Case Report

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Candida utilis candidaemia in neonatal patients

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In recent years, an evident rise in the frequency of candidaemia caused by non-*albicans* *Candida* species has been reported. In this paper we present three cases of clinically manifested candidaemia caused by *Candida utilis* in neonatal patients hospitalized in the same neonatal intensive care unit within a 6 month period. To the authors' knowledge, only two cases of *C. utilis* candidaemia have been reported in the literature to date, but neither of these involved newborns. Clinical resolution and elimination of *C. utilis* from the blood were achieved using liposomal amphotericin B or caspofungin in all patients.

Introduction

The frequency of candidaemia has been on the rise throughout the last few decades (Arendrup, 2010; Asciglu *et al.*, 2002; Hajjeh *et al.*, 2004). The reason for this is the improvement in medical treatment especially of immunocompromised individuals. Within the child population, the incidence of candidaemia is the highest among infants younger than 3 years of age (Abelson *et al.*, 2005), particularly in newborns (Arendrup, 2010; Hajjeh *et al.*, 2004). Children at the greatest risk of candidaemia are premature infants, especially those with very low birth weight (<1500 g) and extremely low birth weight (<1000 g) hospitalized in neonatal intensive care units (NICUs) (Benjamin *et al.*, 2006; Saiman *et al.*, 2001). *Candida albicans* is the most common causative agent of candidaemia, although there is an evident rise in frequency of candidaemia caused by other, less-virulent species of *Candida* (Colombo *et al.*, 2003; Hajjeh *et al.*, 2004; Hawkins & Baddour, 2003; Hazen 1995; Jabra-Rizk *et al.*, 2005).

Case reports

Case 1

A male premature baby of 890 g birth weight was delivered vaginally in the 27th week of gestation. Mechanical

ventilation was begun immediately due to infant respiratory distress syndrome and Curosurf [1.5 ml (120 mg) once] was given. Antibiotic therapy also started on the first day of the infant's life (3 mg gentamicin daily, 10 mg amoxicillin-clavulanic acid twice daily). On the fifth day of life, due to sepsis of unknown cause, the gentamicin was replaced with 20 mg meropenem twice daily. On the same day, the infant developed a tension pneumothorax and so thoracic drainage was set up. From the seventh day of life, due to the isolation of *C. albicans* from tracheal aspirate and stomach contents, prophylactic fluconazole (6 mg kg⁻¹ twice per week) was given for the next 23 days. Enteral nutrition with mother's milk was gradually introduced alongside total parenteral nutrition given by umbilical catheter for 15 days, then afterwards by central venous catheter (CVC) placed in the subclavian vein for another 15 days. Anti-ulcer prophylaxis was carried out with H₂ blockers (1 mg twice daily) for 25 days. On the 30th day of life, the infant's clinical condition worsened and he became septic (25 mg C-reactive protein (CRP) l⁻¹, thrombocytopenia 59 × 10⁹ platelets l⁻¹). Blood samples were drawn for bacterial and fungal cultures, and the CVC was removed. Fluconazole treatment was discontinued, and a trial of vancomycin (15 mg daily) and liposomal amphotericin B (1 mg kg⁻¹) commenced. Blood cultures revealed the presence of *Enterococcus faecalis* (vancomycin was replaced with 15 mg amoxicillin-clavulanic acid three times daily) and *Candida utilis*. By the third day of this therapy, the blood cultures were negative. The infant was much improved clinically by this point and treatment with

Abbreviations: CRP, C-reactive protein; CVC, central venous catheter; NICU, neonatal intensive care unit; RAPD, random amplification of polymorphic DNA.

liposomal amphotericin B (3 mg kg^{-1}) was continued for another 4 days (7 days in total). During hospitalization in the NICU *C. utilis* was never isolated from any surveillance culture of this patient.

