Original Article

RFP Indian Journal of Hospital Infection
Volume 1 Number 1, January - June 2019

Fungemia by Trichosporon asahii in a Neonatal Intensive Care Unit in a Tertiary Care Hospital in Delhi- An Epidemiological Investigation

Gitali Bhagawati[1], Sangeeta Gupta[2], Sanjay Misra[3], Sanjay Singhal[4]

Abstract

Introduction: Neonatal Intensive Care Units (NICUs) have a high rate of nosocomial infections. Invasive fungal infections are increasingly being reported from ICUs. *Trichosporon asahii* is an uncommon emerging cause of sepsis and life-threatening opportunistic pathogen. The present study was to investigate the epidemiological factors related to invasive infection by *Trichosporon asahii* among neonates in a tertiary care hospital in Delhi.

Methods: Seven isolates of *Trichosporon asahii* were reported from routine blood cultures (BacT/ALERT) during a period of two and a half months. Identification and Antifungal Susceptibility testing was done by Vitek 2 Compact System. Outbreak investigations and infection control measures were initiated after the detection of 3rd case. Forty five samples from environment and health care workers were collected and processed to find out the source and infection control measures were strictly implemented.

Results: A total of 7 cases of fungemia by *Trichosporon asahii* occurred in neonates that were mostly preterm and low birth weight. All of them were on broad spectrum antibacterial agents. Environmental samples were positive for this fungus from I.V. cannulae of 4 neonates out of which one developed infection. Strict implementation of Infection control practices resulted in cessation of the outbreak.

Conclusion: The exact source of infection could not be identified. However, the epidemiology of the disease was pointing towards an exogenous source and spread through hands of HCWs. Use of broad spectrum antibacterial agents, preterm and low birth weight were predisposing factors. The strict and prompt actions taken to control the outbreak were fruitful showing that breach in infection control practices resulted in the outbreak.

Keywords: Neonatal Intensive Care Units (NICUs), *Trichosporon asahii*, Pre-term, Blood cultures.

Introduction

Neonatal Intensive Care Units (NICUs) have a high rate of nosocomial infections. Although the initial source of colonization and/or infection of the newborn is the mother, subsequently, the newborn is a potential source as well as recipient of microorganisms from other sources (Arnaldo et al., 2011) Late onset sepsis (>3 days of birth) mostly occurs via nosocomial transmission where skin or mucosal colonization by potential pathogens may be acquired from the hands of health care workers (HCWs), water used in incubator, ventilator humidification system or from the fomites such as stethoscopes. After colonization, the organisms get access into the bloodstream through breaks in the skin or mucosa, by gastrointestinal translocation or via invasive devices such as vascular catheters, endotracheal tubes or feeding tubes (Chagas-Neto et al., 2009)

The invasive fungal infections are increasingly being reported from all types of ICUs due to use of broad spectrum antibacterial agents and NICUs are no exception. The risk for invasive fungal infection is high in very low birth weight (VLBW) infants (< 1500 g) and highest for infants born at the youngest gestational ages who survive past the immediate postnatal period (Chagas-Neto et al.,

Author Affiliation: [1]Senior Resident [2,3]Assistant Professor [4]Professor and Head, Department of microbiology, ESI-PGIMSR, ESI-Hospital, Basaidarapur, New Delhi- 110015, India.

Corresponding Author: Sanjay Singhal, Professor and Head, Department of microbiology, ESI-PGIMSR, ESI-Hospital, Basaidarapur, New Delhi- 110015, India.

Email: gitalibhagawati32@gmail.com

2009; Chagas-Neto et al., 2008) The most common fungal pathogens are various Candida species like *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida lusitaniae*, and *Candida glabrata* (Chowdhary et al., 2004). *Trichosporon* belongs to the genus of anamorphic basidiomycetes that is widely distributed in nature. *Trichosporon asahii* is an uncommon cause of fungal sepsis and now emerging as an important life-threatening opportunistic systemic pathogen. Although *Trichosporonemia* represents a small percentage of all invasive fungal infections, *Trichosporon* spp. have been reported as the second or third most common agents of yeast fungemia (CLST, 2012).

