Clinical Microbiology and Infection xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Research note

# *Candida blankii*: an emerging yeast in an outbreak of fungaemia in neonates in Delhi, India

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ARTICLE INFO

*Article history:*
Received 3 October 2019
Received in revised form
11 December 2019
Accepted 1 January 2020
Available online xxx

Editor: E. Roilides

*Keywords:*
*Candida blankii*
India
Neonates
Outbreak
Whole-genome sequencing

ABSTRACT

*Objective:* Outbreaks of fungal sepsis due to emerging and rare multidrug-resistant *Candida* species are increasingly reported in health-care settings. We report an outbreak of fungaemia due to the rare multidrug-resistant yeast *Candida blankii* in an Indian neonatal unit.
*Materials and methods:* Blood cultures grew *C. blankii* in nine neonates in the Neonatal Intensive Care Unit of a multispecialty hospital in Delhi during a period of 7 months. Isolates were identified by internal transcribed spacer and D1/D2 region sequencing. Antifungal susceptibility testing was performed by M27-A3 CLSI broth microdilution. To determine genetic relatedness among *C. blankii* isolates we undertook amplified fragment length polymorphism analysis and DNA sequencing using the Illumina NextSeq500 platform.
*Results: Candida blankii* fungaemia occurred at 2–3 postnatal days in nine low birthweight/very low birthweight neonates. All neonates were treated with fluconazole and four of the nine neonates died, resulting in a case fatality rate of 45%. *Candida blankii* was misidentified or not identified by automated identification systems. Fluconazole had higher geometric mean (GM) MICs (8 mg/L) than the other azoles. Also, anidulafungin (GM-MIC 2 mg/L) had high MICs. Genome sequencing confirmed transmission of genetically mostly indistinguishable strains. The *C. blankii* genome showed an altered 1,3-β-D-glucan synthase protein and several larger deletions in the echinocandin target *FKS1* gene, suggesting potential for development of antifungal resistance.
*Conclusions:* The study emphasizes the emergence of a rare and uncommon yeast, *C. blankii*, with reduced susceptibility to one or more antifungal agents, in nosocomial fungaemia. Genomic insights of this rare yeast are reported using whole-genome sequence typing. **A. Chowdhary, Clin Microbiol Infect 2020;■:1**
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## Introduction

Healthcare-associated fungal outbreaks due to uncommon and new species of fungi have been frequently described in recent years [1]. *Candida* species were the third most common cause of bloodstream infections in a recent point prevalence survey among hospitalized adults and children in the USA [2]. In the last decade, outbreaks of fungal sepsis due to emerging and rare *Candida* species, particularly multidrug-resistant *Candida auris*, have gained ample attention among health-care personnel [1,3]. All outbreaks emphasized the importance of heightened awareness, rapid diagnostic methods and molecular typing tools such as whole-genome sequencing, to promptly investigate and implement aggressive interventions in controlling and preventing these infections in health-care settings [3]. We report a cluster of nosocomial fungaemia in a neonatal intensive care unit of a multispecialty hospital due to the hitherto rare pathogen *Candida blankii* over a period of 8 months.

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https://doi.org/10.1016/j.cmi.2020.01.001
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Please cite this article as: Chowdhary A et al., *Candida blankii*: an emerging yeast in an outbreak of fungaemia in neonates in Delhi, India, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2020.01.001

## Materials and methods

During June 2016 to January 2017, *C. blankii* fungaemia occurred in nine neonates in the neonatal intensive care unit of a multi-specialty hospital in Delhi, India. Blood samples grew yeasts that were identified as *C. blankii* by sequencing of internal transcribed spacer and D1/D2 regions. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) Biotyper (Bruker Daltonics, Bremen, Germany) yielded no identification of yeasts and therefore an in-house data base was created using the first two molecularly identified strains as described previously [4]. The methods for identification, antifungal susceptibility testing, amplified fragment length polymorphism and whole-genome sequencing are detailed in the Supplementary material, Appendix S (Tables S1–S4) [5–7]. No ethical approval was required given the retrospective nature of the study and the focus on the pathogen.

