

## Research Article

# Recurring Events of *Candida krusei* Septicaemia: First Report from an ICU

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Resurgence of nonalbicans *Candida* spp. was observed thrice at the same location in our hospital over a period of four years. In two of these outbreaks it was identified up to the molecular level as *Candida krusei*. This fungus was traced to some environmental source in all three episodes. Prompt infection control measures were initiated which helped to control the outbreak every time. To the best of our knowledge, this is the first series of recurring *Candida krusei* infections at the same site in a hospital with successful control of each episode.

## 1. Introduction

*Candida* is the fourth leading cause of blood stream infections in hospitalized patients, with an associated mortality of 40–50% [1]. Outbreaks of candidemia have been linked with contaminated milk bottles, parenteral nutrition, glycerine suppositories, contaminated intravenous fluids, syringe reutilization, health care worker (HCW) hand-colonization, and long term indwelling vascular devices [2]. It has been recognized as a difficult to treat fungal pathogen due to fluconazole resistance and decreased susceptibility to flucytosine and amphotericin B [2].

We present a report in which resurgence of nonalbicans *Candida* spp. was observed thrice at the same place over a period of four years after successful control each time. It was identified as the same strain of *Candida krusei* in the second and third outbreaks. In all the outbreaks the source could be traced and immediate control was achieved.

## 2. Methods

The outbreaks took place in September 2011, May 2013, and October 2014 in the neonatal ICU of our tertiary care

hospital. Blood culture samples were processed by standard laboratory procedures. Cultures yielding growth of yeast like organisms were subjected to morphological identification on corn meal agar, germ tube test, and sugar assimilation test (SAT) by 4% carbohydrate discs (HiMedia, India) on yeast nitrogen base agar. Antifungal susceptibility testing (AFST) was done by broth microdilution as per CLSI Document M27-A3 [3]. *C. krusei* ATCC 6258 was used as standard strain. For molecular analysis genomic DNA from *Candida krusei* isolates was subjected to RAPD analysis using M13 Primer (5'GAGGGTGGCGGTCT3'). The reactions were performed in a final volume of 50  $\mu$ L with 30 ng of *Candida* genomic DNA, 1  $\mu$ L Primer (1x sigma), 1.32  $\mu$ L Taq DNA polymerase (Bangalore Genei) (3 U/ $\mu$ L), and 5  $\mu$ L MgCl<sub>2</sub> buffer (provided by Bangalore Genei with Taq DNA polymerase). The amplification was performed for ten cycles at low stringency, 95°C—3 minutes and 95°C—1 minute (denaturation), 35°C—1 minute (annealing), and 72°C—1 minute (extension) and for 30 cycles at high stringency, followed by 95°C—1 minute (denaturation), 55°C—1 minute (annealing), 72°C—1 minute (extension), and 72°C—5 minutes (final extension). The amplification products were separated on 1.4% agarose gel and stained by ethidium bromide at a concentration of



FIGURE 1: Growth on Sabouraud dextrose agar.



FIGURE 2: Growth on cornmeal agar 100x.

10 mg/mL stock to a final concentration of 0.1  $\mu\text{g/mL}$  in 1X TBE (Tris-Borate-EDTA) for 5 hours at 3 V/cm and observed under UV light (Gel Doc) [4].

A case definition was established for patients who were likely to have been exposed during this time period having similar risk factors and location. This included any neonate admitted in the neonatal ICU and presenting with signs of infection. Case control study was undertaken to establish risk factors for infection and to help elucidate potential source of contamination. The hospital authorities were alerted and the suspected samples and fluids were sent to Department of Microbiology for culture. Antifungal susceptibility patterns were reviewed followed by molecular analysis to establish the relatedness of suspected organisms. A thorough review of potential breaches in infection control practices in the ICU and at the point of infusate delivery was done. Cultures of the potential point source contaminants in the environment was performed, including intravenous medications administered to patients and processed likewise.

### 3. Results

In the first outbreak, the isolates were identified as nonalbicans *Candida* based on colony characteristics, morphology, and negative germ tube test (unpublished data). Further speciation, antifungal susceptibility, and molecular characterization could not be done. In the second outbreak, seven blood culture samples from patients in the neonatal ICU revealed growth of *Candida krusei* [5]. After 18 months of successful control, *Candida krusei* was isolated again from four patients in the neonatal ICU. All the positive samples were from neonates who had been admitted to the ICU for more than 72 hours.

