Multidrug-resistant *Trichosporon*: an unusual fungal sepsis in preterm neonates

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We report a cluster of three extremely-low birth weight (ELBW), preterm neonates who developed late-onset sepsis (LOS) by *Trichosporon asahii* within a span of 1 week period. Two of these cases had the initial diagnosis of respiratory distress syndrome and the third one was admitted for low birth weight and prematurity. Initial sepsis screen was negative and blood culture was sterile in all. Late-onset sepsis was developed after the first week of life and the presenting features were lethargy, feeding intolerance, bleeding manifestations, positive sepsis screen and severe thrombocytopenia. The isolates were sensitive to voriconazole but resistant to both amphotericin-B and fluconazole on all occasions. All the infants were treated with liposomal amphotericin-B before the availability of culture reports but the clinical deterioration was rapid and all three neonates succumbed to death before we could procure voriconazole. The source of the outbreak could not be identified from multiple surface cultures from the unit and screening of the health care staffs. We emphasise the need for high index of suspicion for unusual fungal pathogens, resistant to conventional antifungal drugs while treating preterm neonates with LOS.

Keywords: Amphotericin-B, Extremely-low birth weight, Fluconazole, Fungal infection, Neonate, *Trichosporon asahii*

The most common cause of invasive fungal infection in preterm, extremely-low birth weight (ELBW) neonates is *Candida* species, accounting for 9–13% of such infection episodes.¹ Over the past two decades, several studies have reported opportunistic and nosocomial sepsis by other uncommon fungal spp. such as, *Malassezia*, *Trichosporon*, *Rhodotorula* and *Hansenula* in immunocompromised patients and rarely in newborns.² *Trichosporon* spp., a ubiquitous yeast found in soil, is commonly known to cause infection of hair shafts and onychomycosis.³ Intermittent outbreaks of *Trichosporon* spp. are reported in extremely preterm infants causing serious infection with high mortality.^{4,5} Early recognition of these unusual pathogens are important because most of these infections have unpredictable antifungal susceptibility and they carry a poor prognosis.

We report late-onset sepsis (LOS) with *Trichosporon asahii* in a cluster of three preterm, ELBW neonates within a period of 1 week, which was fatal to all of them. On all occasions, the organisms were found to be sensitive to voriconazole but resistant to amphotericin-B and fluconazole.

Case Reports

Three preterm, ELBW neonates developed LOS by *T. asahii* in our Neonatal Intensive Care Unit (NICU) within a period of 1 week. Details of clinical features, laboratory investigations and outcome have been summarised in Table 1. *Trichosporon* spp. was grown in blood culture on two occasions in all of them. Sabouraud dextrose agar was used as culture medium. All isolates were resistant to amphotericin-B and fluconazole but sensitive to voriconazole. Sensitivity against echinocandins was not tested. Antibiotic susceptibility was determined by the Clinical Laboratory Standards Institute (CLSI) disk diffusion testing (document M44-A). Commercially available paper discs for amphotericin-B (100 U/disc), fluconazole (25 µg/disc) and voriconazole (1 µg/disc) were used. The interpretive breakpoints (zone diameters) were defined as sensitive (≥ 15 mm for amphotericin-B, ≥ 19 mm for fluconazole and ≥ 17 mm for voriconazole) and resistant (≤ 10 mm for amphotericin-B, ≤ 14 mm for fluconazole and ≤ 13 mm for voriconazole).

Management was done as per our unit protocol. None of the infants were on antifungal prophylaxis from the beginning. Intravenous (IV) ampicillin and amikacin were started as empirical antibiotics soon after birth in Cases 1 and 2, but no antibiotic was started in Case 3, as she was asymptomatic.

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Table 1 Details of the patients

Parameter	Case 1	Case 2	Case 3
Gestational age (weeks)	27	27	28
Birth weight (g)	920	980	900
Gender	Male	Male	Female
Maternal age (years)	24	28	30
Mode of delivery	SVD	SVD	SVD
PROM	Yes	Yes	Yes
Other maternal problem	Placenta previa		Known case of SLE
Receipt of antenatal betamethasone	No	Yes	Yes
Required resuscitation at birth	Yes	Yes	No
Apgar score 1 minute	4	4	7
5 minutes	6	7	8
diagnosis RDS	RDS	RDS	ELBW and prematurity
Mechanical ventilation	Yes	No	No
CPAP	No	Yes	No
Receipt of surfactant	Yes	Yes	No
Sepsis screen and blood culture at birth	Negative	Negative	Negative
Antibiotics started at admission	Ampicillin + amikacin		Nil
Umbilical venous cannulation	Yes	Yes	No
Parenteral nutrition (10% amino acid, 20% lipid)	Yes	Yes	10% amino acid only
Orogastric feeding with expressed breast milk	Started at 12 hours	Started at 12 hours	Started at birth
LOS developed on	Day 9	Day 8	Day 11
Initial presenting features of LOS	Lethargy, feeding intolerance	Lethargy, feeding intolerance, purpuric spots	Lethargy, feeding intolerance, haemorrhagic gastric aspirate
Repeat sepsis screen	Day 9	Day 8	Day 11
TLC/cumm	4200	4400	3800
CRP (mg/l)	48	82	60
Platelet count/cumm	44 000	23 000	35 000
Blood culture	Day 9 and 12: <i>Trichosporon asahii</i> grown	Day 8 and 12: <i>Trichosporon asahii</i> grown	Day 11 and 14: <i>Trichosporon asahii</i> grown; <i>Trichosporon asahii</i> + <i>Klebsiella pneumoniae</i> grown on day 14
UVC tip/urine/CSF culture	Sterile	Sterile	Sterile
Electrolytes, liver function and renal function test	Remained normal	Remained normal	Remained normal
Antibiotics change	Day 9: vancomycin + meropenem + fluconazole	Day 8: vancomycin + meropenem + fluconazole	Day 11: ampicillin + amikacin + fluconazole
Expired on	Day 15	Day 15	Day 21