## Results

The first case of *C. blankii* fungaemia occurred in a very low birthweight (VLBW) neonate with severe asphyxia. The infant developed symptoms of sepsis on day 2 after birth and blood culture grew *C. blankii* 2 days after collection. Fluconazole treatment was initiated with a loading dose of 12 mg/kg body weight followed by 6 mg/kg body weight, however, the infant died on day 10. The time span between the first and second patient was 6 weeks. The second case also occurred in a low birthweight (LBW), preterm neonate whose blood culture was collected at day 2 after birth and was positive for *C. blankii* on postnatal day 4. An epidemiological investigation was conducted after the third patient was identified. Overall, nine neonates had *C. blankii* fungaemia within a period of 7 months (Table 1). All neonates had LBW and age at onset of fungaemia ranged from 2 to 3 postnatal days. Of nine neonates, five had a vaginal delivery and four were born by caesarean section. They had one or more common risk factors, i.e. preterm delivery, LBW/VLBW, and six had a central venous line and required ventilator support. All were treated with fluconazole and four of nine infants died, resulting in a case fatality rate of 45%. After the second case of fungaemia was detected, enhanced infection control measures, including strict compliance with standard precautions in particular hand hygiene, and contact isolation were instituted. Surveillance cultures from all health-care personnel in contact with neonates in paediatric and

**Table 1**
Clinical details of nine neonates with *Candida blankii* fungaemia

| Patient | Isolate ID | DOB/DOA (day/month/year) | GA (weeks)/ sex/mode of delivery | BW (g) | Risk factors | Antifungal therapy | NICU stay/outcome |
|---|---|---|---|---|---|---|---|
| 01 | VPCI 936/P/16[b] | 05/06/2016 | 41/M/VD | 1200 | VLBW, IUGR, thrombocytopenia, CVC, severe asphyxia, sepsis, mechanical ventilation | FLU[c] for 10 days Van + Mero for 7 days | 10 days/Death |
| 02 | VPCI 1130/P/16[b] | 19/07/2016 | 37/M/VD | 1900 | LBW, IUGR, sepsis, thrombocytopenia | FLU for 14 days Van + Mero for 10 days | 15 days/Survived |
| 03 | VPCI 1168/P16[b] | 02/08/2016 | 35/F/VD | 1780 | PT, LBW, sepsis, thrombocytopenia | FLU 14 days Van + Mero 14 days | 23 days/Survived |
| 04 | VPCI 1175/P/16[b] | 06/08/2016 | 36/F/LSCS | 1500 | PT, LBW, IUGR, thrombocytopenia, maternal history of preeclampsia, antepartum haemorrhage | FLU for 12 days Ami + Cip for 10 days | 14 days/Survived |
| 05 | VPCI 2237/P/16 | 04/09/2016 | 38/F/LSCS | 1100 | VLBW, Persistent hypoglycaemia, severe asphyxia, sepsis, CVC mechanical ventilation | FLU for 6 days Van + Mero for 6 days | 6 days/Death |
| 06 | VPCI 2251/P/16 | 05/12/2016 | 24/M/VD | 855 | Extremely PT, ELBW, Severe asphyxia, CVC, sepsis, mechanical ventilation | FLU for 10 days | 12 days/Death |
| 07[a] | VPCI 3415/P/16[b] & VPCI 54/P/17 | 21/12/2016 | 24/M/LSCS | 1035 | Extremely PT, VLBW, sepsis, persistent hypoglycaemia, severe asphyxia, CVC, mechanical ventilation | FLU for 10 days Van + Mero for 7 days | 18 days/Survived |
| 08 | VPCI 3429/P/16 | 26/12/2016 | 40/M/LSCS | 3440 | Severe asphyxia, CVC, thrombocytopenia, persistent hypoglycaemia, mechanical ventilation | FLU for 5 days Van + Mero for 5 days | 5 days/Death |
| 09 | VPCI 45/P/17[b] | 09/01/2017 | 28/M/LSCS | 855 | Very preterm, ELBW, severe asphyxia, thrombocytopenia, sepsis, CVC, mechanical ventilation | FLU for 21 days Van + Mero for 14 days | 25 days/Survived |

Abbreviations: Ami, amikacin; BW, birthweight; Cip, ciprofloxacin; CVC, central venous catheter; DOA, date of admission; DOB, date of birth; ELBW, extremely low birthweight; F, female; FLU, fluconazole; GA, gestational age; IUGR, intrauterine growth restriction, LBW, low birthweight; LSCS, lower segment cesarean section; M, male; Mero, meropenem; NICU, neonatal intensive care unit; PT, preterm; Van, vancomycin; VD, vaginal delivery; VLBW, very low birthweight.