Case 2

A female newborn of 2740 g birth weight born vaginally in the 39th week of gestation was diagnosed with intestinal atresia. On the third day of life, surgical reparation of the ileum was performed while on the fourth day of life a CVC was placed into her subclavian vein. Anti-ulcer prophylaxis was conducted using H2 blockers (3 mg three times daily) for 16 days. Antibiotic therapy commenced on the second day of life (7 mg gentamicin twice daily and 20 mg metronidazole twice daily). On the eighth day of life, the newborn became clinically septic (29 mg CRP l^{-1} , thrombocytes (platelets) dropped to $116 \times 10^9 \text{ l}^{-1}$). Multiple blood cultures were drawn for testing for bacteria and fungi, and were found to be positive for extended-spectrum β -lactamase-producing *Klebsiella pneumoniae*. Gentamicin treatment was discontinued and meropenem treatment (75 mg three times daily) was instituted. On the ninth day of treatment, methicillin-resistant *Staphylococcus epidermidis* was isolated from a blood culture, and therapy with meropenem and metronidazole was continued. On the 11th day of treatment with this regimen, *C. utilis* was isolated from the blood. On the 12th day she became clinically worse. The CVC was removed, and vancomycin and fluconazole (12 mg kg^{-1} daily) were added to therapy. Considering the persistently high CRP, thrombocytopenia, intermittent fevers and repeated positive blood cultures for *C. utilis* despite a 6 day therapy with fluconazole, this antifungal drug was replaced by liposomal amphotericin B (gradually up to 3 mg kg^{-1}). The fifth day of this new treatment brought clinical improvement. Therapy with liposomal amphotericin B was continued for another 15 days (21 days in total). During hospitalization in the NICU, yet before the institution of antifungal therapy, *C. utilis* was isolated from a surveillance culture of the infant's groin.

Case 3

A female newborn of 2800 g birth weight born vaginally in the 38th week of gestation was diagnosed with gastro-schisis. Operative treatment by placement of a Silastic bag was conducted on the same day, followed by abdominal wall reconstruction on the 10th day. The infant was mechanically ventilated for 11 days. From the second day of life she had a CVC placed (for a total of 16 days) into her subclavian vein. Anti-ulcer prophylaxis was carried out with H2 blockers (3 mg three times daily) for 15 days. From the first until the 12th day of life, antibiotic therapy was carried out as well (7 mg gentamicin twice daily and 100 mg ampicillin three times daily). On the 10th day fluconazole (6 mg kg^{-1} every 2 days) was instituted. On the 11th day of life, the infant became clinically septic

($145 \text{ mg CRP l}^{-1}$, without thrombocytopenia). Empiric antibiotic treatment was stopped and meropenem (75 mg three times daily) was introduced, though blood cultures remained sterile. Gradually, clinical appearance and laboratory results indicated improvement, but *C. utilis* was isolated from the blood sample taken on the 17th day of life. The dose of fluconazole was raised to 12 mg kg^{-1} daily, and the CVC was removed. Throughout this time, the infant was clinically stable and without thrombocytopenia, but the CRP level was on the rise. On the 24th day of life the infant's clinical condition worsened and she became febrile with a CRP of 102 and a drop in thrombocyte count from 400×10^9 to $225 \times 10^9 \text{ l}^{-1}$. The blood cultures taken on the 22nd and 24th day were still positive for *C. utilis*, and caspofungin (1 mg kg^{-1}) was introduced into the treatment regimen. During the treatment with caspofungin improvement was noted both clinically and on laboratory tests. After 6 days of caspofungin treatment the blood cultures became sterile and caspofungin treatment was maintained for another 15 days (21 days in total). During hospitalization in the NICU, *C. utilis* was not found in any surveillance culture.

Methods

Blood cultures drawn from the patients were cultured in a BACTEC 9050 system (Becton Dickinson) using BACTEC PedsPlus/F culture vials. Positive vials were Gram-stained and subcultured for organism identification on horse's blood and Sabouraud glucose agars. Preliminary identification of *C. albicans* was done by germ tube test in the clinical microbiological laboratory (Microbiological Laboratory, Department of Diagnostic Laboratories, Children's Hospital Zagreb, Zagreb, Croatia).

Yeast isolates were routinely sent for confirmation of identification, or identification and *in vitro* antifungal susceptibility testing in the National Reference Centre for Diagnosis of Systemic Mycoses. Isolates were identified using the ID 32 C (bioMérieux) yeast identification system and by morphology on cornmeal agar. *In vitro* antifungal susceptibility testing was performed by the ATB FUNGUS 3 (bioMérieux) microdilution method and the results were interpreted according to Clinical and Laboratory Standards Institute recommendations.

Isolation of yeast genomic DNA for random amplification of polymorphic DNA (RAPD) analysis was performed starting from a 3 ml stationary-phase culture grown in Sabouraud glucose medium using the procedure described by Philippsen *et al.* (1991). The extracted DNA was then used as a template in RAPD-PCRs, which were performed according to Gardiner *et al.* (2002). The PCR products were analysed on a 1.5% (w/v) agarose gel with $1 \times$ TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA) and ethidium bromide (0.5 mg l^{-1}) staining. A DNA of bacteriophage λ cut with *Hind*III was used as a molecular size standard.