**3.1. Characterization of the Isolates.** The isolates from patients and the environmental samples in all the three outbreaks showed negative germ tube test and morphology consistent with *Candida krusei* on corn meal agar. The colonies were

white to cream colored, smooth, and glabrous on Sabouraud's dextrose agar. The environmental isolate obtained from the suction bottle had a rough and rugose surface compared to the clinical isolates (Figure 1). Microscopically, small elongated to ovoid budding yeast-like cells, 2-3  $\times$  4-10  $\mu\text{m}$  in size, were seen. Dalmau plate culture on cornmeal agar after 72 hours of incubation at 25°C showed abundant long, wavy, branched pseudohyphae with elongated to ovoid blastoconidia in verticillate branches; chlamydo spores were not seen (Figure 2). Sugar assimilation test and further speciation of the nonalbicans *Candida* were done for isolates in the second and third outbreaks. They were positive for dextrose but negative for lactose, sucrose, inositol, dulcitol, trehalose, maltose, mellibiose, cellobiose, galactose, and xylose.

**3.2. Antifungal Susceptibility Testing.** All *C. krusei* isolates exhibited similar pattern of antifungal susceptibility. They were resistant to fluconazole but susceptible to amphotericin B, caspofungin, and micafungin. Antifungal therapy was started with amphotericin B 1 mg/kg/day intravenously for 2 weeks.

**3.3. Molecular Analysis.** Relatedness among the *C. krusei* isolates was established after matching the RAPD (Rapid Amplified Polymorphic DNA Analysis) profiles. RAPD pattern of isolates showed similar band patterns of *Candida krusei* using M13 Primer in the latter two outbreaks (Figure 3).

**3.4. Investigation of Outbreak and Infection Control.** Relatedness among the clinical and the environmental isolates of *Candida krusei* was established by similar antifungal susceptibility and molecular profiles. The source of infection was found to be multielectrolyte dextrose solution in the first and second outbreaks and suction apparatus in the third outbreak. The brand of multielectrolyte dextrose solution was different in both outbreaks and in both cases yeast was isolated from unopened bottles also. They were immediately withdrawn from the ICU and replaced with another stock.

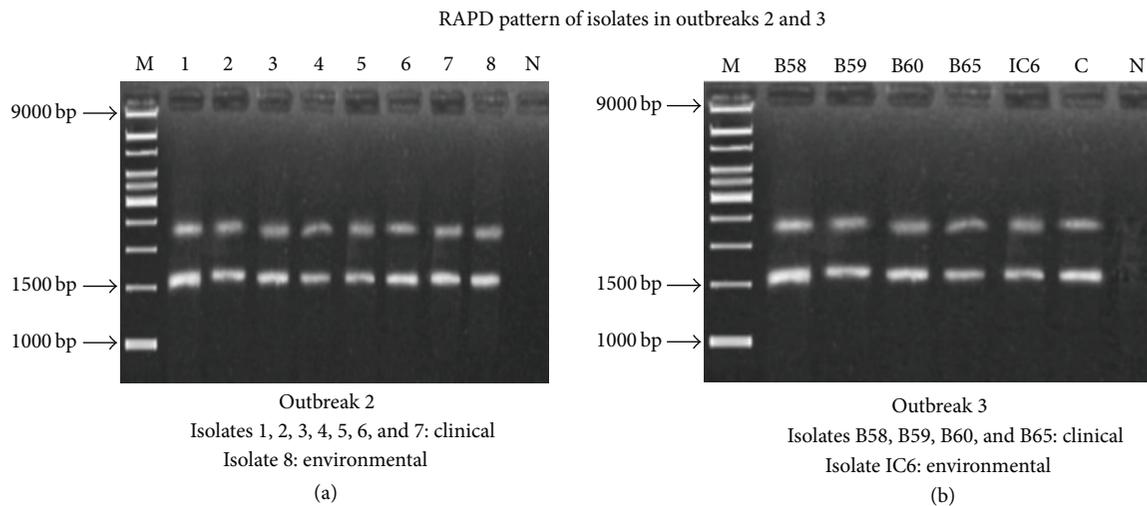


FIGURE 3: Clonality of isolates in outbreaks 2 and 3. (a) Random amplified polymorphic DNA analysis with M13 Primer; Lanes 1 to 8 represent the *Candida krusei* band pattern and Lane M represents the molecular weight markers (1 kb). Lane N represents the negative control. (b) Random amplified polymorphic DNA analysis with M13 Primer; Lanes B58 to IC6 represent the *Candida krusei* band pattern, Lane M represents the molecular weight markers (1 kb), Lane C represents the positive control, and Lane N represents the negative control.

The suction apparatus was properly cleaned, scrubbed, and disinfected; and hand hygiene protocols were reinforced. Infection control surveillance activities were reinforced in the ICU and the health care staff was advised to keep a strict vigil on all the fluids administered. Regular surveillance culture of the environmental fluids and the environmental surfaces were done. No fungus was isolated during the intervening surveillance period.

Though the increase in virulence or pathogenicity was not tested in each episode of fungaemia, it was observed that morbidity and mortality were more in the last outbreak (Table 1). Also the fact that the yeast was recovered from an environmental surface to which the strain had adapted was appreciable.