[a] Two isolates from two blood cultures were obtained from the same patient.

[b] Isolates selected for whole genome sequencing.

[c] All neonates were given fluconazole 12 mg/kg body weight (loading dose) followed by 6 mg/kg body weight.

Please cite this article as: Chowdhary A et al., *Candida blankii*: an emerging yeast in an outbreak of fungaemia in neonates in Delhi, India, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2020.01.001

maternity units were obtained. The sampling included screening for hand carriage and sampling of fomites, equipment, disinfectants and vials. Considering that all patients developed early-onset sepsis, maternal high vaginal swabs were also cultured. However, all environmental and high vaginal swab cultures were negative for *C. blankii*.

*Candida blankii* showed elongated to oval budding yeast cells and grew at 37°C and 45°C as white to cream-coloured colonies on Sabouraud dextrose agar. VITEK-2 (bioMérieux, Marcy l'Étoile, France) misidentified five *C. blankii* isolates as *Candida cifferi* and another five as *Trichosporon asahii*. An in-house *C. blankii* database ($n = 2$) in MALDI Biotyper correctly identified the remaining eight isolates with score values of 2–2.2. Antifungal susceptibility testing showed that fluconazole had higher MICs (geometric mean (GM) MIC 8 μg/mL) than the other azoles, i.e. isavuconazole (GM MIC 0.07 mg/L), posaconazole (GM MIC 0.13 mg/L), itraconazole (GM MIC 0.18 mg/L) and voriconazole (GM MIC 0.25 mg/L). Anidulafungin (GM MIC 2 mg/L) had high MICs whereas micafungin (GM MIC 0.06 mg/L) exhibited potent activity (Table 2).

The average draft genome sequence size of *C. blankii* is 13.65 Mb with an average G + C content of 54.3%. The six assemblies ranged between 1706 and 11176 contigs and encoded an average of 4370 protein-coding genes. The qualitatively best genome contained 4626 gene models, which appears to be a reasonable prediction when compared with the closest available sister species, *Tortispora caseinolytica* encoding 4657 protein-coding gene models. Overall, 47 gene models from other *Candida* species were used to determine particular genes involved in antifungal resistance. Such genes showed partially expected homologies (*ERG11*) to other *Candida* species but were also contrasted by relatedness (*FKS1*) to unrelated black yeasts (*Cladophialophora* and *Fonsecaea*). Clonality of outbreak isolates was determined by read mapping against the qualitatively best reference genomes assembled within this study, which yielded a maximum total distance between genomes of 1.17% and a minimum distance of 0.34%, respectively (see Supplementary material, Table S4). A single nucleotide polymorphism analysis indicated high clonality of the investigated strain set, except for one isolate (VPCI_963/P/16) differing in approximately 69 000 total polymorphic variants whereas strain-to-strain variation between all remaining isolates was between 50 and 277 variants in total. The next-generation sequencing data together with the amplified fragment length polymorphism analysis of the Indian *C. blankii* isolated clearly showed a clonal origin for most isolates that was genotypically distinct from the type strain (amplified fragment length polymorphism pattern; Fig. 1).

