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Genome Note

Delay in effective therapy in anidulafungin-resistant *Candida tropicalis* fungaemia: Potential for rapid prediction of antifungal resistance with whole-genome-sequencing

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ABSTRACT

Objectives: This study investigated the feasibility of using whole-genome sequencing (WGS) for the prediction of antifungal resistance in anidulafungin-resistant *Candida tropicalis* candidaemia isolates.
Methods: Next-generation sequencing was performed for three anidulafungin-resistant *C. tropicalis* isolates on an Illumina MiSeq system with in-house bioinformatics analysis.
Results: Mutations in Fks1p associated with anidulafungin resistance were identified. Other mutations associated with varying levels of phenotypic resistance to fluconazole were also identified.
Conclusions: These data demonstrate the potential to predict antifungal resistance using WGS. With improving technology, real-time WGS may be used for tailoring effective antifungal therapy in patients with candidaemia.

Candidaemia commonly occurs in immunocompromised patients. Current guidelines recommend an echinocandin as first-line treatment for candidaemia [1]. The rise of echinocandin resistance is problematic and therapeutic failure is linked to resistance-associated mutations in hotspot regions of the Fks1p subunit of the β-d-1,3-glucan synthase complex. Whilst *Candida albicans* is the most commonly identified agent of candidaemia, other non-*albicans Candida* spp. such as *Candida tropicalis* are clinically relevant in Asia and South America [2]. In this study, we reviewed our laboratory records of *C. tropicalis* fungaemia and identified three patients with echinocandin-resistant *C. tropicalis* isolates between 2015–2018. All three patients had underlying haematological malignancies and were neutropenic at the time of infection. All three patients died from sepsis, with blood cultures remaining positive for *C. tropicalis* and as well as concurrent *Trichosporon asahii*, *Candida glabrata* and *Enterobacter cloacae* complex infections, respectively (Table 1). Although mortality was multifactorial, with the underlying primary haematological malignancy playing a significant role, these cases highlight the threat of increasing antifungal resistance in *Candida* spp. The clinical utility of whole-genome sequencing (WGS) to comprehensively detect antimicrobial resistance in *Candida* spp. is very limited. Here we performed a pilot study exploring the utility of WGS for the prediction of phenotypic susceptibility using these three clinical *C. tropicalis* isolates.

Blood cultures were incubated in BACT/ALERT VIRTUO® automated blood culture machine (bioMérieux, Marcy-l'Étoile, France). The isolated yeasts were later identified using a MALDI® Biotyper matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-TOF/MS) system (Bruker Daltonik GmbH, Bremen, Germany), with antimicrobial susceptibility testing performed by Etest (bioMérieux) and interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. Next-generation sequencing was performed on the three echinocandin-resistant *C. tropicalis* isolates (isolates 1, 2 and 3 corresponding to Patients 1, 2 and 3) on a MiSeq system (Illumina Inc., San Diego, CA) to generate 300-bp paired-end reads. Raw reads were trimmed using Trimmomatic v.0.36 [3] to remove adaptors and low-quality bases and were assembled with ABySS v.2.1.0 [4]. The de novo assemblies were further

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processed using SSPACE [5] and GapFiller [6] to improve assembly quality. An average sequencing depth of 40× was achieved for the genomes. *Candida tropicalis* multilocus sequence typing (MLST) was determined from the genomes using the de novo assemblies (https://pubmlst.org/ctropicalis/) as well as read mapping using the housekeeping genes *ICL1*, *MDR1*, *SAPT2*, *SAPT4*, *XYR1* and *ZWF1a*, respectively. Variant calling for resistance-associated loci was performed using CLC Genomics Workbench 9.5 (CLC Bio, Aarhus, Denmark) with a minimum read coverage of 30 and single nucleotide polymorphisms occurring at a >95% frequency.

All three isolates had a unique MLST profile and likely represented novel diploid sequence types (DSTs) as there were no matching DST assignments in the *C. tropicalis* MLST database (Table 1). In silico typing confirmed that the three isolates had mutations associated with echinocandin and azole resistance (Table 1). The Fks1p mutations detected were different in each isolate (Table 1). These mutations have been previously documented to be associated with elevated echinocandin minimum inhibitory concentration (MICs). In isolate 1, a novel Fks1p M1235I mutation was detected. This mutation has yet to be described and its role in affecting resistance remains unvalidated. Patients infected with Fksp mutants may fail to respond to echinocandin therapy despite phenotypic susceptibility. This highlights the advantage of genomic prediction of echinocandin resistance.

In isolates 2 and 1, the Y257H mutation and the double mutation Y132F and S154F, respectively, were observed in Erg11p. These mutations are associated with fluconazole resistance [7], in particular co-occurring Y132F and S154F mutations being the predominant mutations locally that contribute to high fluconazole MICs (>256 μg/mL) [7]. With the exception of isolate 1 that carried a P448L mutation in Mdr1p (encoding a multidrug efflux pump of the major facilitator superfamily), no mutations were observed in Mdr1p and Cdr1p or in expression regulators Upc2p of the other isolates. The P448L mutation has not been described previously and it would be interesting to determine whether it plays a role in azole resistance.

It is well documented that acquisition of resistance mutations in *C. albicans* leads to a loss of fitness [8,9]. However, these drug-resistant isolates are able to overcome the fitness cost through compensatory mutations [10]. It is likely that similar mechanisms of fitness recovery exist in *C. tropicalis*, although this has yet to be documented. In this study, we were limited in our ability to investigate the presence of compensatory mutations as such work requires matched isolate pairs or isogenic strains in order to follow the development of mutations.

