ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 6, Issue – 3, May – 2021 , Page No. : 39 - 45

Study of Fungal pathogens causing Septicaemia in Neonatal Intensive Care Unit in Tertiary care hospital

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Citation this Article: Dr.V.Lakshmanakumar, Dr.M.R.Vasanthapriyan, Dr.J.Suriakumar, "Study of Fungal pathogens causing Septicaemia in Neonatal Intensive Care Unit in Tertiary care hospital", IJMSIR- May - 2021, Vol – 6, Issue - 3, P. No. 39 – 45.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Introduction

Newborn health is the key marker of child health. India accounts for 20% of global births and Infant mortality rate 30 per 1000 live birth, but neonatal mortality accounts for 23 per 1000 live births. Preterm birth, intrapartum-related complications (birth asphyxia or lack of breathing at birth), infections and birth defects cause most neonatal deaths. Infections (33%), asphyxia (21%) and prematurity (15%) are the leading causes of neonatal deaths in India.[1] A newborn is deficient in various components of humoral and cellular immunity. Neonatal infections are unique in several ways. Neonatal sepsis is mostly caused by bacteria and fungi[8]**.** **Early onset of sepsis (EOS)** presents within the first 72 hours of life. **Late Onset Sepsis (LOS)** presents after 72 hours of age.[5] This fungal sepsis is mostly nosocomial and known hazard of prolong life support techniques like ventilator care, venous catheterization, exchange blood transfusions and total parenteral nutrition (TPN), prolonged antibiotic administration for bacterial sepsis.[10]

Aims and Objective of this study are

1. To study the Prevalence of neonatal fungal sepsis.
2. To identify the risk factors for development of fungal sepsis in neonates.
3. Characterization and speciation of the fungal isolates and test the antifungal drugs susceptibility pattern.
4. To guide the clinician for the appropriate treatment with antifungal drugs and help to improve the clinical outcome.

Material and Methods: This study was carried out in the tertiary care NICU and Department of Microbiology of Government Sivagangai Medical College, Sivagangai, TamilNadu. from July 2016 to December 2017.

Sample Collection:[6] Sample of blood, CSF and urine were collected from the clinically selected cases for fungal culture**.** About 0.5- 1ml of blood was collected

and it was added to the Brain Heart Infusion (BHI) broth bottles (5 ml BHI in each bottle) for conventional blood culture**.** Under strict aseptic precautions, CSF samples collected by lumbar puncture method (L3-L4 level of spinal card) in babies suspected meningitis cases. Urine sample was collected aseptically by insertion of sterile urinary catheter or infant feeding tube and it removed immediately after the adequate volume of urine (10 -20 ml) collection in suspected septicaemia cases of newborn.

Processing of samples:[3,4] Direct wet mount and KOH mount: The specimens like centrifuged CSF and Urine deposits were examined under direct wet mount. Gram's stain is done to detect yeast fungal pathogens directly from specimen. Emmons' modification of Sabouraud Dextrose Agar (SDA) was used for culture. The media supplemented with antibiotics, such as Gentamicin and Chloramphenical**.** SDA slants were prepared in a sterile screw capped bottle and sample was inoculated in to two bottles of SDA, and they were incubated at two different temperature i.e 37℃ and 25℃. Colony morphology identified after proper growth.

Identification methods:[2] Candida yeast cells are small oval cell with single budding and Gram positive. On SDA cream-coloured pasty colonies seen after 24-48 hours at 35-37 ℃. It had a distinctive yeast smell. Lacto Phenol Cotton Blue (LPCB) mounts: LPCB mount effective for detection of fungal morphology especially in moulds.

Speciation of candida:[2]For identification and speciation of Candida, Germ Tube Test (GTT), Dalmau plate method on Corn Meal Agar (CMA), urease test, sugar fermentation and assimilation tests (auxanogram), were used. A germ tube is defined as a filamentous extension from a yeast cell and a hyphal structure and there is no constriction at the neck. GTT, **Reynolds-Brande** Phenomenon was used for presumptive identification of C.albicans and C. dubliniensis.. Morphological features of yeast on Corn meal agar (CMA) **DALMAU PLATE METHOD** often allow tentative identification of species. The growth was examined microscopically after removing the lid and placing the culture plate directly under the low and high-power lens. CHROME agar Candida is a rapid plate-based method, contains substrate of fluorochrome dyes which produce different colours for different species of Candida growth to the media. Media was brought from HiMedia laboratories Pvt. Limited, Mumbai (HiCHROM Candida Differential Agar).