## Discussion

The study emphasizes the emergence of *C. blankii* in nosocomial fungaemia and reports for the first time the genomic insights of this rare yeast using whole-genome sequencing typing in an outbreak setting. *Candida blankii* was described for the first time in 1968 from the blood of a mink in Canada [8]. This yeast had rarely been isolated from human samples before this outbreak [9–13]. In a

**Table 2**
Antifungal susceptibility profile of *Candida blankii* isolates (n = 10)

| Isolate no. | Drugs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | FLU | ITC | VRC | ISA | PSC | AMB | CAS | MFG | AFG | FC |
| VPCI 936/P/16 | 8 | 0.25 | 0.25 | 0.06 | 0.25 | 0.5 | 1 | 0.125 | 2 | 0.125 |
| VPCI 1130/P/16 | 8 | 0.125 | 0.25 | 0.125 | 0.125 | 0.25 | 1 | 0.06 | 2 | 0.125 |
| VPCI 1168/P/16 | 8 | 0.25 | 0.25 | 0.06 | 0.06 | 0.25 | 1 | 0.06 | 2 | 0.125 |
| VPCI 1175/P/16 | 8 | 0.25 | 0.25 | 0.125 | 0.25 | 0.25 | 1 | 0.06 | 2 | 0.125 |
| VPCI 2237/P/16 | 8 | 0.125 | 0.25 | 0.06 | 0.125 | 0.25 | 1 | 0.06 | 2 | 0.125 |
| VPCI 2251/P/16 | 8 | 0.25 | 0.25 | 0.03 | 0.06 | 0.25 | 2 | 0.06 | 2 | 0.125 |
| VPCI 3415/P/16 | 8 | 0.125 | 0.25 | 0.125 | 0.125 | 0.25 | 2 | 0.06 | 2 | 0.125 |
| VPCI 3429/P/16 | 8 | 0.125 | 0.25 | 0.03 | 0.125 | 0.25 | 1 | 0.06 | 2 | 0.125 |
| VPCI 45/P/17 | 8 | 0.25 | 0.25 | 0.125 | 0.125 | 0.25 | 2 | 0.06 | 2 | 0.125 |
| VPCI 54/P/17 | 8 | 0.25 | 0.25 | 0.06 | 0.25 | 0.5 | 2 | 0.125 | 2 | 0.125 |
| Range | 8 | 0.125–0.25 | 0.25 | 0.03–0.125 | 0.06–0.25 | 0.25–0.5 | 1–2 | 0.06–0.125 | 2 | 0.125 |
| $MIC_{50}$ | 8 | 0.25 | 0.25 | 0.06 | 0.125 | 0.25 | 1 | 0.06 | 2 | 0.125 |
| $MIC_{90}$ | 8 | 0.25 | 0.25 | 0.125 | 0.25 | 0.5 | 2 | 0.125 | 2 | 0.125 |
| GM | 8 | 0.18 | 0.25 | 0.07 | 0.13 | 0.28 | 1.31 | 0.06 | 2 | 0.12 |

Abbreviations: AFG, anidulafungin; AMB, amphotericin B; CAS, caspofungin; FC, 5-flucytocine; FLU, fluconazole; ISA, isavuconazole; ITC, itraconazole; MFG, micafungin; PSC, posaconazole; VRC, voriconazole.