#### 4. Discussion

*C. krusei* has emerged as a notable pathogen, especially in compromised patient groups in nosocomial settings. Widespread use of triazoles in these patients has contributed to a selective increase in *C. krusei* infection [6].

**4.1. Clinical Manifestations.** Neonates with invasive *Candida* infections usually present with nonspecific signs of infection. There may be temperature and glucose instability, respiratory distress, increase in oxygen requirements, necrotizing enterocolitis (NEC), feeding difficulties, lethargy, abdominal distension, and vomiting. It is difficult to ascertain whether the pathogen is fungus or bacteria, leading to delay in starting the appropriate treatment. There is an inverse relationship between gestational age and the acquisition of fungal sepsis. It has been reported that fungal sepsis is more common if the patient's gestational age is <25 weeks as seen among ELBW (extremely low birth weight) and VLBW (very low birth weight) groups [7]. In our study, multiple risk factors were

present in the affected neonates including lower Apgar score and low birth weight.

**4.2. Laboratory Investigations.** Laboratory parameters like high C-reactive protein and thrombocytopenia are suggestive of septicemia but positive blood culture is the gold standard for diagnosis. Even if a single yeast cell is visualized in gram-stained smear of a positive blood culture, it should be enough to alert the microbiologist and clinicians of a possible candidemia. Hence prompt treatment with amphotericin B should begin pending the yeast identification and susceptibility results. *Candida krusei* can be identified by negative germ tube test and characteristic colony morphology on corn meal and chromogenic agar media. Glucose is fermented as well as assimilated. Lactose, sucrose, galactose, maltose, and trehalose are neither fermented nor assimilated [8]. This could explain its persistence and growth in the multielectrolyte dextrose solution.

**4.3. Virulence Factors.** These include adherence to host cells, production of phospholipase, proteinase, and formation of hyphae. Few studies have been conducted to understand the various factors of *Candida krusei* in man and laboratory animals. Cytopathic effects of various *Candida* spp. have been studied in cell cultures. *C. krusei*, *C. kefyr*, and *C. parapsilosis* can destroy cultured mouse renal epithelial cells in 48–72 h, whereas the relatively more pathogenic *C. albicans*, *C. tropicalis*, and *C. stellatoidea* induce degenerative changes rapidly and destroy the cultures in a much shorter period of 24 h [9]. Howlett determined the pathogenicity of *C. krusei* by an in vitro organ culture system comprising the dorsal tongue mucosa of neonatal Sprague Dawley rats. In the tissues infected with *C. krusei*, the fungus grew in both the yeast and mycelial phases but generally showed less invasiveness than *C. albicans* or *C. tropicalis* [10]. Cell-surface hydrophobicity

TABLE 1: Differential characteristics in three successive outbreaks.

| Characteristic features     | Outbreak 1   | Outbreak 2 [5]   | Outbreak 3  |
|-----------------------------|--|--|---|
| Causative agent             | <i>Candida nonalbicans</i>   | <i>Candida krusei</i>                                  | <i>Candida krusei</i>   |
| Duration of outbreak        | 2 days   | 11 days  | 10 days   |
| Number of affected neonates | 2  | 7  | 4   |
| Gestational age at delivery | 28 weeks (1)<br>30 weeks (1)   | 28 weeks (5)<br>30 weeks (1)<br>36 weeks (1)           | 26 weeks (1)<br>28 weeks (1)<br>37 weeks (2)                          |
| Host factors                | Prematurity, low birth weight, asphyxia, and TPN                       | Prematurity, asphyxia, low birth weight, IUGR, and TPN | VLBW, TPN, Apnea of prematurity, HIE III, and meconium stained liquor |
| Susceptible antifungals     | Not tested, but patients responded after treatment with amphotericin B | Amphotericin B, caspofungin, micafungin, anidulafungin | Amphotericin B, caspofungin, micafungin, and posaconazole             |
| Resistant antifungals       | Not tested   | Fluconazole  | Fluconazole, flucytosine  |
| Outcome                     | No attributable mortality  | No attributable mortality                              | One recovered, three expired  |

HIE: Hypoxic Ischemic Encephalopathy, VLBW: very low birth weight, IUGR: Intrauterine Growth Retardation, and TPN: Total Parenteral Nutrition.

together with adherence may have clinical implications in fungal infections related to plastic devices such as implants and catheters. A study on 20 oral isolates of *C. krusei* showed 5 times greater hydrophobicity with biphasic assay system compared to *C. albicans* isolates. On polyvinyl chloride catheter disks *C. krusei* produced the most extensive biofilm [6].