Antifungal susceptibility tests done by **Disk diffusion**[7] CLSI M44-A2 document guidelines for antifungal disk diffusion susceptibility testing of Candida species**.** Agar medium used was Mueller-Hinton agar + 2% dextrose and 0.5 µg of methylene blue dye/ml. Disks used with contents of Fluconazole 25 mcg; Itraconazole 10mcg; Voriconazole 1mcg, Amphoteracin B 20 mcg and Nystatin 50mcg. All the 5 discs were placed on to agar medium and incubated for 20-24 h at room temperature,

Results

On analysing 200 cases of neonates, the sex distribution of selected cases was found to be male 99 (49.5%) and female 101 (50.5%).

Table 1: Sex wise distribution of 200 selected cases.

Gender	No. of cases	Percentage
Male	99	49.5%
Female	101	50.5%
Total	200	100%

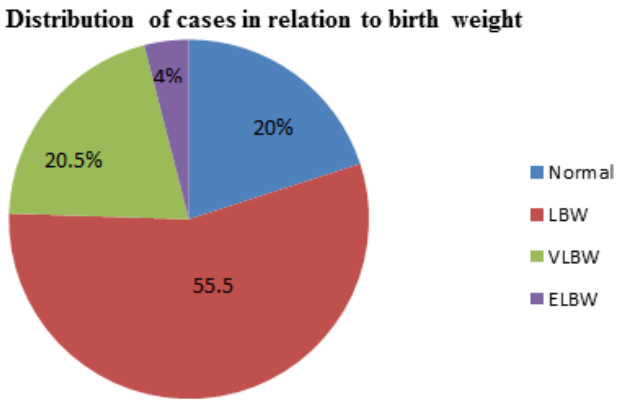
The cases were further analyzed by in relation to place of birth, cases selected from Inborn ward (institutional

delivery babies admitting unit-NICU) was 94(47%), referred from outside (referral cases from PHC, other Government Hospitals, private hospitals) was 104(53%).

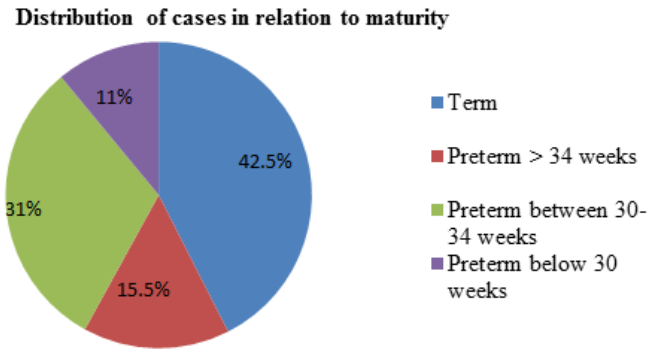
Table 2: Distribution of cases in relation to place of birth.

Birth place	No of Cases	Percentage
Inborn	94	47%
Outborn	104	53%
Total	200	100%

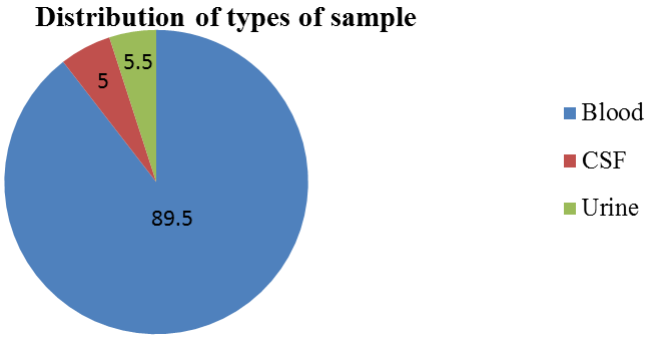
The above cases were further studied for their birth weight. There were 40(20%) cases of normal weight (N >2.5kg), 111(55.5%) cases of low birth weight (LBW <2.5 kg), 41(20.5%) cases of very low birth (VLBW <1.5 kg) and 8(4%) cases of Extremely low birth (ELBW <1.0 kg).



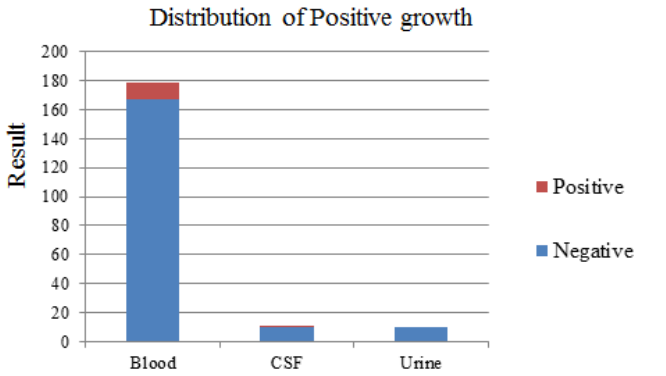
Maturity at birth of each neonates were categorized into term and preterm (below 37 weeks). Term neonates were 85(42.5%) and preterm neonates were 115(47.5). Among the 115 preterm babies, 31(15.5%) cases were late preterm (>34 weeks), 62(31%) cases were preterm between 30-34 weeks and 22(11%) cases preterm below 30 weeks.