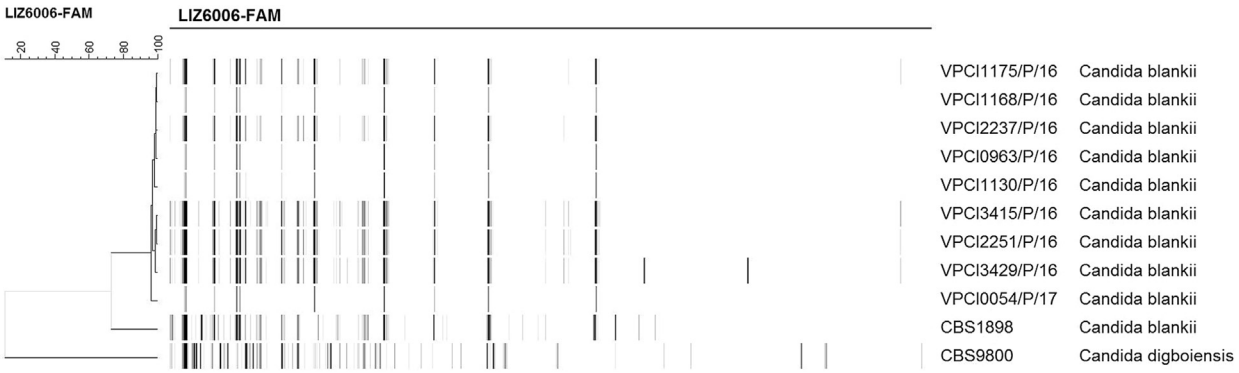


**Fig. 1.** Amplified fragment length polymorphism analysis showing all Indian *Candida blankii* strains had a clonal origin and was genotypically distinct from the *C. blankii* type strain from Canada (CBS1898). The dendrogram was constructed by using UPGMA (unweighted pair group method with averages) in combination with the Pearson correlation coefficient and was restricted to fragments of 60–400 bp. Scale bar indicates the percentage similarity.

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prospective candidaemia study in Norway a single isolate of *C. blankii* was identified [9]. In 2000, *C. blankii* was isolated from the hands of a 2-month-old infant with atopic dermatitis in Russia [10]. Subsequently it was isolated from respiratory samples of a 14-year-old individual with cystic fibrosis in Argentina [11]. So far only two cases of candidaemia with clinical details have been recorded in a 16-year-old girl with cystic fibrosis in Brazil who underwent lung transplantation and in a 27-week-old preterm neonate in Kuwait [12,13]. Notable findings in both cases indicated that *C. blankii* has reduced susceptibility to azoles and echinocandins. Despite the finding that all isolates recovered during this outbreak exhibited high MICs of fluconazole, five neonates treated with high doses of fluconazole for 10–21 days survived and had clearance of fungemia. The lack of susceptibility breakpoints for *C. blankii* and limited therapeutic data preclude the use of any specific antifungals in neonatal sepsis.

It is not known if *C. blankii* exist on human skin or mucosa but its isolation from the skin of an individual with atopic dermatitis suggests that it may colonize damaged skin [10]. Like *C. auris*, this yeast grows at elevated temperature and is tolerant to high salt concentrations and so has appropriate attributes to be a human colonizer and pathogen. Killing assays in a *Galleria mellonella* model has previously shown variable strain pathogenicity of *C. blankii* [12]. Remarkably, a strongly altered 1,3-β-D-glucan synthase protein sequence was inferred from the *C. blankii* genome. A large deletion through almost the entire *FKS1* region was identified together with multiple larger deletions in the glucan synthase subunit. Interestingly for the evolutionary relation with respect to *FKS1*, the cytochrome P450 superfamily was affiliated to black yeasts in the order Chaetothyriales that are often associated with hydrocarbon-rich environments and are pathogenic to humans (e.g. *Cladophialophora bantiana*) [14]. Notably, *C. blankii* and its sister species such as *Candida digboiensis* and *Candida bituminiphila* occur in similar hydrocarbon-rich environments, suggesting a probable link between two unrelated higher-level taxonomic entities. Earlier, in India, *C. blankii* was identified as the most frequently isolated species (4.7%) from nectary gland samples of flowers [15]. In the present outbreak, all isolates, except for one were genotypically indistinguishable with very high genome-to-genome identity, suggesting at least a single, but undetermined, origin and route of transmission. The study is limited by the fact that, despite thorough investigations, the source of the *C. blankii* could not be traced. This report acts as a warning for the importance of the rare and uncommon yeast *C. blankii* that exhibit reduced susceptibility to one or more antifungal agents and has the potential to cause serious invasive infections warranting a thorough understanding of its epidemiology and transmission.

## Funding

This work was supported in part by a research grant from the India Council of Medical Research (F. No. OMI/13/2014-ECD-I to AC), Government of India, New Delhi, India.

## Transparency declaration

JFM has received grants from F2G and Pulmozyme; has been a consultant to Scynexis; and has received speaker's fees from United Medical, TEVA and Gilead. JBS is an employee of Thermo Fisher Scientific, Landsmeer, the Netherlands. All other authors have no conflicts of interest to declare.

## Acknowledgement

We thank Mr. Pradip Roy (Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India), Dr. Ferry Hagen (presently Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands) and Dr. Theun de Groot (Department of Medical Microbiology and Infectious Diseases, Canisius-Wilhelmina Hospital (CWZ), Nijmegen, The Netherlands) for technical assistance.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2020.01.001.

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