin non-susceptible strain of *C. albicans* that harboured a *FKS1* gene mutation and also had enhanced chitin levels.

**Case report**

A 51-year-old female was admitted to the intensive care unit (ICU) with pyrexia, acidosis, hypoglycaemia and shock with multi-organ failure. She had advanced liver disease secondary to alcohol intake. She was put on ventilatory support, inotropic support and renal replacement therapy. The patient was clinically septic with a raised C-reactive protein (51 mg l$^{-1}$) in relation to the extent of her liver failure and leukocytosis (18.8 $\times$ 10$^9$ l$^{-1}$) with neutrophilia (17.8 $\times$ 10$^9$ l$^{-1}$), very high serum transaminases (aspartate aminotransferase 19388 U l$^{-1}$, alanine aminotransferase 8542 U l$^{-1}$), elevated serum bilirubin (72 $\mu$mol l$^{-1}$) and normal alkaline phosphatase (132 U l$^{-1}$). She had a history of hepatitis B infection 15 years prior to her admission, but had recovered and seroconverted (hepatitis B surface antigen and envelope antigen negative and envelope antibodies positive). She tested negative for hepatitis A, C and E, cytomegalovirus, Epstein–Barr virus, adenovirus and leptospirosis. The extent of her liver injury led to hepatorenal syndrome with further rise in serum bilirubin (473 $\mu$mol l$^{-1}$), increased urea (22.6 mmol l$^{-1}$) and creatinine (288 $\mu$mol l$^{-1}$) and an estimated glomerular filtration rate of only 13 ml min$^{-1}$. Seven days following admission, she was empirically commenced on intravenous piperacillin–tazobactam and caspofungin (70 mg loading dose, followed by 35 mg once daily as per recommendations for patients with severe hepatic insufficiency), both of which were discontinued after 1 week. Two weeks following discontinuation of caspofungin, cultures from the tracheal aspirate grew *Staphylococcus aureus*, *Aspergillus versicolor* and *Candida* species. Given the extent of her sepsis, she was started on a second course of caspofungin for suspected ventilator-associated chest infection in order to target both *A. versicolor* and *Candida* even though we were not sure about the significance of these fungi in the context of her illness. Caspofungin was given for 14 days at dosages mentioned above during this second course (Fig. 1).

After a 5 week stay in the ICU, her general condition improved slightly. She was transferred to the renal high dependency unit for dialysis support. During her stay in the high dependency unit, she remained intermittently pyrexial and continued to show features consistent with hepatorenal failure. Blood cultures from the internal jugular line (peripheral cultures were not done) 2 weeks after stopping the second course of caspofungin grew yeasts (Fig. 1).

Despite the fact that the patient was severely ill, we commenced intravenous fluconazole (200 mg once daily) rather than caspofungin. Our decision was based on the recent

**Abbreviations:** ICU, intensive care unit; IDSA, Infectious Diseases Society of America; PKC, protein kinase C.
Caspofungin resistance is uncommon in *Candida*. A major national mycology reference centre in France identified only 20 caspofungin-resistant isolates over an 8 year period (2004–2011) despite using the lower breakpoints recommended by the European Committee for Antimicrobial Susceptibility Testing (0.5 mg l\(^{-1}\) rather than 2 mg l\(^{-1}\) as in our report) (Dannaoui et al., 2012). *FKS1* gene mutations are well known to be associated with echinocandin resistance but increased chitin synthesis as a compensatory mechanism has not been widely investigated. The mutation identified in the hotspot region 1 of the *FKS1* gene in our isolate has been previously described (Walker et al., 2010). Mutations in the *FKS2* gene have also been reported in *Candida glabrata* and *Candida guilliermondii*. In contrast to the *FKS1* mutations, the compensatory rise in chitin synthesis is related to the cell wall salvage pathway involving protein kinase C (PKC) signalling (Walker et al., 2008). The compensatory mechanism of the chitin-rescue phenomenon is not yet fully understood. When the *C. albicans* cell wall is defective, *C. albicans* cells activate signalling pathways, such as the PKC pathway, to maintain cell wall integrity. *C. albicans* cells with an Fks1 mutation are likely to have higher basal levels of PKC1 phosphorylation compared to non-Fks1 mutants, but this has not been measured in this study (K. K. Lee & C. A. Munro, personal communication).

In addition, strains with *FKS1* gene mutations associated with echinocandin resistance, and which also show elevated chitin levels, tend to have reduced fitness and virulence (Ben-Ami et al., 2011). This should, hopefully, limit their epidemiological and clinical impact.

Acquired echinocandin resistance in *C. albicans* bloodstream infections remains rare and sporadic. In the seven cases reported by Koffertidis et al. (2010), only two were caused by *C. albicans*. The authors did not investigate the specific mechanism of reduced susceptibility in any of the strains. Garcia-Effron et al. (2010) also reported three cases of candidaemia with non-*albicans* Candida species harbouring novel *FKS* gene mutations. Enhanced chitin synthesis following pre-treatment of *Candida* with caspofungin leading to reduced killing has been reported in vitro