We hereby report a cluster of cases of fungemia by *Trichosporon asahii* and the epidemiological investigation related to it.

Materials and Methods

Seven strains of phenotypically similar *Trichosporon asahii* were isolated from the blood of neonates admitted in Neonatal Intensive Care Unit (NICU) at a tertiary care hospital in Delhi over a period of three months, from November 2013 to January 2014. Clustering of Late onset sepsis (>3 days after birth) by this fungus prompted us to evaluate the emergence of an outbreak in NICU. Identification of the fungus, susceptibility testing and environmental sampling were done for the outbreak investigation.

Identification of *Trichosporon asahii*

The samples for blood culture were routinely inoculated into BacT/ALERT blood culture bottles under aseptic and antiseptic precautions. Paired blood culture (two Aerobic blood culture bottles) bottles from each patient were incubated until evidence of positive culture up to 5 days. Any positive blood culture bottle showing arthoconidia, blastoconidia, septate pseudohyphae, hyphae on Gram stain was sub-cultured on Blood agar, Chocolate agar and Sabouraud's dextrose agar (SDA). Two tubes of SDA were used; one tube was incubated at 28°C and the other at °37C for up to 72 hours. Colonies that grow at both the temperature are cream coloured cerebriform type. [Fig. 1] Identification and susceptibility testing were done by Vitek 2 Compact System (bioMérieux, Inc. Durham, North Carolina/USA). For susceptibility testing, the CLSI 2012 guideline criteria for *Candida albicans* were considered [6]. Final identification was done by DNA amplification and sequencing method. D1/D2 region of 26S rDNA were amplified with NL-1(5-GCATATCAATAAGCGGAGGAAAAG) and NL-4(5-GGTCCGTGTTTCAAGACGG) primers. Amplifications were performed in a T-Personal thermocycler (Biometra, Germany). PCR condition was 35 PCR cycles with annealing at 52°C, extension at 72°C for 2 min, and denaturation at 94°C for 1 min. 26S amplified band of all Trichosporons shows same size and position.

Fig. 1: Dry, rough, cream coloured cerebriform colonies of Trichosporon asahii in Sabouroud's Dextrose Agar (SDA)

Environmental sampling

These cases were labeled as an outbreak when three consecutive blood culture positive new born infants were identified. Thereafter environmental samples were collected from NICU in December 2013. A total 45 environmental samples were collected and processed in search of the source of infection. Samples were collected from the cradles, humidifiers, feeding trolley, medicine trolley, floor, linen, intra-venous fluids, water tap, umbilical cord stumps and cannula hubs of neonates and the hands of health care workers in NICU. All the samples were directly plated on Blood Agar and SDA. Plates were incubated at 37°C for 48 hours. In-use test was done for the disinfectants such as sodium hypochlorite and phenol used in NICU as described (Denise et al., 2009)

Measures taken to control the outbreak

Various steps that were taken promptly to control this outbreak are as follows:

- Training on Hand hygiene, Sterilization and disinfection, Biomedical waste management were given to the staff of NICU

- Emphasis was given of Hand hygiene practices and to improve the compliance of the same.
- Accessibility of alcohol based hand rubs (ABHR) that contains 70% Alcohol and Chlorhexidine Gluoconate was checked routinely.
- Continued Medical Education (CME) was conducted to involve all health care workers of the hospital to promote 5-Moments of Hand Hygiene and Proper steps of Hand Hygiene as per WHO guideline [8].
- Focus was also given on rigorous cleaning of the floors and other surfaces with disinfectants at every shift of the hospital staff.
- Cleaning of the incubators and bassinets were done twice in a day.
- Re-emphasis was given on cleaning of the equipments before and after every use.
- Reviewed antibiotic policy of NICU; Discouraged overindulgence of broad spectrum antibiotics and stop antibiotics if not necessary.
- Restriction was done for frequent entry of hospital staff and visitors to NICU.
- Frequent changing of the staff, specially the House keeping staff, was prohibited.