Varies categories of risk factors were analyzed in this study. About 78 (39%) cases were stayed more than 7 days in wards at the time of sample collection. Mechanical ventilator support was given for 47 (28.5%) cases. Umbilical catheter was inserted to 13 (6.5%) neonates, who underwent exchange blood transfusion and it was done for neonatal jaundice. No neonate had on central venous catheter insertion. Surfactant therapy was administered for 20(10%) cases, mostly to the VLBW and ELBW neonates. Among the 200 selected cases 45(22.5%) cases were transfused with blood Fresh Frozen Plasma (FFP) and platelet. Out of the200 selected neonates, 87(43.5) cases received broad spectrum antibiotics beyond 5 days. About 9 cases (4.5%) underwent major surgical intervention and they were in post-operative care.



Out of 200 samples inoculated on to SDA, 13 samples showed growth. In that 12 were from blood sample inoculations and 1 from CSF sample. All urine samples were culture negative for fungus.



Germ tube test were positive in 4 isolates out of 13 growths. In CHROM agar, colour production of each candida species were noted and according to the colour, species of Candida were identified. The growth morphology of all isolates in Corn meal agar was examined under microscope.

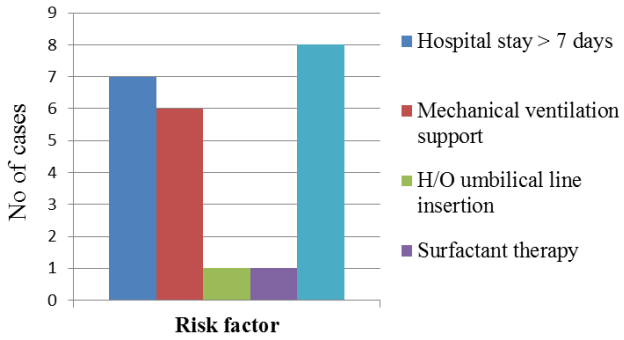
From above special methods and biochemical test, all 13 isolates of candida were differentiated to species level, as C.albicans 3 in number, C.guilliermondii 3 in number, C.glabrata 2 in number and C.parapsilosis, C.dubliniensis, C.tropicalis, C.krusei, C.kefyr each one in number. In that 12 were isolated from blood samples and one C.albicans was from CSF.

Table 11: Species level distribution of Candida

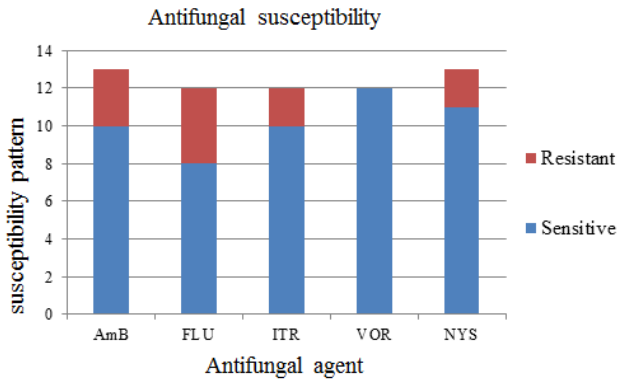
S.No	Name of the species	Number of isolates
1	C.albicans	3
2	C.guillermondi	3
3	C.glabrata	2
4	C.krusei	1
5	C.parapsilosis	1
6	C.tropicalis	1
7	C.kefyr	1
8	C.dubliniensis	1
	Total	13

Out of 13 fungal pathogen 7 were recovered from male neonates. Remaining 6 were female neonates. Fungal sepsis neonates analyzed according to place of birth and 8 cases were from out born cases. Distribution of positive cases in relation to place of birth given in Table 13. Low birth weight neonates were more infected than normal weight neonates. A total of 10 cases of LBW were affected by fungal sepsis and in that 5 of them were VLBW and 1 was ELBW neonates.There were more sepsis cases in preterm neonates. About 9 cases were in preterm category. Among that, one was < 30 weeks of gestational age and three were late preterm > 34 weeks.

Risk factors associated with fungal sepsis cases



Analyzing the Antifungal susceptibility pattern, Amphotericin B resistance was noticed in three isolates. One C.albicans, C.tropicalis and C.krusei resistant to Amphotericin B. Azole drugs tested only in 12 isolates. C.kursei have intrinsic resistance to Azoles. Fluconazole resistance was noticed in 4 isolates, One C.glabrata, one C.albicans, C.tropicalis and C.kefyr. Itraconazole resistant was noticed in 2 isolates. One was C.albicans and another one C.tropicalis. Voriconazole was sensitive to 12 Candida species and among them one C.albicans was in Susceptible Dose Dependent (SDD) range. SDD was taken as sensitive. For Nystatin 11 out of 13 isolates were sensitive, among them 2 were in SDD range and 2 were resistant.