Table 1. Results of *in vitro* antifungal susceptibility testing of *C. utilis* isolates

Case	Isolate	Origin	Antifungal drug MIC ($\mu\text{g ml}^{-1}$)				
			Amphotericin B	Flucytosine	Fluconazole	Itraconazole	Voriconazole
1	1	BC	<0.5	<4	<1	<0.125	<0.063
	2	BC	<0.5	<4	<1	<0.125	<0.063
2	1	BC	<0.5	<4	<1	0.25	<0.063
	2	SC – groin	<0.5	<4	<1	0.25	<0.063
	3	BC	<0.5	<4	<1	0.25	<0.063
	4	BC	<0.5	<4	<1	0.25	<0.063
	5	BC	<0.5	<4	<1	0.25	<0.063
	6	BC	<0.5	<4	<1	0.25	<0.063
3	1	BC	<0.5	<4	<1	0.5	<0.063
	2	BC	<0.5	<4	<1	0.5	<0.063
	3	BC	<0.5	<4	<1	0.5	<0.063
	4	BC	<0.5	<4	<1	0.5	<0.063
	5	BC	<0.5	<4	<1	0.5	<0.063

BC, Blood culture; SC, surveillance culture.

Discussion

C. utilis has long been known for its industrial applications (Prior *et al.*, 1980), but it has rarely been described as an infectious agent in humans (Alsina *et al.*, 1988; Bougnoux *et al.*, 1993; Hazen *et al.*, 1999; Shih *et al.*, 1999). To the best of our knowledge, only two cases of *C. utilis* candidaemia have been reported in the literature to date (Alsina *et al.*, 1988; Bougnoux *et al.*, 1993). The first patient was a 5-year-old male with AIDS who had developed a CVC-associated transient candidaemia, and the second patient was a 68-year-old immunocompetent man without a CVC. Other reported clinical manifestations of infection

with this pathogen are chronic urinary tract infections (Hazen *et al.*, 1999) and keratitis (Shih *et al.*, 1999).

We present three cases of transient *C. utilis* candidaemia in newborns hospitalized in the same NICU, all within a 6 month period in 2008. In all of the patients, a significant number of risk factors for the development of candidaemia co-existed. They were all newborns, all were on antibiotic therapy, parenteral nutrition and anti-ulcer prophylaxis, all had a CVC in place, underwent a surgical procedure at birth, and two of them were on artificial ventilation. Other authors have reported candidaemia cases in neonatal patients associated with the same risk factors, pointing out prematurity and low birth weight as risk factors either for acquiring infection or for a bad outcome of infection (Asticcioli *et al.*, 2007; Benjamin *et al.*, 2003, 2006; Fridkin *et al.*, 2006).

C. utilis was the single cause of the bloodstream infection only in one newborn. In the other two newborns, *C. utilis* candidaemia was preceded by bacteraemia caused by Gram-negative and Gram-positive micro-organisms or *C. utilis* and Gram-positive bacteria caused simultaneous bloodstream infection. The duration of candidaemia in our patients differed in length from 2 to 14 days. All previously discussed facts would suggest low virulence of this *Candida* species.

Clinically manifested candidaemia caused by *C. utilis* developed regardless of the treatment with fluconazole, either prophylactically or therapeutically. Although all bloodstream isolates of *C. utilis* were susceptible to fluconazole *in vitro* (Table 1), clinical resolution and elimination of fungi from the blood were only achieved using liposomal amphotericin B or caspofungin. A very similar situation was described in the literature, when the sterility of blood cultures was obtained using amphotericin

Fig. 1. RAPD fingerprints of *C. utilis* isolates. 1–13, *C. utilis* isolates; 14, *C. albicans* (ATCC 16231); 15, *Candida parapsilosis* (ATCC 22109); S, molecular size standard.

B, despite the fact that *C. utilis* isolates showed *in vitro* susceptibility to fluconazole (Bougnoux *et al.*, 1993).

C. utilis was not isolated from any surveillance culture from the digestive, respiratory and urogenital tract, nor from the removed CVCs, except from the surveillance culture from one patient's groin skin. All 13 *C. utilis* isolates from our patients (12 blood culture isolates and 1 from the surveillance culture) showed the same RAPD-PCR banding pattern (Fig. 1), indicating them as being clonally related. These findings could suggest the environment and/or hospital staff as the potential source of the infection among our patients (Asticcioli *et al.*, 2007).

There was a time span of 73 to 86 days between each of the three cases of candidaemia. Given this circumstance, we did not search for the source of infection. During the observed 6 month period, other newborn babies with very similar risk factors for developing candidaemia were also hospitalized, and were nursed by the same hospital staff. The efficient application of infection control measures could be the reason why these three cases remained sporadic. By presenting these cases, our intention is to point out *C. utilis* as a possible cause of candidaemia among the hospitalized newborns, as well as our experience in successful treatment of the patients with liposomal amphotericin B or caspofungin.

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