SVD: spontaneous vaginal delivery; RDS: respiratory distress syndrome; ELBW: extremely-low birth weight; CPAP: continuous positive airway pressure; LOS: late-onset sepsis; TLC: total leucocyte count; CRP: C-reactive protein; UVC: umbilical venous catheter; CSF: cerebrospinal fluid.

Subsequently, at the clinical suspicion of LOS, antibiotics were changed and fluconazole was added. When the growth of *Trichosporon* spp. was communicated from the Microbiology Department, fluconazole was changed to liposomal amphotericin-B, but no clinical improvement was documented within next couple of days. After the sensitivity pattern was available, we tried to procure voriconazole, which was not readily available. The condition of the neonates deteriorated progressively. All of them developed hyperglycaemia (blood glucose >180 mg/dl) and major bleeding manifestations in the form of intraventricular haemorrhage/pulmonary haemorrhage/frank gastrointestinal bleed before they succumbed to death and we could start voriconazole. None of them had any evidence of meningitis or

fungal hyphae in urine. Ultrasonography abdomen, echocardiography and ocular examination were normal in all.

A thorough microbial surveillance was done for identification of the possible source of outbreak. Multiple swabs were taken from skin, hands including nails and finger webs, anterior nares and throat of all health care staffs and other persons who had access to NICU. Surface cultures were taken from floors, walls, wash basins, warmers, cots, phototherapy units, monitors, syringe pumps, resuscitation equipments, ventilators, CPAPs, furnitures, freeze, weighing machines, infantometers, measuring tapes, stethoscopes, telephones, air-conditioner ducts, air filters and all other electronic equipments and non-disposable items of all the cubicles. Air samples and

Table 2 Details of studies reporting neonatal infections with *Trichosporon* species

S. No.	Published articles	Case details
<i>Trichosporon asahii</i>		
1.	Panagopoulou et al. ³	The authors reported a 26-week-old female neonate weighing 890 g, who had severe respiratory distress syndrome and late-onset sepsis with <i>Klebsiella pneumoniae</i> and <i>T. asahii</i> on sixth day of life. The infant was successfully treated with conventional amphotericin-B.
2.	Yildiran et al. ⁹	The authors reported a 27-week-old female neonate with late-onset sepsis and pneumonia. <i>T. asahii</i> was grown on blood and urine culture. The baby responded to conventional amphotericin-B in a dose of 1 mg/kg, q8h for 3 weeks.
3.	Girmenia et al. ¹⁰	The authors reported late-onset sepsis in a premature male infant who responded to flucytosine and conventional amphotericin-B. Blood culture grew <i>T. asahii</i> , <i>Escherichia coli</i> and <i>Klebsiella</i> spp.
4.	Tellez-Castillo et al. ¹¹	The authors reported a fatal case of <i>T. asahii</i> infection in a premature newborn of 685 g. <i>T. asahii</i> was isolated from endovascular catheters and endotracheal tube.
5.	Pereira et al. ¹²	The authors reported disseminated <i>T. asahii</i> infection in a newborn infant weighing 815 g.
6.	Vashishtha et al. ⁴	The authors reported an outbreak of <i>T. asahii</i> infection in eight newborn infants with sepsis. The infection was fatal in six of them. The organism was sensitive to amphotericin-B but resistant to fluconazole. The source of the outbreak was suspected to be the laminar flow system of the unit.
<i>Trichosporon beigelii</i>		
7.	Giacola et al. ¹³	The author reported a case of neonatal sepsis caused by <i>T. beigelii</i> in a granulocytopenic premature twin infant.
8.	Fisher et al. ⁵	The authors reported a cluster of four cases of <i>T. beigelii</i> infections. Three cases were very low birth weight premature infants (23–25 weeks), two of whom died. The organism was isolated from urine, skin, blood, tracheal aspirate or central venous catheter tip culture. The last case was a full-term infant with respiratory distress syndrome. <i>T. beigelii</i> was grown from central venous catheter tip with no clinical evidence of infection. The isolates were inhibited but not killed by usually achievable concentrations of amphotericin-B.
9.	Yoss et al. ¹⁴	The authors reported two cases of neonatal <i>T. beigelii</i> sepsis and two cases of neonatal <i>T. beigelii</i> colonisation. The first one was a 23-week-gestation male who developed clinical evidence of sepsis on day 10 with <i>T. beigelii</i> isolated in blood culture on day 12. He expired within 2 days despite receiving amphotericin-B. The second one was a 23-week gestation female developing <i>T. beigelii</i> septicaemia in second week of life. <i>T. beigelii</i> grew from suprapubic urine, tracheal aspirate and umbilical catheter tip cultures. She also died 2 days later despite therapy with amphotericin-B. Two other infants were found to have colonisation of central vascular catheters with <i>T. beigelii</i> , without any evidence of invasive disease.
10.	Sweet et al. ¹⁵	The authors reported a case in a 25-week gestation, 950 g female infant who had evidence of disseminated infection with <i>T. beigelii</i> . The infant survived after treatment with liposomal amphotericin-B.
<i>Trichosporon asteroides</i>		
11.	Herbrecht et al. ¹⁶	The clinical and mycological features of late-onset sepsis with <i>T. asteroides</i> are described in a preterm low birth weight neonate. The infant responded to amphotericin-B treatment.
<i>Trichosporon mucoides</i>		
12.	Gokahmetoglu et al. ¹⁷	In this study, the authors reported <i>T. mucoides</i> infections in three premature newborns.