**Fig. 1.** Detailed timeline from admission to the onset of candidaemia.
Fig. 2. Patient isolate Fks1 amino acid sequence analysis and cell wall composition. (a) Alignment of the deduced amino acid sequences of the Fks1 hotspot 1 (HS1) region of \textit{C. albicans} SC5314 (S645, caspofungin-sensitive) and the patient isolate using the CLUSTAL W Multiple Sequence Alignment program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The dashed rectangular box indicates amino acids 640–650, with the shaded amino acids indicating either a silent or missense mutation. (b) Increased chitin in caspofungin-resistant isolates. Formalin-fixed cells of SC5314, the patient isolate and a caspofungin-resistant isolate (205) (Garcia-Effron et al., 2009) were stained with 25 \( \mu \)g Calcofluor White ml\(^{-1}\) and fluorescent images were obtained to visualize cell wall chitin. SC5314 and isolate 205 were included for comparison. Scale bars, 10 \( \mu \)m. (c) Cell wall composition of the patient isolate. Total cell wall was extracted from cells grown in YPD for 5 h and freeze-dried. Isolate 205 was included for comparison. The dried cell wall was acid-hydrolysed, washed and resuspended in deionized water. Chitin, glucan and mannan levels were measured by acid-hydrolysing the cell wall polymers and quantifying the amounts of the released monosaccharide (glucosamine, glucose and mannose) by high performance anion-exchange chromatography with pulsed amperometric detection (Lee et al., 2012). The results are presented as a percentage of total dried cell wall. Mean \( \pm \) SEM (n=2).
(Walker et al., 2008), but has been less well studied in clinical isolates. In an experimental murine model, infection with C. albicans with elevated chitin levels was associated with reduced survival following treatment with caspofungin (Lee et al., 2012).

In our patient, the C. albicans isolate recovered from the bloodstream had both FKS1 gene mutations and elevated chitin levels. The exact contribution made by these two independent mechanisms is undefined at present although recent data suggest that elevated cell wall chitin in C. albicans could provide cells with a window of opportunity to acquire an FKS1 mutation, even without exposure to caspofungin (Lee et al., 2012). Clinical isolates with reduced echinocandin susceptibility should be investigated for both FKS mutations and cell wall alterations. In the case described by Costa-de-Oliveira et al. (2011), the C. glabrata strain had a mutation in the FKS region but the chitin content was unspecified. Understanding the mechanisms of resistance will help promote further research into chitin synthase inhibitors which are known to act synergistically with the echinocandins (Walker et al., 2008). To our knowledge, this is the first report of a Candida isolated from bloodstream with both FKS mutation and altered cell wall chitin content.

This case has several clinically relevant learning points. The Infectious Diseases Society of America (IDSA) recommends the use of echinocandins in the treatment of candidaemia in patients with a history of azole use (Pappas et al., 2009). Whether the scope of this recommendation should be extended to antifungal agents other than azoles, i.e. patients with recent exposure to echinocandins should be treated empirically with an agent belonging to a different drug class, requires more evidence but it is worth noting that the German recommendations do not limit the scope to a particular class of drugs (Ruhnke et al., 2011). Empirical and early use of echinocandins for invasive candidiasis is supported by major international guidelines (Pappas et al., 2009). However, occasional strains such as the one we report may not be susceptible to echinocandins. Clinicians should remain alert to this possibility, especially if there is a history of prior echinocandin exposure. It is likely that with the widespread use of echinocandins, the prevalence of non-susceptible strains could increase. The Sensititre YeastOne (YO10) colorimetric microdilution test is easy to use and reliable as are other commercially available tests such as MIC-gradient strips. Laboratories should determine echinocandin susceptibility of all invasive Candida strains so that patients are effectively managed and a switch to an alternative therapy can be considered early in illness. In a recent UK-wide survey, Schelenz et al. (2009) found that only 38% of laboratories provided on-site antifungal susceptibility testing. Routine testing of antifungal susceptibility for C. albicans is not recommended by the IDSA guidance and this too may need revisiting because the current guidance recommends step-down therapy to fluconazole in patients with C. albicans bloodstream infection only when they are clinically stable and not on the basis of speciation alone (Pappas et al., 2009). Inappropriate therapy of invasive candidiasis is associated with high mortality (Parkins et al., 2007). Secondly, the issue of empirical therapy of suspected invasive candidiasis needs to be addressed, even though in this instance there is no evidence to link empirical caspofungin use and subsequent isolation of caspofungin non-susceptible C. albicans from the bloodstream. Schuster et al. (2008) found no benefit from empirical use of fluconazole in ICU patients. However, in our experience, the use of antifungal agents (fluconazole, as well as caspofungin) continues to be indiscriminate and widespread despite evidence to the contrary. We recently reported that more than 80% of ICUs in the UK use these agents empirically (Chalmers & Bal, 2011). Finally, it is interesting that in our patient, a brief exposure (21 days covering two non-continuous prescriptions) to caspofungin was followed by candidaemia caused by a caspofungin non-susceptible C. albicans. Whether the lower dose used due to hepatic insufficiency led to a reduced and possibly suboptimal drug exposure is unknown. Although bloodstream infections with caspofungin non-susceptible Candida have been reported, such infections have usually occurred after prolonged exposure to echinocandins or in neutropenic patients. A majority of strains in these settings have been Candida parapsilosis, which is known to be constitutively less susceptible to echinocandins (Pfeiffer et al., 2010). We believe that prudent antifungal prescribing has not drawn the same attention as antibiotic prescribing but perhaps it is time to have closer monitoring of antifungal usage in hospitals. To the best of our knowledge, this is the first case of caspofungin-resistant C. albicans bloodstream infection reported from the UK.

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References