**4.4. Antifungal Therapy.** The antifungals effective against *C. krusei* include amphotericin B and echinocandins. These should be continued for two weeks after documented clearance of *Candida* from the blood stream [11]. Amphotericin B disrupts the fungal cell membrane and kills the fungus while echinocandins act at fungal cell wall by irreversibly inhibiting (1, 3)- $\alpha$ -D-glucan synthase. *C. krusei* isolates are intrinsically resistant to fluconazole as seen among our isolates also. Voriconazole may remain effective due to more efficient binding to the cytochrome P-450 isoenzyme. Resistance to azoles may develop due to alteration in the target enzyme, 14 $\alpha$ -demethylase, or its inhibition by deficiency of C5 desaturase enzyme [12]. This leads to accumulation of C14 methylated sterols and disrupt membrane structure. Another mechanism is decreased drug accumulation, mediated by either diminished uptake or increased efflux of the drug. Decreased susceptibility to amphotericin B and flucytosine has been reported in some *C. krusei* isolates. Hence susceptibility testing of *C. krusei* is warranted to help guide therapeutic decisions. Recovery from candidemia is defined as resolution of all clinical manifestations and documented clearance 2 weeks after therapy [13].

Since *C. krusei* is present in the environment, potential environmental sources of infection must be screened when this isolate grows in culture from sterile sites. Fortunately, in all these outbreaks, finding a source helped to effectively

curb the outbreak in time. However, during the intervening periods, we could never isolate *Candida* from any of the routine surveillance cultures. Reappearance of this fungus despite stringent infection control in the affected area of the hospital remains an enigma. Though we could not speculate the exact reason for their resurgence, it can be said that these outbreaks usually occur when sterilization, disinfection, or other infection control practices are compromised. It may indicate an inanimate shelter including dextrose fluids or hospital environment niche where it can remain dormant over prolonged periods till an opportunity strikes.

Though an environmental source was always traced, we feel that cultures to identify fungal carriage on the hands of health care workers could have been done to find sustenance of this fungus during the interoutbreak periods. We also feel that dedicated trained health care staff posted in intensive care units should not be changed frequently. Training schedules should be intensified, especially for the staff in high risk areas, and they should be sensitized to hand hygiene and other infection control practices in their work area. Hand hygiene protocols should also be followed by the relatives, especially mothers of neonates who frequently handle them for feeding or fondling. Also intravenous fluids should be in smaller pack sizes for the neonates to be consumed during single use. Though the bottles are received as sealed packs, breaches in quality control practices starting from the point of manufacturing to the distributor, supplier, and consumer cannot be ruled out.

Based on literature search, we could find five articles in the medical literature that report six isolated cases and an outbreak of seven cases of *C. krusei* fungemia amongst neonates (Table 2) [14–18]. There are no previous reports of repeated isolation of a fungal pathogen from the same area. This is the first study reporting on recurring *Candida krusei* outbreaks from a tertiary care hospital in Delhi, India.

TABLE 2: Cases of *Candida krusei* blood stream infections cited in the literature.

| References                        | Number of patients | Gestational age at birth   | Risk factors   | Culture origin           | Therapy                     | Outcome   |
|-----------------------------------|--------------------|----------------------------|--|--------------------------|-----------------------------|-----------|
| Wanjari et al., 2008 [14]         | 01                 | 28 wks                     | Prematurity, congenital tuberculosis                               | Gastric blood            | Fluconazole, amphotericin B | Recovered |
| Patted et al., 2009 [15]          | 01                 | 34 wks                     | Prematurity, broad spectrum antibiotics                            | Right ventricular mass   | Not mentioned               | Expired   |
| Natale et al., 2009 [16]          | 01                 | 26 wks                     | Prematurity, thrombocytopenia, and fluconazole prophylaxis         | Peripheral blood culture | Amphotericin B, caspofungin | Recovered |
| Amaral-Lopes and Moura, 2012 [17] | 03                 | 25 wks<br>31 wks<br>29 wks | Premature pneumoperitoneum abdominal distension, and hyperglycemia | Blood culture            | Liposomal amphotericin B    | Recovered |
| Handrick et al., 1989 [18]        | 07                 | Full term                  | Perinatal problems (Not specified)                                 | Blood culture            | Miconazole/ketoconazole     | Recovered |
| Rongpharpi et al., 2014 [5]       | 07                 | 28–36 wks                  | Preterm, low birth weight  | Blood culture            | Amphotericin B              | Recovered |

## 5. Conclusion

*C. krusei* is an uncommon cause of blood stream infections. Detection of simultaneous episodes in the neonatal ICU of our hospital led us to consider the possibility of an outbreak, which was further confirmed after environmental sampling. Continued infection control surveillance activities coupled with molecular analysis of the source and clinical isolates can bring out important information relating to nosocomial infections. Hence recognition, diagnosis, and prompt action are key to infection control. Breaches in infection control protocols pave the way for impending outbreaks but timely intervention including source tracing is the key to effective control.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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