Table 1
Clinical description of *Candida tropicalis* fungaemias and characterisation of the isolates.

	Patient/isolate 1	Patient/isolate 2	Patient/isolate 3
Clinical description			
Primary haematological malignancy	Relapsed refractory B-cell acute lymphoblastic leukaemia	Relapsed stage IVb diffuse large B-cell lymphoma	Acute myeloid leukaemia
Antifungal therapy	Initially i.v. anidulafungin followed by addition of i.v. voriconazole	Initially i.v. anidulafungin followed by switch to i.v. amphotericin B	Initially i.v. anidulafungin followed by switch to i.v. amphotericin B and i.v. fluconazole
Clinical course	Died of sepsis. Concurrent *Trichosporon asahii* fungaemia	Died of sepsis. Concurrent *Candida glabrata* fungaemia	Died of sepsis. Concurrent *Enterobacter cloacae* bacteraemia
Microbiological and molecular characterisation of clinical *C. tropicalis* isolates			
Length of time taken for:			
Blood culture positivity	19 h	26 h	16 h
Species identification	44 h	39 h	29 h
AST results	72 h	109 h	69 h
Antimicrobial MICs (μg/mL) [susceptibility interpretation][a]			
Fluconazole	>256 [R]	4 [I]	0.5 [S]
Voriconazole	12 [R]	0.25 [I]	0.06 [S]
Anidulafungin	0.5 [R]	0.25 [R]	0.5 [R]
Amphotericin B	0.25 [S]	0.5 [S]	0.12 [S]
Mutations in resistance-associated loci[b]			
Fks1p	F641L, M1235I	D648V	S645P
Erg3p	–	–	–
Erg11p	Y132F, S154F	Y257H	–
Cdr1p	–	–	–
Cdr2p	–	–	–
Mdr1p	P448L	–	–
Upc2p promoter	–	–	–
Upc2p	–	–	–
Allele profile of genes used for MLST			
ICL1	1	1	5
MDR1	7	117	7
SAPT2	22	4	4
SAPT4	46	7	Closest match 5[c]
XYR1	Closest match 141[d]	92	92
ZWF1a	22	1	2

i.v., intravenous; AST, antimicrobial susceptibility testing; MIC, minimum inhibitory concentration; MLST, multilocus sequence typing.
[a] Interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints: S, susceptible; I, intermediate; R, resistant.
[b] All mutations were homozygous.
[c] Three differences compared with reference allele SAPT4_5 (43M → 43C; 130C → 130T; and 133G → 133A).
[d] Two differences compared with reference allele XYR1_141 (83Y → C; 242C → T).

Using phenotypic methods, there may be significant delays in identification and antimicrobial susceptibility testing owing to the growth rates of certain micro-organisms. Echinocandin resistance was recognised at ca. 72 h for two patients and past the 96-h mark for one patient (Table 1). Delayed recognition of drug resistance may contribute to poor patient outcomes. As the cost of WGS decreases over time and with software advances to facilitate yeast genome assembly, we envision a shortened turnaround time (ca. 2 days), especially with real-time sequencing technologies such as nanopore sequencing (Oxford Nanopore Technologies), allowing specific antifungal treatment to be more efficiently tailored for invasive *Candida* infections.

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Competing interests

None declared.

Ethical approval

Ethical approval was obtained from the National Healthcare Group Domain Specific Review Board [NHG DSRB ref. 2018/00580].

References

[1] Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 2016;62:e1–e50.
[2] Zuza-Alves DL, Silva-Rocha WP, Chaves GM. An update on *Candida tropicalis* based on basic and clinical approaches. Front Microbiol 2017;8:1927.
[3] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.
[4] Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJM, Birol I. ABySS: a parallel assembler for short read sequence data. Genome Res 2009;19:1117–23.
[5] Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. Scaffolding pre-assembled contigs using SSPACE. Bioinformatics 2011;27:578–9.
[6] Nadalin F, Vezzi F, Policriti A. GapFiller: a de novo assembly approach to fill the gap within paired reads. BMC Bioinformatics 2012;13(Suppl. 14):S8.
[7] Chew KL, Cheng JWS, Jureen R, Lin RTP, Teo JWP. ERG11 mutations are associated with high-level azole resistance in clinical *Candida tropicalis* isolates, a Singapore study. Mycoscience 2017;58:111–5, doi:http://dx.doi.org/10.1016/j.myc.2016.11.001.
[8] Hill JA, O'Meara TR, Cowen LE. Fitness trade-offs associated with the evolution of resistance to antifungal drug combinations. Cell Rep 2015;10:809–19, doi:http://dx.doi.org/10.1016/j.celrep.2015.01.009.
[9] Sasse C, Dunkel N, Schäfer T, Schneider S, Dierolf F, Ohlsen K, et al. The stepwise acquisition of fluconazole resistance mutations causes a gradual loss of fitness in *Candida albicans*. Mol Microbiol 2012;86:539–56.
[10] Ford CB, Funt JM, Abbey D, Issi L, Guiducci C, Martinez DA, et al. The evolution of drug resistance in clinical isolates of *Candida albicans*. eLife 2015;4:e00662, doi:http://dx.doi.org/10.7554/eLife.00662.