Results

Cases

Seven cases of *Trichosporonosis* were isolated from the admitted cases in NICU over a period of three months [Table 1]. The first case of fungemia by *Trichosporon asahii* was detected on 9th November, 2013 from the blood of a female baby who was delivered by Lower Segment Caeserian Section (LSCS) and admitted in NICU due to preterm delivery with moderate birth asphyxia and early onset sepsis (EOS).

Out of total seven cases, five neonates (71.43%) were preterm (<37 weeks of gestation). Among seven cases, three neonates (43%) were Low Birth Weight (LBW, birth weight <2,500 grams) and three (43%) were Very Low Birth Weight (VLBW, birth weight <1,500 grams). Out of the three VLBW babies, two babies could not survive (Table1). The other predisposing conditions observed among all these cases were Early Onset Sepsis (EOS) (100%) and Mechanical ventilation due to respiratory failure (71.43%). However, no patient was on central line.

On the basis of clinical signs and symptoms of EOS, C - reactive protein (CRP) and procalcitonin level, all the cases were put on Cefotaxime and Amikacin. Later, upon isolation of Matello B-Lactamase (MBL) producing *Acinetobacter* spp. and their antimicrobial susceptibility report, two of the cases (Case 1 and Case 3) were put on Colistin, Netilmycin and Tigecycline. Blood culture of the second case was positive for Methicillin Resistant *Staphylococcus aureus* (MRSA) and thus the patient was put on linezolid, vancomycin and colistin to cover both MRSA and Gram negative spectrum. On the basis of low Agar score, birth weight and suspected EOS, three of the cases (Case 4, 5 and 6) were empirically put on meropenem and vancomycin. The presenting signs and symptoms were non-specific. Low grade pyrexia (100%), respiratory distress (71.43%), feeding intolerance (86%), thrombocytopenia (57%) and hypoglycaemia (71.43%) were common presentations among these cases. No baby had any congenital anomaly except one that had Cytomegalo virus (CMV) infection (Case 4).

On the basis of CLSI guideline for *Candida albicans*, all the isolates were found to be susceptible to fluconazole (MIC ≤ 2) and voriconazole (MIC ≤ 0.12) while intermediate to amphotericin B (MIC = 2) and resistant to caspofungin (MIC ≥ 4). Five of the cases were put on Fluconazole. Since the length of stay was long in Case 4 and Case 5 and both were not responding clinically to Fluconazole, Amphotericine B was also added to their treatment. Out of all these cases, two VLBW babies, Case 3 and Case 6 expired on Day 12 and Day 4 of birth respectively [Table1].

Results of Environmental Sampling: The exact source of infection could not be isolated. Pyogenic as well as fungal culture of the environmental samples (cradle, humidifier, feeding trolley, medicine trolley, floor, linen, intra-venous fluids, water tap etc.) did not show any growth of *Trichosporon asahii*. In-use test also showed no growth of this fungus in the disinfectants that were being used in NICU. However, the culture of cannula hubs from four newborn infants showed growth of *Trichosporon asahii*. Out of these four infants, blood culture was found to be positive for *Trichosporon asahii* in one of them (Case 4). Case 4, who had fungemia by this fungus was a LBW baby (1,800 grams) and had a prolonged stay of 45 days in NICU. The rest three babies who were colonized by this fungus, but not infected, had birth weight more than 2,500 grams.

Results of the measures taken to control this outbreak: The prompt and strict actions that were taken to control this outbreak showed a great response within a short span of time. No new case had

Table 1: Clinical and microbiological findings of the newborns with Trichosporonosis