Discussion

The study on Characterization and speciation of fungal isolates in neonatal sepsis in a tertiary care hospital revealed the following finding.

1) Among the 200 selected cases of neonates, fungal pathogens were isolated from 13 cases and the incidence of fungal sepsis was 6.5%

2) More cases were among out born neonates referred from varies centres like PHC, Government HQ hospitals, Private hospitals. There were more chances for exposure to pathogens in outside delivery, while handling and transportation to the tertiary care hospital.

3) Among the fungal sepsis cases, 7 were male neonates, 6 were female, female have a better resistance to infection by possessing double X chromosome which are the dominant factor in immune development.

4) Prematurity and low birth weight was major risk factors for fungal sepsis. Immature immune system among them was main factor of concern.

5) Among the risk factors, administration of broad-spectrum antibiotics prolonged hospital stays for more than 7 days and transfusion had major contributions.

Intervention like mechanical ventilator care, umbilical catheter insertion and surfactant therapy were also implicated as risk factors. The causative role of the above factors is modifiable one by creating awareness and giving better training for all health care workers.

6) Thrombocytopenia (platelet count <1,00,000 cells/ cmm), elevated I/T ratio > 0.2 and positive CRP were the relevant septic screen markers. If the neonates with suspected sepsis have positive for above markers it gives a clue to rule out the fungal sepsis.

7) Conventional culture methods for fungal isolation, SDA with supplemented antibiotics were the primary media. Germ tube test, cornmeal agar morphology, sugar fermentation & assimilation tests were used for speciation of Candida.

8) All the fungal pathogen isolated from samples was belonging to Candida species. Most common species of Candida isolated were C.albicans (3), C. guillermandi (3), C.glabrata (2), each one of C.tropicalis, C.parapsilosis, C.kefyr, C.krusei, C.dubliniensis were also isolated.

9)Resistant pattern was more for Fluconazole (30%), Amphotericin B (23%), followed by Itraconazole (16%), Nystatin (15%). Voriconazole was the least resistant one. Voriconazole is the second line drug for most of the resistant fungi but echinocandins a newer class of drugs are in the next line. Appropriate usage and adequate dosing of antifungal agents will reduce the emergence of resistance. Antifungal susceptibility testing and MIC determination are now recommended for all the isolated fungal pathogens. Micro broth dilution method done in microtitre well, is the standardized method for MIC determination for antifungal agents.

10) Proper care for preterm neonates, restricted usage of broad-spectrum antibiotics, early removal or replacement of indwelling catheters or devices, reducing the unnecessary intervention, early initiation of enteral feeding and earlier discharge are

recommended for controlling the nosocomial spread of fungal pathogen.

11) Prevention of fungal sepsis starts from antenatal period. Proper screening for genital infection and treatment during ANC period is the first step. Despite the place, standard recommendation should be followed for a clean delivery. Transit of sick neonates with all precaution is helpful to avoid exposure of pathogens. Avoidance of unwanted admissions, early discharge of healthy neonates and following a universal precaution will reduce the disease burden.

12) Evaluation for suspected neonatal sepsis is recommended for both bacterial and fungal pathogens. Studies about screening and surveillance of fungal pathogen in neonatal sepsis are helpful for better outcome in neonatal mortality and morbidity.

Summary

The study on Characterization and speciation of fungal isolates in neonatal sepsis in a tertiary care hospital reveals an incidence of 6.5% fungal sepsis. The important risk factors are male preponderance (54%), prematurity (69%), and low birth weight (77%). Most of the sepsis cases are presented as late onset sepsis (75%). Modifiable risk factors like administration of broad-spectrum antibiotics (62%), prolonged hospital stay (54%) and mechanical ventilation care (46%) have an important causative role for colonization and infection. Blood transfusion, surfactant therapy and umbilical catheterization also have associated with risk factors for transmission. Thrombocytopenia is the important marker for fungal sepsis along with increased I/T ratio, positive CRP and reduced total cell count. In fungal culture, only Candida species are isolated from the culture positive specimens. Among them C.albicans, and C.gullermondii are the common pathogens. Antifungal susceptibility pattern showed more resistance for Fluconazole and Amphotericin B.

A conventional fungal culture method was done to identify causative organism. Preliminary observation of samples with wet mount, Gram staining, and culture growth observation lead to a presumptive identification of Candida species. Further processing of growth with microscopic methods like germ tube test and corn meal agar were done for the speciation of the organism. Sugar fermentation and sugar assimilation tests were performed for the conformation. Rapid kit-based identification test and CHROM agar growth were the additional tests that improved the outcome. Antifungal susceptibility testing was done by disc diffusion technique. Micro dilution method is the standardized method to evaluate the MIC of the antifungal agents.

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