q8h: 8-hourly.

samples from all antiseptic solutions used for disinfection and cleaning and water used in humidifiers were also taken. No yield of organism was detected in any of these cultures.

Discussion

Difficulty in diagnosis, resistance to conventional antifungal drugs and association with high mortality rates were the main features in the present series of *Trichosporon* sepsis. The clinical presentations were similar in all three cases and there was no clue to differentiate from LOS of other fungal aetiology. None of the infants was very sick initially but the condition progressively deteriorated over time. Possible risk factors for *Trichosporon* infection could be extreme prematurity, prolonged hospital stay, presence of central venous line and use of broad spectrum antibiotics (Cases 1 and 2).

As per literature, though trichosporonemia represents a small percentage of all invasive fungal infections, *Trichosporon* spp. is the second or third most commonly reported agents of yeast fungemia.⁶ Most of the reports are from cancer patients with central venous catheters or from patients suffering from chronic illness with disruption of skin and mucous membranes.^{6,7} Reports of neonatal infections are not very common. A Spanish multicentre prospective survey analysing 1357 fungemia episodes found the incidence of neonatal sepsis by *Trichosporon* spp. to be 0.36%.⁸ Several other authors reported occasional outbreaks of trichosporonemia from different NICUs involving single or a small number of patients.^{3–5,9} Commonly isolated *Trichosporon* spp. in all age groups include *T. asahii*, *T. beigeli*, *T. asteroides*, *T. mucoides*, *T. coremiiforme* and *T. dermatis*.¹⁰

A review of published articles reporting *Trichosporon* infections in neonates has been summarised in Table 2. *T. asahii* and *T. beigeli* are reported more commonly in preterm neonates with sepsis. *T. asteroides* and *T. mucoides* infections are rarely reported. Most of the reported cases were extremely preterm infants, weighing <1000 g and presented as LOS.^{3,4,9–17} Fisher *et al.*⁵ reported one full-term infant growing *T. beigeli* from central venous catheter tip without any clinical evidence of sepsis. Common culture specimens used for isolation were blood, urine, central venous catheter, endotracheal tube, skin swab or tracheal aspirate where *Trichosporon* spp. grew either alone or as a part of polymicrobial flora.³ The largest series reported from India is by Vashishtha *et al.*,⁴ who reported an outbreak of *T. asahii* infection in eight newborn infants. The possible source of the outbreak was the laminar flow system of the unit.

In some of these reports,^{3–5,9} the organism was found to be resistant to fluconazole but sensitive to amphotericin-B, whereas in our series, the organism

was resistant to both the drugs. Large multicentric studies addressing *Trichosporon* invasive infections in all age groups demonstrated high *in vitro* susceptibility to triazoles (voriconazole being the most effective antifungal agent), but poor susceptibility to amphotericin-B, flucytosine and caspofungin.^{18–20} Resistance to amphotericin-B and caspofungin is alarming, as amphotericin-B is the most commonly used systemic antifungal agent in neonates and in recent past, echinocandins are being increasingly used as the first-line agent to treat culture proven cases.²¹ A high incidence of mortality was observed in most of the series.

We emphasise the need for a high index of suspicion for unusual fungal pathogens with prompt availability of culture and sensitivity report to guide appropriate antifungal therapy to salvage these pre-term newborns with LOS.

Disclaimer Statements

Contributors Sriparna Basu – has taken part in conceptualization and design of the study, collection of data, patient management, critical literature review and drafting of the manuscript. She will act as the guarantor of the paper. Ragini Tilak – has taken part in concept and study design, microbiological analysis, literature review and drafting of the manuscript. Ashok Kumar – has taken part in concept and study design, patient management, critical literature review and drafting of the manuscript. All authors have approved the final version of the manuscript.

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