Case no.	SEX	Date of positive blood culture for T. asahii /Day of Birth	Previous blood culture report	Date of admission	Mode of delivery	Birth wt. (grams)	Indication for hospital admission	Mechanical ventilation	Central line	Antibiotics prior to isolation of T. asahii	Antifungal after isolation of T. asahii	Length of stay (Days)	Final Outcome
1	F	9/11/13/ Day 14	NG	25/10/13	LSCS	2400	Preterm, LBW, RF, EOS	YES	NO	TGC, CT, NET	FLU	37	Improved
2	M	11/11/13/ Day 25	NG	16/10/13	LSCS	1400	Preterm, SGA,VLBW, Delayed cry, BA, EOS, RF, Persistent Hypoglycaemia, Congenital pneumonia	YES	NO	LZ, VAN, NET, CT	FLU	36	Improved
3	M	02/12/13/ Day 7	NG	25/11/13	PT Breech	1100	Preterm, VLBW, BA, EOS, Apnea, RF, Thrombocytopenia, NEC	YES	NO	CTX, AK, NET, CT, TGC	FLU	12	Expired
4	M	20/12/13/ Day 9	NG	10/12/13	FTND	1800	Term, IUGR, Hypoglycaemia, CMV IgM Positive with hepatitis, thrombocytopenia, COA with PDA	NO	NO	CTX, AK, VA, MEM	FLU, AMP.B	45	Improved
5	M	23/12/13/ Day3 8	NG	15/11/13	FTND	2400	Term, IUGR, RF	YES	NO	CTX, AK, VA, MEM	FLU, AMP.B	46	Improved
6	M	17/01/14/ Day 3	ND	14/01/14	PTND	1000	Preterm, VLBW, EOS, Pulmonary haemorrage, NEC	YES	NO	CTX, AK, VA, MEM	FLU	4	Expired
7	F	18/01/14 Day 3	ND	15/01/14	LSCS	3250	Post-dated, EOS, Persistent hypoglycaemia	NO	NO	CTX, AK, LZ, PIP TAZ	FLU	13	Improved

Note: NG: no growth, ND: not done, LSCS: Lower segment caesarian section, PT: preterm, FTND: full term normal delivery, EOS: early onset sepsis, SGA: small for gestational age, LBW: low birth weight, VLBW: very low birth weight, BA: Birth Asphyxia, RF: respiratory failure, IUGR: Intra uterine growth retardation, COA: Coarctation of aorta, PDA: Patent ductus arteriosus, NEC: Necrotising enterocolitis, CMV: Cytomegalovirus, BAL: Bronchoalveolar lavage, AFB: Acid fast bacilli, ATT: antitubercular therapy, LZ: linezolid, CTX: Cefotaxime, Net: netilmycin, VAN: Vancomycine, CT: Colistin, AK: Amikacin, MEM: meropenem, TGC: Tigecycline, PIP TAZ: Piperacillin Tazobactum,FLU: Fluconazole, AMPB: Amphotericine B.

immerged after the last case that was identified in January, 2014. Surveillance is still going on for the identification of such cases.

Discussion

Trichosporon asahii is emerging yeast that causes disseminated and nosocomial infections in neonatal ICU (Chowdhary et al., 2004; Girmenia et al., 2005). In literature, first description as a cause of invasive disease was found in 1970; *which was* recognized as a cause of systemic illness in immune compromised patients. Hematologic malignancies were the most common (63%) risk factors for *Trichosporonosis* among all the reported cases (Kaufan, 2010; Niki et al., 2002; Ruan et al., 2009). *Trichosporonosis* is usually an insidious disease and its diagnosis is likely to be missed because of lack of awareness about the fungus and lack of specific diagnostic features associated with the etiologic agent (Ryan and Maves, 2018).

A search on internet in the main databases (MEDLINE, LILACS, and SciELO) returned only few articles reporting neonatal infection by *Trichosporon asahii* in preterm newborns. Most of these weighed less than 1,000 g at birth and only one weighed more than 1,500 g at birth. The studies revealed death of 6 neonates out of total 14 preterm newborns. All deaths occurred in the extremely low birth weight group (Chowdhary et al., 2014). In an Indian study, it was found that the infected preterm infants were not extremely premature and had comparatively higher weights, and even full term neonates were affected by *Trichosporon asahii*; the study mentioned death of 6 neonates out of total 8 cases of sepsis by this fungus (Girmenia et al., 2005) In our series, out of total seven cases, three newborns were pre-term, VLBW weighed less than 1,500g; two cases were term, IUGR; one newborn was pre-term, LBW and the rest one was term with normal weight, 3,250g. In this series, out of total 7 neonates, 2 babies expired. Both of them were VLBW babies.

In regard to *Trichosporon* infection, gastrointestinal colonization and further translocation throughout the gut may be considered the source of infection in immune compromised patients. Morever, from the colonized skin, *Trichosporon* spp. may enter the bloodstream via the percutaneous route through intravascular catheter. In vaginal delivery, up to 14% of woman may harbor this organism in their vulvo-vaginal region and neonates may get colonized. Neonatal skin colonization by *Trichosporon* can also be acquired from the hands of health-care workers. (CLST, 2012; Salazar and Campbell, 2002; Sanjay sanghal, 2013) showed that most of the cases of *Trichosporonosis* were subjected to treatment with broad-spectrum antibiotics and multiple invasive medical procedures before developing fungemia (Shih Ta shang et al., 2010; Tashiro et al.,1994) In our study, the source of infection was presumed to be exogenous. Since the first case, a pre-term, LBW, immune compromised baby was delivered by LSCS, there was less probability of infection via vulvo-vaginal route. Moreover, the baby's previous blood culture was sterile. The baby had sepsis with Metallo-beta-lactamase (MBL) producing *Acinetobacter* spp., for which she was put on broad spectrum antibiotics. The second case, a preterm, VLBW baby was also delivered by LSCS. Both the babies were put on mechanical ventilation but no one was on central line catheter. Both the cases were detected almost simultaneously, with a gap of two days duration. Another reason for labeling the source as exogenous was the positive culture report of one of the environmental samples. The cannula hubs of four babies were found to be positive for *Trichosporon asahii*; one of these babies developed fungemia (Case 5).

The isolates were found to be susceptible to fluconazole and voriconazole while intermediate to amphotericin B and resistant to caspofungin. Susceptibilities to antifungal drugs within different species of the genus *Trichosporon* are largely still not investigated. Triazoles were found to have better antifungal activity both *in vivo* and *in vitro* as compared to amphotericine-B (CLST, 2012; Vipin et al., 2012; Watson and Kallichurum, 1970) In many studies, it was mentioned that although the minimum inhibitory concentrations (MIC) of amphotericine-B may suggest susceptibility, fungicidal activity may not been observed for *Trichosopron asahii* (CLST, 2012; Shih-Ta Shang et al., 2010; White, 2008) As for azoles, miconazole, itraconazole, ketoconazole and voriconazole had higher *in vitro* activity than amphotericin B (Niki et al., 2002; White, 2008) Many authors have suggested combination therapy of high-dose amphotericin B (deoxycholate or liposomal) with either or both 5-flucytosine or voriconazole (CLST, 2012; Ruan et al., 2009; Wolf et al., 2001) Advanced nature of underlying condition and intrinsic resistance to various antifungals explains the high mortality rate (77%) by disseminated *Trichosporonosis* (White, 2008; Wolf et al., 2001; WHO, 2009).

Conclusion

There is no typical pathognomonic clinical

feature for the diagnosis of *Trichosporonosis*. It is still a challenge for the diagnosis and species identification. High index of clinical suspicion is into consideration when dealing with low birth weight preterm infants, particularly those with prolonged hospital stay or with nosocomial sepsis having cocktail of broad spectrum antibiotics but still with unfavorable clinical progress.

The exact source of infection could not be identified. However, the epidemiology of the disease was pointing towards an exogenous source and spread through hands of HCWs. The strict and prompt infection control measures, specially focusing in hand hygiene were truthful to control the outbreak.

Funding: none

Potential conflicts of interest: no conflicts.

References

1. Arnaldo L. Colombo, Ana Carolina B. Padovan, and Guilherme M. Chaves. Current Knowledge of Trichosporon spp. and Trichosporonosis. Clinical Microbiology Reviews. 2011;24(4):682–700.
2. Chagas-Neto, T.C., G.M. Chaves, A.S. Melo, and A.L. Colombo. Bloodstream infections due to Trichosporon spp.: species distribution, Trichosporon asahii genotypes determined on the basis of ribosomal DNA intergenic spacer 1 sequencing, and antifungal susceptibility testing. Journal of Clinical Microbiology 2009;47:1074–81.
3. Chagas-Neto, T.C., G.M. Chaves, and A.L. Colombo. Update on the genus Trichosporon. Mycopathologia 2008;166:121–32.
4. Chowdhary A, Ahmad S, Khan ZU, Doval DC, Randhawa HS. Trichosporon asahii as an emerging etiologic agent of disseminated trichosporonosis: A case report and an update. Indian Journal of Medical Microbiology. 2004;22:16-22
5. Clinical and Laboratory standard institute. M27-S4. 2012;32:11-13.
6. David Kaufman, Karen D. Fairchild. Clinical Microbiology of Bacterial and Fungal Sepsis in Very Low Birth Weight Infants. Clinical Microbiology Reviews. 2004;17(3):638-80.
7. Denise N. Pereira, Silvana S. Nader, Paulo Nader, Patrícia G. Martins, Silvana P. Furlan, Cláudia R. Hentges. Disseminated Trichosporon spp. infection in preterm newborns: a case report. Jornal de Pediatria. 2009;85(5):459-61.
8. Ellner K., McBride M. E., Rosen T., Berman D. Prevalence of Trichosporon beigelii. Colonization of normal perigenital skin. J. Med. Vet. Mycol. 1991;(29):99–103
9. Girmenia C, Pagano L, Martino B et al. Invasive infections caused by Trichosporon species and Geotrichum capitatum in patients with hematological malignancies: A Retrospective Multicenter Study from Italy and Review of The Literature. J. Clin. Microbiol. 2005;43:1818–28.
10. Kaufman DA. Challenging issues in Neonatal Candidiasis. Current Medical Research and Opinion 2010;26(7):1769-78. [Medline].
11. Niki I. Paphitou, Luis Ostrosky-Zeichner, Victor L. Paetznick, Jose R. Rodriguez, Enuo Chen and John H. Rex. In Vitro Antifungal Susceptibilities of Trichosporon Species. Antimicrobial Agents and Chemotherapy. 2002;46(4):1144-6.
12. Ruan, S.Y., J.Y. Chien, P.R. Hsueh. Invasive trichosporonosis caused by Trichosporon asahii and other unusual Trichosporon species at a medical center in Taiwan. Clinical Infecious Diseases. 2009;(49):11–17.
13. Ryan C Maves. Trichosporon Infections. Drugs and diseases: infectious disease. Feb 12, 2018. Medscape.
14. Salazar G.E., Campbell J.R. Trichosporonosis, an unusual fungal infection in neonates. Pediatr. Infect. Dis. J. 2002;(21).pp.161–5
15. Sanjay Singhal. Infection Control in Nursery and NICU. In: Handbook of Hospital Infection Control, 1st ed. CBS Publishers & Distributors Pvt. Ltd. 2013. pp.90-91.
16. Shih-Ta Shang, Ya-Sung Yang Ming-Yieh Peng. Nosocomial Trichosporon asahii Fungemia in a Patient with Secondary Hemochromatosis: A Rare Case Report. Journal of Microbiology, Immunology and Infection. 2010;43(1):77–80
17. Tashiro T, Nagai H, Kamberi P, et al. Disseminated Trichosporon beigelii infection in patients with malignant diseases: immunohistochemical study and review. Eur J Clin Microbiol Infect Dis. 1994 Mar.;13(3):218-24
18. Vipin M Vashishtha, Amol Mittal, Amit Garg. A Fatal Outbreak of *Trichosporon asahii S*epsis in a Neonatal Intensive Care Unit. Indian Pediatrics. 2012;49.pp.745-7
19. Watson KC, Kallichurum S. Brain abscess due to Trichosporon cutaneum. *J Med Microbiol*. Feb 1970;(3):191-3
20. White A.B.. Sterilization and disinfection in the laboratory. In: JG Collee, AG Frase, BP Marmion, A Simmons, Eds. Mackie and Mc Cartney Practical Medical Microbiology, 14th Edn. CHURCHILL LIVINGSTONE 2008.pp.813-34.
21. Wolf DG, Falk R, Hacham M, Theelen B, Boekhout T, Scorzetti G, et al. Multidrug-resistant Trichosporon asahii infection of non-granulocytopenic patients in three intensive care units. Journal of Clinical Microbiology. 2001;39:4420–5.
22. World Health Organisation: Patient Safety: A World Alliance For Safer Health Care: Save lives Clean your Hands. 2009.pp.1-7.